

Protocol for Enhanced *In Situ* Bioremediation Using Emulsified Edible Oil



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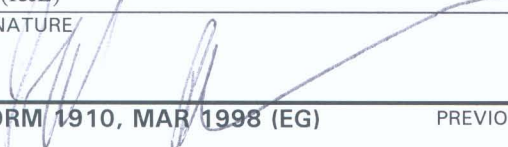
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This protocol focuses on the application of edible oil emulsions to provide a long-lived carbon source to enhance anaerobic bioremediation of contaminants in groundwater. The use of emulsified oils for groundwater remediation is patented by US Patent No. 6,398,9060. International patents are pending.

To discuss applications of this technology please contact:

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APPENDICES

Appendix A	Substrate Calculations
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LIST OF ABBREVIATIONS

°C	degrees Celsius
°F	degrees Fahrenheit
µg/L	micrograms per liter
ρ _B	aquifer bulk density (g/cm ³)
AFB	Air Force Base
AFCEE	Air Force Center for Environmental Excellence
atm	atmospheres
BOD	biological oxygen demand
BTEX	benzene, toluene, ethylbenzene, and xylenes
CAHs	chlorinated aliphatic hydrocarbons
CD	chlorite dismutase
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CH ₄	methane
<i>cis</i> -DCE	<i>cis</i> -1,2-dichloroethene
Cl ⁻	chloride ion
CM	chloromethane
cm ³	cubic centimeters
CO ₂	carbon dioxide
COD	chemical oxygen demand
cP	centiPoises
CPT	cone penetrometer testing
CT	carbon tetrachloride
CSM	conceptual site model
cSt	centiStokes
DCA	dichloroethane
DCE	dichloroethene
DNAPL	dense nonaqueous-phase liquid
DO	dissolved oxygen
DOC	dissolved organic carbon
DOD	Department of Defense
DQOs	data quality objectives
dynes/cm	dynes per centimeter
Eh	measure of oxidation-reduction potential
EOFA	edible oil fatty acids
EOS [®]	Emulsified (edible) Oil Substrate
ESTCP	Environmental Security Technology Certification Program
f _o	fraction of oil in the sediment (g/g)
Fe(II)	ferrous iron
Fe(III)	ferric iron
g	grams
g/ml	grams per milliliter

GMO	glycerol monooleate
GRAS	generally recognized as safe
H ₂	hydrogen
H ₂ O	water
HMX	high melting explosive; octahydro-1,3,5,7-tetranitro-1,3,5,7 tetrazocine
hr ⁻¹	per hour
K _{ow}	octanol-water partition coefficient
K _p	oil-water partition coefficient (mL/g)
K _s (H ₂)	Monod half-saturation constant (for hydrogen)
LIF	laser-induced fluorescence
M	moles
MSDS	material safety data sheet
MC	methylene chloride
MCL	maximum contaminant level
MBT	molecular biological tool
mg	milligram
mg/L	milligrams per liter
ml	milliliters
ml/sec-cm	milliliters per second per centimeter
MNA	monitored natural attenuation
Mn(II)	manganese (II)
Mn(IV)	manganese (IV)
mV	millivolts
n	porosity
n _e	effective porosity
NaBr	sodium bromide
NAPL	nonaqueous-phase liquid
ND	non-detect
NFESC	Naval Facilities Engineering Service Center
nmol	nanomoles
nM	nanomolar = nmol/L
OD	outside-diameter
O&M	operations and maintenance
ORP	oxidation-reduction potential
PCBs	polychlorinated biphenyls
PCE	perchloroethene, or tetrachloroethene
PRB	permeable reactive barrier
psi	pounds per square foot
PVC	polyvinyl chloride
R	retardation factor
RDX	research department explosive; hexahydro-1,3,5-trinitro-1,3,5 triazine
ROI	radius of influence
RTDF	Remediation Technologies Development Forum

SERDP	Strategic Environmental Research and Development Program
TCA	trichloroethane
TCE	trichloroethene
TDS	total dissolved solids
TIC	total inorganic carbon
TNT	trinitrotoluene
TOC	total organic carbon
TPH	total petroleum hydrocarbons
UIC	Underground Injection Control
USEPA	United States Environmental Protection Agency
VC	vinyl chloride
VFAs	volatile fatty acids
VOCs	volatile organic compounds

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SECTION 1 USING THE EMULSIFIED OIL PROCESS

1.1 INTRODUCTION

Management of groundwater contaminated with chlorinated solvents, perchlorate, and explosives is one of the Department of Defense's (DoD's) greatest environmental challenges. Chlorinated solvents have been used for years in both the military and commercial sectors for cleaning and degreasing many products and equipment including aircraft engines, automobile and truck parts, electronic components, and clothing. Widespread use of these compounds has resulted in impacts to the environment. Because of their physical and chemical properties, most chlorinated solvents are relatively recalcitrant in the subsurface, are more difficult to access once they are in the ground, and take longer to remediate. Similarly, groundwater contaminated with perchlorate has become a major environmental issue for the DoD due to the use, release, and/or disposal of solid rocket fuel and munitions containing ammonium perchlorate. These releases have resulted in extensive contamination of groundwater supplies. Perchlorate is highly soluble in water and poorly sorbs to mineral surfaces.

The Strategic Environmental Research and Development Program (SERDP) and the Environmental Security Technology Certification Program (ESTCP) have funded numerous projects to develop and demonstrate new remediation technologies to address these contaminants. Two of these projects focus on the use of emulsified edible oil to enhance *in situ* anaerobic bioremediation of groundwater contaminants: SERDP Project ER-1205 "Development of Permeable Reactive Barriers Using Edible Oils" and ESTCP Project ER-0221 "Edible Oil Barriers for Treatment of Chlorinated Solvent- and Perchlorate-Contaminated Groundwater." This protocol has been developed based on the information gained from these projects.

The objective of this protocol is to provide guidance on the use of emulsified edible oils for enhanced *in situ* anaerobic bioremediation. Edible oils have been applied at more than 60 commercial and military sites nationwide. Although emulsified oils are well demonstrated in the laboratory and the field, this technology continues to evolve. This protocol is based on the current state of practice at the time of writing.

Several other documents and projects complement this protocol. ESTCP funded development of "A Treatability Test for Evaluating the Potential Applicability of the Reductive Anaerobic Biological *In Situ* Treatment Technology to Remediate Chloroethenes" (i.e., the RABITT Protocol), which aids users in determining the site applicability of enhanced anaerobic bioremediation for chloroethene contamination in groundwater (Morse *et al.*, 1998). The "Principles and Practices of Enhanced Anaerobic Bioremediation of Chlorinated Solvents" (i.e., the Principles and Practices document) published cooperatively by the Air Force Center for Environmental Excellence and the Naval Facilities Engineering Service Center (NFESC) describes the scientific basis of enhanced anaerobic bioremediation and summarizes relevant site selection, design, and performance criteria for various engineered approaches to stimulate and enhance *in situ* biodegradation of chlorinated solvents (AFCEE, 2004). AFCEE is also in the process of publishing a "Protocol for *In situ* Bioremediation of Chlorinated Solvents using Edible Oil" (i.e., the Edible Oils Protocol), which focuses on the application of pure liquid edible oils and edible oil emulsions to provide a long-lived carbon source to enhance anaerobic bioremediation of chlorinated solvents in groundwater. In addition, ESTCP is funding a separate project, ER-0319 "Sequestration of a DNAPL Source with Vegetable Oil," to evaluate the use of pure vegetable oil to physically and chemically sequester DNAPLs in the short-term followed by enhanced biodegradation of dissolved

contaminants in the long-term. These documents and projects are referenced throughout this protocol, as applicable.

1.2 INTENDED USE OF THIS DOCUMENT

Emulsified edible oils have been used to stimulate *in situ* anaerobic biodegradation of groundwater contaminants at commercial, industrial and military sites throughout the US. The procedures and applications of emulsified oils for the anaerobic bioremediation of chlorinated solvents are applicable to numerous other anaerobically biodegradable contaminants like nitrates, perchlorates, and energetics (e.g., RDX, TNT). The protocol presented in this document is intended to assist base managers and project engineers in: (1) determining if the emulsified oil process is appropriate for their site; and (2) designing and implementing this technology. This protocol also provides background information on the development and scientific basis of this technology.

The intended audience for this document is DoD personnel and their contractors, scientists, consultants, regulatory personnel, and others charged with remediating contaminated groundwater. This protocol is intended for use within the established regulatory framework appropriate for selection of a remedy at a particular hazardous waste site. It is not the intent of this protocol to prescribe a course of action, including site characterization, in support of all possible remedial technologies. Instead, this protocol is another tool that allows practitioners to gain an in-depth understanding of the emulsified oil process, decide how best to apply it, and then use the technology for site remediation. This protocol will illustrate how the geochemical, aquifer, and contaminant data collected as part of the site characterization evaluation are critical to the feasibility assessment and design of an emulsified oil application.

This document describes (1) development of the emulsified oil process and its effectiveness for stimulating anaerobic biodegradation, (2) methods for applying the substrate to the subsurface, (3) various configurations that can be applied, (4) site conditions that should be evaluated when considering the use of the emulsified oil process, and (5) hydrogeological and engineering considerations for developing an injection layout. Some information in this protocol overlaps material discussed in greater detail in the RABITT Protocol and the Principles and Practices document. Wherever possible, extensive repetition has been minimized by referring to these documents. However, sufficient information is retained so that the reader of this protocol can understand the background of the emulsified oils process without reading other documents.

This first section of the protocol provides an overview of the emulsified oil process and preliminary screening criteria. Site managers considering use of this process should carefully review the information presented in this first section to determine if the emulsified oils process is appropriate for use at their site. If this preliminary evaluation indicates the site is potentially suitable for use of the emulsified oils process, a more detailed evaluation should be performed following procedures described in the remainder of this document.

1.3 OVERVIEW OF ENHANCED ANAEROBIC BIOREMEDIATION

Research and project experience has shown that many groundwater contaminants can be cost-effectively biodegraded *in situ* by providing a source of biodegradable organic substrate. The application of enhanced anaerobic bioremediation is covered in detail in Principles and Practices. The technology is not effective unless the following criteria can be met:

- The contaminants are anaerobically biodegradable;
- Strongly reducing conditions can be generated;
- A microbial community capable of driving the process is present or can be introduced; and
- An organic substrate can be successfully distributed in the subsurface.

Each of these criteria is briefly discussed in the following subsections.

1.3.1 APPLICABLE CONTAMINANTS

Enhanced anaerobic bioremediation has been primarily used for remediation of chlorinated aliphatic hydrocarbons (CAHs). The most common CAHs released to the environment include tetrachloroethene (PCE, or perchloroethene), trichloroethene (TCE), 1,1,1-trichloroethane (TCA) and carbon tetrachloride (CT). These chlorinated solvents and their chlorinated degradation products fall into the categories of chloroethenes, chloroethanes and chloromethanes. Examples of the degradation pathways for chloroethenes, chloroethanes, and chloromethanes are shown in **Figure 1.1**.

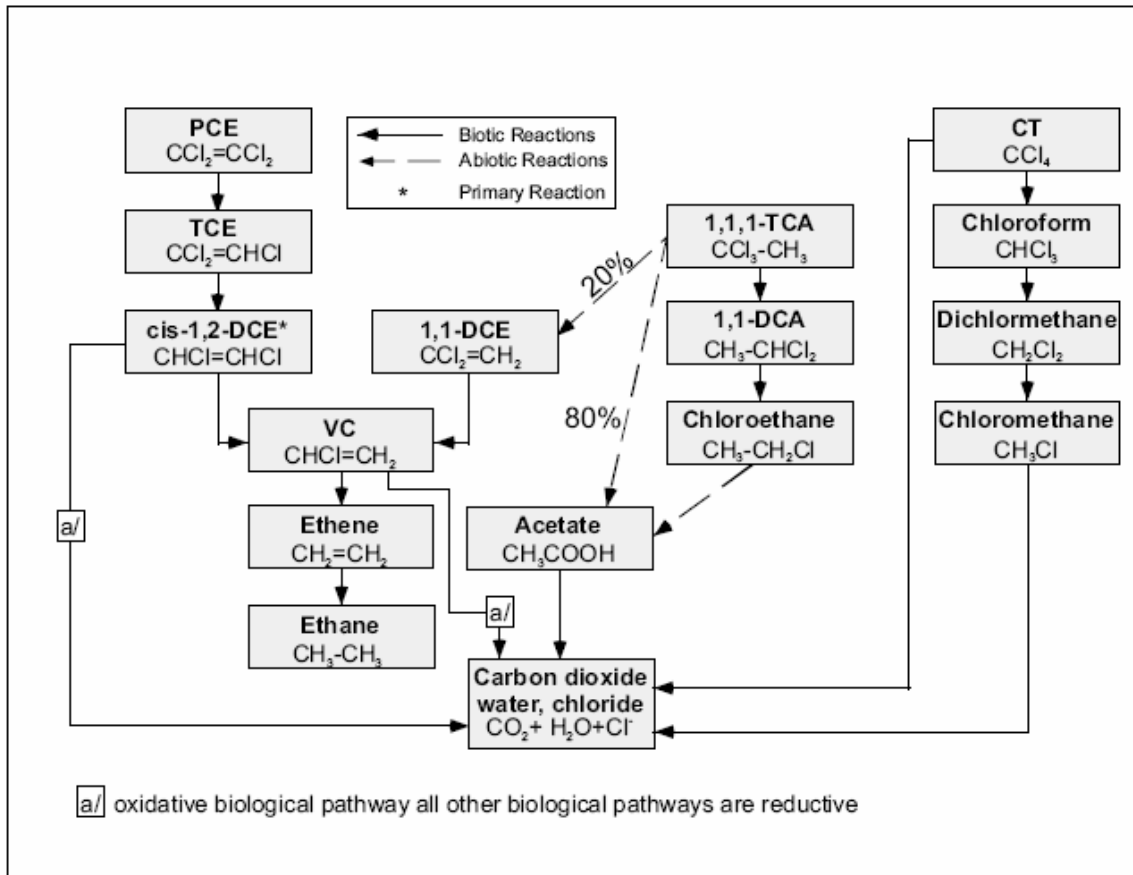


Figure 1.1. Abiotic and Biological Transformation Pathways for Selected Chlorinated Solvents (from Principles and Practices, AFCEE, 2004)

Other groundwater contaminants also subject to enhanced anaerobic bioremediation include the following types of chemicals:

- Chlorobenzenes;
- Chlorinated pesticides (e.g., chlordane), polychlorinated biphenyls (PCBs), and chlorinated cyclic hydrocarbons (e.g., pentachlorophenol);
- Oxidizers such as perchlorate and chlorate;
- Explosive and ordnance compounds (e.g., TNT, RDX, HMX);
- Dissolved metals (e.g., hexavalent chromium); and
- Nitrate and sulfate.

1.3.2 SUITABLE SITE CONDITIONS

In order for the site conditions to be suitable for enhanced anaerobic bioremediation, it must be possible to effectively distribute the organic substrate in the subsurface and generate strongly reducing conditions. Substrate distribution depends primarily on the hydraulic conductivity of the aquifer, the depth to groundwater, and the groundwater flow. In many cases, systems can be designed to overcome difficult hydrogeologic conditions; however, the cost-effectiveness of implementing the technology may be reduced. The ability to generate strongly reducing conditions is dependent on the aquifer geochemistry. Specific factors to consider include dissolved oxygen (DO), oxidation-reduction potential (ORP), iron, sulfate/sulfides, pH, and alkalinity. Section 4.3 provides additional information on how these hydrogeologic and geochemical factors impact the use of emulsified oils.

1.3.3 MICROBIOLOGY

Natural aquifer systems are complex ecosystems populated by a broad and diverse array of microbial communities. The composition and activity of these microbial communities changes continuously as their environment changes. Alterations in aquifer geochemistry and the availability of substrates and nutrients that can be used to generate energy and support growth and reproduction significantly affect microbial activity. The primary objective of injecting food-grade emulsified edible oil into the subsurface is to stimulate the anaerobic biodegradation of the target contaminants. To be successful at a given site, a microbial community capable of driving the process must be present. A brief overview of the microbiology of anaerobic biodegradation for some common groundwater contaminants is provided in Section 2.1.3. An in-depth discussion of the microbiology of reductive dechlorination can be found in the Principles and Practices document (AFCEE, 2004).

If a microbial community capable of degrading the target contaminants is not present, it may be possible to introduce the appropriate microorganisms. This practice is termed bioaugmentation. The ESTCP white paper “Bioaugmentation for Remediation of Chlorinated Solvents: Technology Development, Status, and Research Needs” provides a detailed evaluation of this practice (ESTCP, 2005).

1.3.4 SUBSTRATES

A variety of different substrates have been used to stimulate anaerobic bioremediation. In practice, the added organic substrates are first fermented to hydrogen (H₂) and low-molecular weight fatty acids. These short-chain molecules, such as acetate, lactate, propionate and butyrate, in turn provide carbon and energy for anaerobic bioremediation. The substrates can be broadly categorized into four types: soluble substrates, viscous or low viscosity substrates, solid substrates and miscellaneous experimental substrates. All of these substrates are biodegraded and ultimately yield (or “release”) hydrogen. A thorough

overview and discussion of the application of all these amendments is provided in the Principles and Practices document. The focus to this current protocol is to provide specific guidance on the use and effectiveness of edible oil emulsions for this process.

1.4 THE EMULSIFIED OILS PROCESS

Edible oils have been used in a variety of locations throughout the United States to stimulate anaerobic biodegradation of chlorinated solvents and other contaminants. Methods used to emplace the oil in the subsurface include injection of the oil as a separate non-aqueous phase liquid (NAPL) and as an oil-in-water emulsion. This protocol focuses on distribution of the oil as an oil-in-water emulsion and uses the term “emulsified oil process” to refer to this technology. The emulsified oil process is designed to generate conditions necessary for microbial anaerobic biodegradation (e.g., reductive dechlorination of chlorinated solvents). Hydrophobic (lipophilic) chlorinated solvents will also partition into the oil, reducing aqueous phase concentrations under certain conditions. In this process, known as sequestration, the edible oil can act as a sorbent to quickly reduce chlorinated solvent concentrations in groundwater. As contaminants remaining in the aqueous phase are biodegraded, additional contaminants will be released from the edible oil, and be degraded.

Edible oils are relatively inexpensive, innocuous, food-grade substrates. When properly prepared and injected, edible oils are immobile and slowly biodegraded in most aquifers. A single, low-cost injection can provide sufficient carbon to drive reductive dechlorination for several years. This is expected to significantly lower operation and maintenance (O&M) costs compared to aqueous-phase injection of soluble carbon sources (e.g., lactate and carbohydrates) and will allow addition of slow-release substrates at locations where placement of solid-phase carbon in trenches is not feasible (e.g., large depths, fractured rock). The emulsified oil process can be used either in the contaminant source zone or downgradient as a barrier to contaminant migration.

1.4.1 APPLICATION METHODS

Two general approaches have been used to distribute edible oils in the subsurface: (1) injection of pure liquid oil as a non-aqueous phase liquid (NAPL); and (2) injection of an oil-in-water emulsion. **Table 1.1** provides a brief comparison of these two oil injection approaches.

Table 1.1. Comparison of Injection of Oil as a NAPL Versus as an Oil-in-Water Emulsion

NAPL Oil	Oil-in-Water Emulsions
<p>Characteristics</p> <ul style="list-style-type: none"> ➤ High residual saturation ➤ Large permeability loss ➤ Can sequester chlorinated solvents 	<p>Characteristics</p> <ul style="list-style-type: none"> ➤ Low residual saturation ➤ Low permeability loss ➤ Limited chlorinated solvent sequestration
<p>Strengths</p> <ul style="list-style-type: none"> ➤ Easy to implement ➤ Relatively low cost ➤ Can inject with temporary, direct push points. 	<p>Strengths</p> <ul style="list-style-type: none"> ➤ Easy to implement ➤ Relatively low cost ➤ Can distribute emulsion greater distances from injection point ➤ Much less oil required ➤ Potential to add other co-substrates (e.g., lactate, yeast extract, vitamins)

NAPL Oil	Oil-in-Water Emulsions
<p>Limitations</p> <ul style="list-style-type: none"> ➤ Very limited spread ➤ Requires large amount of oil ➤ Possibility that oil will float 	<p>Limitations</p> <ul style="list-style-type: none"> ➤ Requires large amount of water to distribute/immobilize oil ➤ Requires semi-permanent injection wells. ➤ Emulsion preparation is more complicated.

Pure liquid oil can be injected as a NAPL directly into an aquifer using conventional wells or using temporary direct push points. Injection of the edible oil as a NAPL results in high oil saturations and large reductions in the permeability to water. Typically, oil injected as a NAPL will occupy between 40% and 90% of the aquifer pore space immediately adjoining the injection point. To push the oil farther out away from the injection point, additional oil must be injected. This can require injection of very large volumes of oil (over 20 gallons of oil per cubic yard of treated aquifer). However, these high oil saturations will also ‘sorb’ more of the chlorinated solvents, resulting in a larger decrease in dissolved solvent concentrations. Injection of NAPL oil also dramatically reduces the treated zone permeability to water. A large permeability loss would cause major problems in a reactive barrier system since contaminated groundwater would flow around the barrier, not through it. However, a large permeability reduction in a source area may be an advantage since this will reduce groundwater flow through the injection area/contaminated zone and reduce the mass flux of contaminants discharging to the downgradient aquifer. When considering NAPL injection, care must be taken to evaluate the possibility that excess permeability loss may result in contamination bypassing the treatment zone entirely. The implementation and use of pure NAPL is not discussed in this document. The AFCEE Edible Oils Protocol contains more detailed information on this process.

Edible oils can also be distributed in aquifers as oil-in-water emulsions. In this approach, an oil-in-water emulsion is first prepared using an edible oil (typically soybean oil), food-grade surfactants, and water. Ideally, the emulsion should be stable (e.g., non-coalescing); have small, uniform droplets to allow transport in most aquifers; and have a negative surface charge to reduce droplet capture by the solid surfaces. The emulsion is then injected into the aquifer with water to distribute and immobilize the oil droplets. As oil droplets migrate through the aquifer pore spaces, they collide with sediment surfaces and stick. The sediment surfaces gradually become coated with a thin layer of oil droplets that provides a carbon source for long-term reductive dechlorination. Soluble substrates and nutrients (e.g., lactate, yeast extract, vitamins) can be added to the mixture prior to injection to stimulate rapid growth of desired bacteria. Field and laboratory studies (Borden *et al.*, 2004; Coulibaly and Borden, 2004, Beckwith *et al.*, 2005) have shown that emulsified oils can be transported substantial distances (up to 50 feet) in a variety of aquifer materials with low to moderate oil retention and little permeability loss. As a consequence, emulsified edible oils are more appropriate for use in barriers where minimizing permeability loss is important. The emulsified oil process is the focus of this protocol.

1.4.2 TREATMENT SYSTEM CONFIGURATIONS

Emulsified oils can be used in a variety of different configurations to treat contaminated aquifers including source area treatments and barriers. Source areas can be treated using the emulsified oil process to stimulate anaerobic biodegradation of dissolved, sorbed, and residual non-aqueous phase contaminants (**Figure 1.2a**). The oils will first stimulate rapid biodegradation of dissolved contaminants. Then, as contaminants are slowly released from the aquifer matrix or residual DNAPLs, edible oil will still be present to support biodegradation.

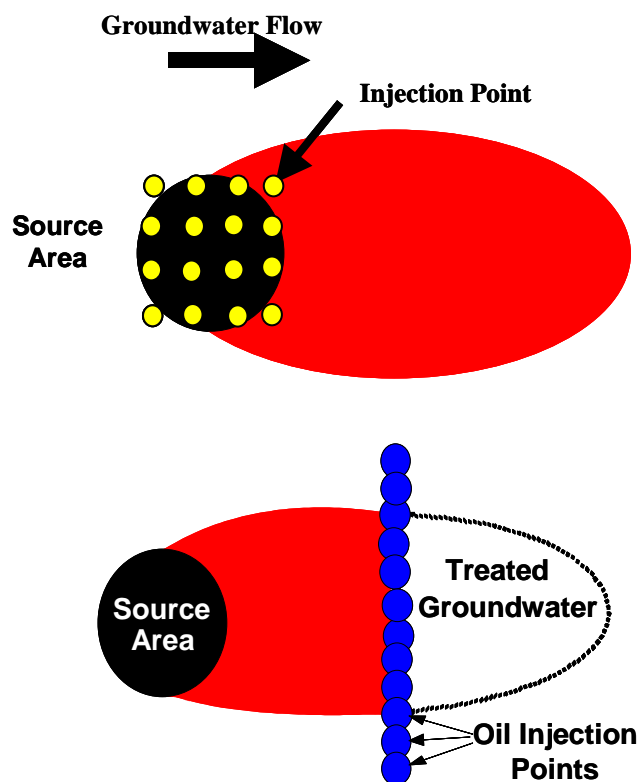


Figure 1.2. Using Edible Oils to Treat Contaminated Groundwater in: (a) Source Areas and (b) Barriers

Emulsified oils are also commonly used to treat contaminated groundwater in a permeable reactive barrier (PRB) configuration by injecting the emulsion through a series of temporary or permanent wells installed perpendicular to groundwater flow (see **Figure 1.2b**). As groundwater moves through the emulsion treated zone under the natural hydraulic gradient, a portion of the trapped oil dissolves providing a carbon and energy source to accelerate anaerobic biodegradation processes. The use of edible oil emulsions minimizes the permeability loss by entrapped oil. If permeability loss were excessive, contaminated groundwater could flow around the barrier and not be treated. Some of the relative advantages and disadvantages of the source area and barrier approaches are summarized in **Table 1.2**.

Table 1.2. Source Area Treatment Versus Permeable Reactive Barrier Designs

Source Area Treatment	Permeable Reactive Barrier
<p>POTENTIAL BENEFITS</p> <ul style="list-style-type: none"> ➤ Controls (i.e., sequestration) or remediates source ➤ Reduces mass flux of dissolved contaminants ➤ Compatible with natural attenuation ➤ Provide post-treatment to other source area treatments (i.e., surfactant flush, resistive heating, etc.) ➤ Potentially more cost-effective than alternative remedial technologies 	<p>POTENTIAL BENEFITS</p> <ul style="list-style-type: none"> ➤ Controls plume migration ➤ Less precise delineation of source area is required ➤ Can be use to remediate extensive dissolved phase plumes (series of barriers) ➤ Compatible with natural attenuation ➤ Helps protect downgradient receptors ➤ Potentially lower cost than other barrier technologies, especially at deeper sites
<p>POTENTIAL DISADVANTAGES</p> <ul style="list-style-type: none"> ➤ Requires more precise delineation of source area ➤ Probably not effective for large volumes of DNAPL ➤ May require decades to fully remediate source area 	<p>POTENTIAL DISADVANTAGES</p> <ul style="list-style-type: none"> ➤ Does not eliminate source ➤ If plume source is not controlled, additional oil injections will be required to maintain performance ➤ If permeability loss is excessive, plume could flow around barrier
<p>TYPICAL DESIGNS</p> <ul style="list-style-type: none"> ➤ Injection points distributed throughout source ➤ Temporary recirculation systems to smear oil thorough out source ➤ Rows of barriers spaced 6 months to 2 years travel time apart 	<p>TYPICAL DESIGNS</p> <ul style="list-style-type: none"> ➤ Row of injection points perpendicular to groundwater flow direction ➤ Multiple barriers can be used to achieve higher removal efficiencies or reduce cleanup time for long plumes

1.5 PROCEEDING WITH THE EMULSIFIED OIL PROCESS

The emulsified oil process can a powerful tool for remediating groundwater contaminated with a wide variety of compounds amenable to anaerobic biodegradation. However, this approach is not appropriate for every site. As discussed in Section 4, project personnel should conduct a preliminary screening to evaluate whether this approach is appropriate for their site. Once this screening is complete, a preliminary conceptual design should be developed for the site and compared against other alternatives. If appropriate, a pilot test should be conducted to evaluate the performance of the edible oils process at the site. Pilot test monitoring results should then be evaluated to determine if performance is acceptable and to develop a full-scale design based on lessons learned. Section 4 of this Emulsified Oils Protocol provides more detailed information on how to implement this process including developing a site conceptual model, designing an emulsified oils project, and implementing the design.

This first section of this protocol has provided an overview of the emulsified oil process. Subsequent sections in this Emulsified Oils Protocol provide greater detail into the scientific and engineering background of the technology. These sections should be used to gain more in-depth understanding of one or more areas of particular interest for the reader. A decision to select enhanced bioremediation as a remedial alternative should be site-specific within the context of engineering feasibility and cost-effectiveness in relation to other technologies.

- Section 2 discusses the impact of edible oils on contaminant transport and fate. It includes information on the chemical, physical, and biological properties of emulsified oils.
 - Section 3 presents information on the injection and distribution of emulsified oils in the subsurface.
 - Section 4 provides procedures for preliminary planning and detailed design of a full-scale emulsified oil project.
 - Section 5 describes the steps required for planning and implementation of an emulsified oil pilot test.
 - Section 6 discusses monitoring and data evaluation.
 - Section 7 presents the references used in preparing this document.
-
- Appendix A contains a commonly used spreadsheet that helps determine the amount of emulsified oil to use for a given application.

SECTION 2

IMPACT OF EMULSIFIED OILS ON CONTAMINANT TRANSPORT AND FATE

Extensive research and field experience has shown that many groundwater contaminants can be cost-effectively treated through injection of emulsified edible oils. The added oil is fermented to hydrogen (H₂) and low-molecular weight fatty acids, providing carbon and energy for anaerobic biodegradation. Aqueous concentrations of some contaminants (e.g., CAHs) can also be reduced by enhanced sorption to trapped residual oil.

This section presents background information on the effect of oil injection on the transport and fate of chlorinated solvents in the subsurface.

- Section 2.1 presents information on: (a) the chemical properties of different edible oils; (b) fermentation of these oils to hydrogen and acetate; and (c) the impact of edible oil addition on reductive dechlorination processes.
- Section 2.2 describes the effect of residual oils on partitioning of contaminants between the aqueous and solid phases and the impact of that partitioning on aqueous concentrations and contaminant migration.
- Section 2.3 briefly summarizes the key points of this chapter.

Readers that are already knowledgeable about these processes may wish to skip directly to Section 3 which provides information on the injection and distribution of emulsified oils or Section 4 which provides a step-by-step guide for designing edible oil remediation systems.

2.1 ENHANCED ANAEROBIC BIOREMEDIATION USING EDIBLE OILS

Extensive research has shown that many groundwater contaminants can be biodegraded *in situ* by providing a source of biodegradable organic substrate. In practice, the added organic substrates are first fermented to hydrogen (H₂) and low-molecular weight fatty acids (e.g., acetate, lactate, propionate and butyrate) providing carbon and energy for anaerobic biodegradation.

A variety of different substrates can be used to generate hydrogen and stimulate anaerobic biodegradation. Soluble substrates including lactate, molasses, and other readily fermented substrates can be very effective in stimulating this process. However, these substrates must be frequently replenished due to rapid biodegradation and/or transport with flowing groundwater. In this section, we describe the use of emulsified oils as a long-lasting, immobile substrate to simulate long-term biodegradation of the target contaminants.

Ideal substrates for use in the emulsified oil process would be: (1) non-toxic, food-grade materials that are sufficiently biodegradable to support complete reductive dechlorination of the target contaminants; (2) slowly biodegradable in order that residual organic amendment can remain effective in the aquifer for an extended period of time (e.g., 5 to 10 years); and (3) a low unit cost. The US Department of Agriculture maintains a list of Generally Recognized as Safe (GRAS) materials approved for direct incorporation into food (21CFR173). This list includes a variety of fats and oils including animal and vegetable fats, paraffin, petrolatum, white mineral oil, and several specialty oils. Petroleum derived oils (e.g., paraffin, petrolatum, and white mineral oil) do not readily ferment to hydrogen and acetate, and consequently would not be good candidates for stimulating reductive dechlorination (He *et al.*, 2002; Borden and Rodriguez, 2005). However, if the target contaminant is nitrate, perchlorate, or another more oxidized

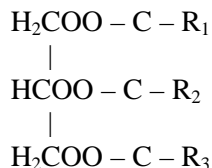
material, the petroleum-derived substrates may be useful. Specialty oils including synthetic fats such as olestra can be used to support reductive dechlorination (Borden and Rodriguez, 2005). However, the high cost of the specialty fats will reduce their widespread use. As a consequence, animal and vegetable oils and fats are often most useful for stimulating anaerobic biodegradation processes.

Some practitioners have considered employing used fats and oils in lieu of virgin vegetable oil. In theory, recycling spent vegetable oil, such as restaurant waste oil or peanut processing oil, for biodegradation would be less expensive. However, in practice, waste oils are often contaminated with other organics from the cooking processes, are of unknown grade, quality or composition (i.e., mono- or polyunsaturated fats), and may not be available in sufficient quantities from any one source to accommodate the particular project needs. Consequently, this approach has not been implemented to date.

While food-grade materials may not be needed in all cases, use of materials approved for direct incorporation into food may aid in gaining regulatory approval. The requirements for gaining approval for injecting substrates vary from state to state. For example, in North Carolina, the initial proposed use of an injectable substrate required approval from both the State Department of Toxicology and Epidemiology as well as a permit from the Underground Injection Control Program. In other states such as Florida, the composition of the injectate must be identified and the fate and transport of the ingredients in the amendment must be described to the satisfaction of the regulatory agency. Unlike some other states, Florida also requires information on the potential impact on secondary drinking water quality and an injection permit is needed. Managers should contact the governing state regulatory agency to determine what approvals or permits, if any, may be required to implement the edible oil process. Where cleanup actions are conducted under CERCLA and/or where the DOD is the lead agent, only substantive requirements need to be met. Users are recommended to consult with personnel experienced in implementing *in situ* bioremediation projects in their respective states.

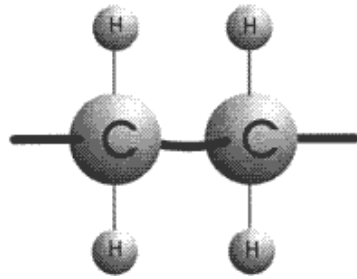
2.1.1 CHEMICAL AND PHYSICAL COMPOSITION OF EDIBLE OILS

All animal and vegetable fats and oils are classified as triglycerides and contain three long chain fatty acids attached (esterified) to a glycerol core. When all of the fatty acids in a triglyceride are identical, it is termed a "simple" triglyceride. The more common forms, however, are "mixed" triglycerides which contain two or three different fatty acids. The molecular structure of a typical mixed triglyceride is shown below.

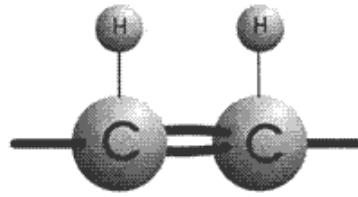


R₁, R₂ and R₃ represent different long-chain fatty acids. Typically, 100 grams of fat or oil will yield about 95 grams of fatty acids. The physical and chemical characteristics of the fatty acids have a major influence of the properties of the resulting fat or oil.

The predominant fatty acids present in animal and vegetable fats and oils contain 16 or 18 carbon atoms arranged in a chain. Fatty acids containing only single carbon-to-carbon bonds are termed "saturated" while fatty acids containing one or more carbon-to-carbon double bonds are termed "unsaturated." Saturated and unsaturated linkages are illustrated in **Figure 2.1**.



Saturated Bond



Unsaturated Bond

Figure 2.1. Single ‘Saturated’ and Double ‘Unsaturated’ Carbon-Carbon Bonds (ISEO, 1999)

When the fatty acid contains one double bond it is called "monounsaturated." If it contains more than one double bond, it is called "polyunsaturated." Properties of common saturated and unsaturated fatty acids in food oils are presented in **Table 2.1**. The melting point of saturated fatty acids increases with chain length. Unsaturated fatty acids often have lower melting points than the corresponding saturated fatty acid. The primary fatty acids present in vegetable oils are lauric, palmitic, stearic, oleic and linoleic. However, different oils contain different proportions of these fatty acids (**Table 2.2**).

The physical properties of the different fats and oils have a significant influence on their transport and distribution in the subsurface. Triglycerides are classified as ‘fats’ if they are solid at room temperature and ‘oils’ if they are liquid at room temperature. In this protocol, the term “oil” refers to the liquid forms since these represent the products that have been documented to date in field demonstrations and project applications.

Oil emulsions are prepared by mixing and blending edible oils with emulsifying agents or surfactants, yielding a smooth blend of small oil droplets suspended in a water matrix (i.e., an oil-in-water emulsion). The types of oil, the percent of oil in the mix, the types of emulsifying agents and other additives all contribute to the appearance and utility of the amendment.

Table 2.1. Common Saturated and Unsaturated Fatty Acids

Systematic Name	Common Name	No. of Carbon Atoms	No. of Double Bonds	Melting Point °C	Typical Fat Source
Ethanoic	Acetic	2	0	--	--
Butanoic	Butyric	4	0	-7.9	Butterfat
Hexanoic	Caproic	6	0	-3.4	Butterfat
Octanoic	Caprylic	8	0	16.7	Coconut oil
Decanoic	Capric	10	0	31.6	Coconut oil
9-Decenoic	Caproleic	10	1	-	Butterfat
Dodecanoic	Lauric	12	0	44.2	Coconut oil
9-Dodecenoic	Lauroleic	12	1	-	Butterfat
Tetradecanoic	Myristic	14	0	54.4	Butterfat, coconut oil
9-Tetradecenoic	Myristoleic	14	1	18.5	Butterfat
Hexadecanoic	Palmitic	16	0	62.9	Most fats and oils
9-Hexadecenoic	Palmitoleic	16	1	-	Some fish oils, beef fat
Octadecanoic	Stearic	18	0	69.6	Most fats and oils
9-Octadecenoic	Oleic	18	1	16.3	Most fats and oils
9-Octadecenoic	Elaidic	18	1	43.7	Partially hydrogenated oils
11-Octadecenoic	Vaccenic	18	1	44	Butterfat
9,12-Octadecadienoic	Linoleic	18	2	-6.5	Most vegetable oils
9,12,15-Octadecatrienoic	Linolenic	18	3	-12.8	Soybean oil, canola oil
Eicosanoic	Arachidic	20	0	75.4	Peanut oil
9-Eicosenoic	Gadoleic	20	1	-	Some fish oils
5,8,11,14-Eicosatetraenoic	Arachidonic	20	4	-49.5	Lard
5,8,11,14,17-Eicosapentaenoic	-	20	5	-	Some fish oils
Docosanoic	Behenic	22	0	80	Peanut oil
13-Docosenoic	Erucic	22	1	33.4	Rapeseed oil
4,7,10,13,16,19-Docosahexaenoic	-	22	6	-	Some fish oils

Source: ISEO, 1999.

Table 2.2. Percent Fatty Acid Compositions for Major Edible Oils (values in mole fraction of fatty acids as a percent)

Fatty Acid	Soy ¹	Corn ¹	Cotton-seed ¹	Palm ¹	Pea-nut ¹	Olive ¹	Canola ₂	Low Linolenic Canola ²	High Oleic Canola ²	Butter-fat ¹	Lard ¹	Beef Tallow ¹
C-4:0, Butyric										4		
C-6:0, Caproic										2		
C-8:0, Caprylic										1		
C-10:0, Capric										3		
C-12:0, Lauric										3		
C-14:0, Myristic			1	1			0.1	0.1	0.1	11	2	3
C-16:0, Palmitic	11	11	22	45	11	13	3.5	3.9	3.4	27	26	24
C-18:0, Stearic	4	2	3	4	2	3	1.5	1.2	2.5	12	14	19
C-20:0, Arachidic					1	1	0.6	0.6	0.9			
C-16:1, Palmitoleic			1			1	0.2	0.2	0.2	2	2	4
C-18:1, Oleic	24	28	19	40	48	71	60.1	61.1	76.8	29	44	43
C-20:1, Gadoleic					2		1.4	1.5	1.6		1	
C18:2, Linoleic	54	58	53	10	32	10	20.1	27.1	7.8	2	10	3
C-18:3, Linolenic	7	1	1			1	9.6	2.1	2.6	1		1
Other	0	0	0	0	4	0	2.9	2.2	4.1	3	1	3

Sources: ¹ ISEO, 1999.
² Przybylski, 2004.

2.1.2 ANAEROBIC FERMENTATION OF EDIBLE OIL

This section describes the scientific and metabolic background for understanding how introduction of oil-in-water emulsions stimulates anaerobic reductive dechlorination. It demonstrates that although there are subtle differences in the composition of the oils, most of the commercially available oils behave similarly. Most practitioners of the edible oil process use soybean oil because of its common availability, good handling characteristics, and relatively low cost.

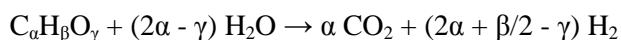
All triglycerides (edible fats and oils) can be anaerobically fermented to hydrogen and organic acids like acetate. Anaerobic fermentation is believed to occur through a two-step process where the ester linkages between the glycerol and the fatty acids are hydrolyzed releasing free fatty acids and glycerol to solution. The glycerol then degrades to 1,3-propanediol and subsequently to acetate. Saturated fatty acids undergo further breakdown by *beta*-oxidation resulting in the formation of two molecules of hydrogen (H₂), one molecule of acetate (C₂H₃O₂⁻), and the original molecule of acid appears as a new acid derivative with two less carbon atoms (Sawyer *et al.*, 1994).



By successive oxidation at the *beta* carbon atom, long-chain fatty acids are whittled into progressively shorter fatty acids and acetic acid. Four hydrogen atoms are released from saturated fatty acids for each acetic acid unit produced (Sawyer *et al.*, 1994). Unsaturated fatty acids undergo the same general process, but release two atoms of hydrogen for each acetic acid unit.

Acetic acid and hydrogen produced in the subsurface by fermentation of edible oils will then be consumed in a variety of different reactions. If high-energy electron acceptors such as oxygen and nitrate are present, the hydrogen and acetic acid will be very rapidly oxidized to carbon dioxide and water. Once these materials are consumed, excess hydrogen and acetate can then be used for reductive dechlorination, or to reduce dissolved sulfate and oxidized forms of manganese and iron in the sediments. Hydrogen and acetic acid may also be fermented to methane. Any hydrogen or acetic acid converted to methane will not be used for reductive dechlorination and can be thought of as 'wasted'. Ideally, one would prefer to minimize methane production to make the most efficient use of the added organic carbon. However, in practice, this does not appear to be feasible. Reducing substrate addition to limit methane production also appears to reduce dechlorination rates. Consequently, excess organic substrate is typically added to provide sufficient substrate for efficient reductive dechlorination and methane production.

The different edible oils do contain different levels of the various fatty acids. As a consequence, one type of oil could potentially be a better electron donor than another. To evaluate this effect, an average chemical formula for each oil was calculated based on the fraction of different fats presented in **Table 2.2**. The electrons released per mole of oil was then calculated according to the following formula,



where: α is the number of carbon atoms per mole of oil

β is the number of hydrogen atoms per mole of oil

γ is the number of oxygen atoms per mole of oil

This formula assumes that any acetate produced in the process will eventually be fermented to hydrogen and carbon dioxide or otherwise beneficially used in the anaerobic biodegradation process. Results of this

analysis are presented in **Table 2.3** and compared with other common substrates (Sawyer *et al.*, 1994). This analysis shows that there is essentially no difference in the amount of reducing power per gram of oil. However, all of the oils have much more reducing power than other common substrates. For example, 100 pounds of oil has about the same reducing power as 270 pounds of acetate or sugar.

Table 2.3. Average Composition of Different Edible Oils and Electrons Released during Anaerobic Fermentation

	Atoms per Mole Substrate			Average Molecular Weight	H ₂ Released per mole Substrate	Moles H ₂ released per gram substrate
	C	H	O			
Acetate	2.0	4.0	2.0	60.1	4.0	0.0666
Lactate	3.0	6.0	3.0	90.1	6.0	0.0666
Glucose	6.0	12.0	6.0	180.2	12.0	0.0666
Soybean	56.3	99.5	6.0	873.1	156.5	0.1792
Corn	56.3	99.9	6.0	873.5	156.6	0.1793
Cottonseed	55.5	99.3	6.0	862.8	154.7	0.1792
Palm	54.2	100.8	6.0	848.5	152.8	0.1800
Peanut	56.8	102.7	6.0	881.4	158.9	0.1803
Olive	56.2	102.7	6.0	875.0	157.8	0.1804
Canola	57.1	102.3	6.0	884.6	159.3	0.1801
Butterfat	50.2	94.0	6.0	793.4	141.4	0.1782
Lard	55.2	102.4	6.0	862.4	155.6	0.1804
Beef Tallow	55.1	102.9	6.0	862.2	155.8	0.1807

Some practitioners have suggested that edible oils high in unsaturated fatty acids (e.g., oleic, linoleic and linolenic) can be used to inhibit methanogenesis, resulting in more efficient use of the added substrate for reductive dechlorination. This approach is based on the work of Lalman and Bagley (2000; 2001) who showed that over 30 mg/L of oleic or linoleic acid will inhibit methane production from acetic acid. However, there is no evidence that use of oils high in unsaturated fats will significantly inhibit methanogenesis under *in situ* conditions. Borden and Rodriguez (2005) monitored methane production from a variety of different fats and oils with varying levels of saturated and unsaturated fatty acids. Soybean oil, which is composed of 96% oleic, linoleic, and linolenic acids (unsaturated fats), was a very efficient carbon source for methane production. The small difference in methane production from different oils is not unexpected, since most vegetable oils are naturally high in unsaturated fats. Increasing the unsaturated fat content from 96% for standard soybean oil to 98% for low linolenic acid canola oil can be expected to have negligible effects on methane production.

An alternative approach to increase substrate life would be to use a hydrogenated oil (e.g., fat) with a higher melting point and lower aqueous solubility. Preliminary studies by Borden and Rodriguez (2005) suggest that highly saturated oils do biodegrade somewhat more slowly. However, the benefits of using saturated fats appear to be minor compared to the increased complexity of injecting materials that are solids at ambient temperatures.

In summary, all edible oils are fermentable to hydrogen and acetate by common subsurface microorganisms. The hydrogen yield (i.e., reducing equivalents) from all common oils is similar and much higher than more oxidized substrates (e.g., acetate, lactate, glucose, etc.). As a consequence, there is no reason to expect that one type of oil would be a significantly better substrate for anaerobic bioremediation than any other oil. When selecting an oil for anaerobic bioremediation, the primary factors to consider are cost, availability, and material handling characteristics (melting point and viscosity). Soybean oil is most commonly used, because of its availability, good handling characteristics, and relatively low cost.

2.1.3 ENHANCED ANAEROBIC BIOREMEDIATION

The primary objective of injecting food-grade emulsified edible oil into the subsurface is to stimulate the anaerobic biodegradation of the target contaminants. To be successful at a given site, a microbial community capable of driving the process must be present. This section provides a brief overview of the microbiology of anaerobic biodegradation for some common groundwater contaminants and the use of emulsified oils to stimulate this process.

2.1.3.1 Reductive Dechlorination of Chlorinated Ethenes

2.1.3.1.1 Microbiology of Reductive Dechlorination

The most important process for the anaerobic biodegradation of chlorinated ethenes is reductive dechlorination. The process of anaerobic reductive dechlorination has been well documented. Recent discussions of the overall process can be found in Wiedemeier *et al.*, 1999; USEPA, 2000; and Suthersan, 2001. During the reductive dechlorination process, the chlorinated hydrocarbon is used as an electron acceptor and a chlorine atom is removed and replaced with a hydrogen atom. In general, reductive dechlorination occurs by sequential dechlorination transforming PCE to TCE to DCE to VC to ethene. If the bacteria are able to obtain metabolically useful energy from reductive dechlorination, this process is referred to as halorespiration. Depending upon environmental conditions and presence/absence of suitable microbes, this sequence may be interrupted, with other processes acting upon the degradation products. A more comprehensive review of the different environmental factors affecting anaerobic reductive dechlorination is presented in AFCEE (2004).

Anaerobic reductive dechlorination is carried out by only a few metabolic classifications of bacteria, including methanogens, sulfate-reducing bacteria, and dechlorinating bacteria. In practice, microorganisms capable of degrading PCE and TCE to *cis*-DCE should be considered ubiquitous in the subsurface environment (AFCEE, 2004). However, dechlorination of *cis*-DCE and VC to ethene appears to be limited only to dechlorinating bacteria, which may not be ubiquitous in the environment (He *et al.*, 2003). The complete degradation of PCE all the way to ethene has only been demonstrated for the species *Dehalococcoides ethenogenes*, the absence of which has been implicated in the persistence of *cis*-DCE and VC in groundwater. Nonetheless, Flynn *et al.* (2000) demonstrated complete dechlorination of PCE to ethene with a mixed culture that did not contain the *Dehalococcoides* species.

Reductive dechlorination occurs under sulfate-reducing and methanogenic conditions. Because chlorinated compounds are used as electron acceptors during reductive dechlorination, there must be an appropriate electron donor present. The electron donor used by most dechlorinating microbes is molecular hydrogen, which may be produced via primary or secondary fermentation of a variety of organic substrates. Potential sources of molecular hydrogen include natural organic matter, fuel hydrocarbons, landfill leachate, or added organic substrates. Hydrogen is generated by fermentation of non-chlorinated organic substrates including fuels, naturally-occurring organic carbon, and a variety of other compounds, such as carbohydrates, sugars, alcohols, volatile fatty acids (VFAs), and edible oils. Fermentation produces hydrogen that is the primary electron donor utilized for reductive dechlorination of chlorinated solvents.

Chlorinated ethenes can also be biodegraded via cometabolism, where the degradation is catalyzed by an enzyme or cofactor that is fortuitously produced by the organisms for other purposes. The organism receives no known benefit from the degradation of the chlorinated compound. Rather, the cometabolic degradation of the chlorinated compound may in fact be harmful to the microorganism responsible for the production of the enzyme or cofactor (McCarty and Semprini, 1994). While cometabolism is best documented in aerobic environments, it also may occur under anaerobic conditions. Anaerobic cometabolic dechlorination has most often been observed in the presence of acetogenic and methanogenic bacteria (Suthersan, 2001). In the field, it is often difficult to distinguish between cometabolic dechlorination and metabolic dechlorination (halorespiration). Because the organisms that cause anaerobic cometabolic dechlorination are ubiquitous in the subsurface, cometabolic dechlorination is likely responsible for some degradation of chlorinated compounds (Gossett and Zinder, 1996).

2.1.3.1.2 Laboratory Studies of Reductive Dechlorination Using Edible Oils

Edible oils have now been used to stimulate enhanced anaerobic biodegradation of chlorinated solvents and related contaminants in small scale pilot studies and large scale remediation projects at over one hundred sites (Harkness and Farnum, 2004; Lieberman *et al.*, 2005; Lieberman *et al.*, 2003; Lindow, 2004; Lee *et al.*, 2003; Zawtocki, 2005; Zawtocki *et al.*, 2004; Parsons, 2002; Boulicault *et al.*, 2000). In this section, we summarize results from a single laboratory microcosm study that evaluated the effect of soybean oil addition on reductive dechlorination of TCE and *cis*-DCE (Zenker *et al.*, 2000). Additional information on laboratory studies evaluating the effect of edible oil addition on reductive dechlorination is presented by Sin Chit To (2001), Long (2004), and Rodriguez (2004).

Zenker *et al.* (2000) presents results of an early laboratory microcosm study evaluating the use of edible oils for stimulating reductive dechlorination (**Figure 2.2**). The microcosms were constructed with aquifer material and groundwater from a chlorinated solvent-contaminated site in the North Carolina coastal plain and amended with 500 mg/L of liquid soybean oil. **Figure 2.2a** shows that TCE and *cis*-DCE were biodegraded within 50 days to VC. The VC was then transformed to ethene after about 90 days. The microcosms were then repeatedly spiked with additional PCE, but without any additional soybean oil. **Figure 2.2b** shows the results from respiking of 90 $\mu\text{mole/L}$ (15 mg/L) PCE on day 1072. The PCE concentrations fell to $\sim 11 \mu\text{mole/L}$ or 1.9 mg/L due to sorption to the oil. The dissolved and sorbed PCE were then transformed to TCE, *cis*-DCE, VC, and ethene. However, as the dissolved PCE was depleted, additional PCE desorbed from the oil and was degraded. By day 1225, all chlorinated solvent concentrations were below analytical detection limits and close to 90% of the injected PCE had been recovered as ethene.

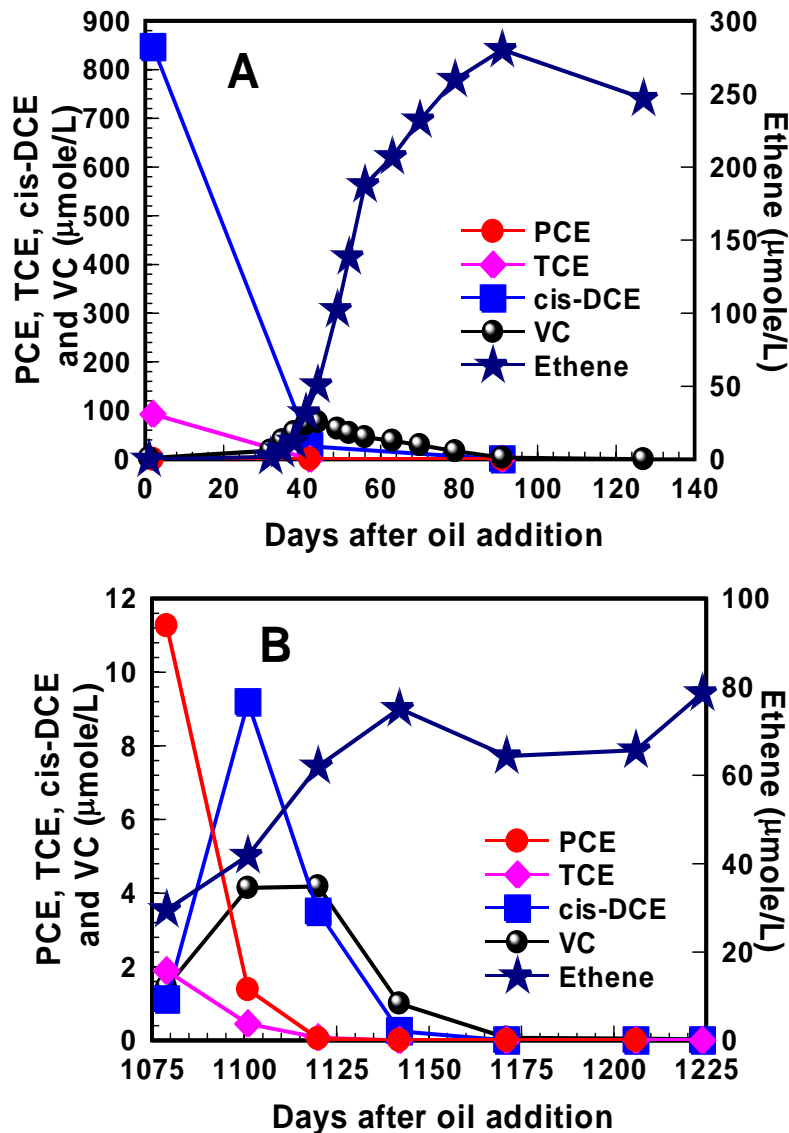


Figure 2.2. Chlorinated solvent reductive dechlorination over time from one addition of 500 mg/L liquid soybean oil: (A) shortly after microcosm construction; and (B) after repeatedly re-spiking with additional PCE over three years.

2.1.3.2 Anaerobic Biodegradation of Perchlorate

2.1.3.2.1 Microbiology of Perchlorate Degradation

In recent years, an extensive body of information has been developed demonstrating that a large and diverse population of microorganisms can degrade perchlorate to chloride and oxygen (Coates *et al.* 1999; Coates and Pollock 2003). Perchlorate-reducing organisms are widespread in the environment (Coates *et al.* 1999; Logan, 2001) and can use a variety of different organic substrates (e.g., acetate, propionate, lactate, etc.) as electron donors for perchlorate reduction (Herman and Frankenberger 1998; Coates *et al.* 1999). Perchlorate biodegradation can occur under strict anaerobic conditions as well as facultative

anaerobic conditions. Facultative anaerobic microorganisms are capable of both aerobic respiration under low oxygen tension and fermentation when anaerobic conditions prevail. This metabolic versatility opens up the possibility that environments exist that can support a variety of perchlorate-reducing microbial populations.

The perchlorate biodegradation pathway is illustrated in **Figure 2.3**. Work by Coates *et al.* (1999), Chaudhuri *et al.* (2002), and Bender *et al.* (2002) indicate that the Dechloromonas and Dechlorosoma groups represent the primary chlorate and perchlorate reducing bacteria in the environment, but more than 30 different strains of perchlorate-reducing microbes have been identified (USEPA, 2005). The rate-limiting step in the three-step degradation process is the conversion of perchlorate to chlorate by a perchlorate reductase enzyme. Subsequent conversion of chlorate to chlorite is also catalyzed by a perchlorate reductase enzyme. Chlorite removal by the chlorite dismutase (CD) enzyme is the final step in perchlorate reduction. Its specificity may be useful as an indicator of perchlorate biodegradation.

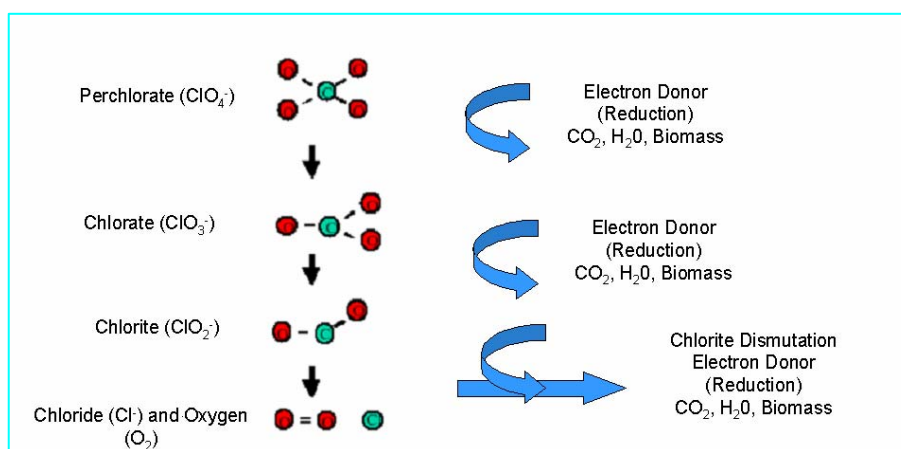


Figure 2.3. Perchlorate Biodegradation Pathway

2.1.3.2.2 Laboratory and Field Studies of Perchlorate Degradation Using Emulsified Oils

Solutions-IES conducted laboratory and field studies to evaluate the use of emulsified oil substrate (EOS^{®1}) for stimulating biodegradation of perchlorate (ESTCP Project ER-0221). Laboratory microcosms were created in triplicate using aquifer sediments and groundwater from a site in Maryland to evaluate the ability of EOS[®] to support perchlorate biodegradation. As shown in **Figure 2.4**, perchlorate degradation was rapid and complete in all microcosms treated with EOS[®].

¹ EOS[®] is a registered trademark of EOS Remediation, Inc.

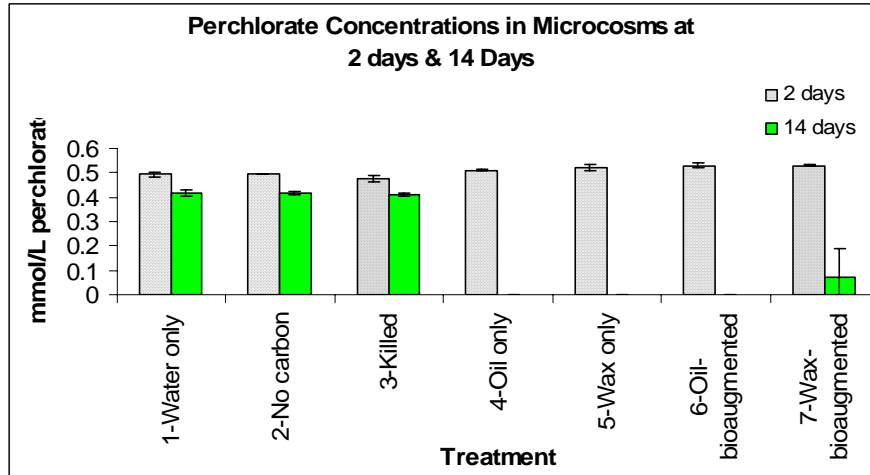


Figure 2.4. Perchlorate Biodegradation in Laboratory Microcosms

Based on the results of the laboratory studies, a pilot study was designed to demonstrate the effectiveness of EOS[®] in the field. The pilot study consisted of installing ten 1-inch diameter direct-push injection wells spaced 5 feet on center perpendicular to groundwater flow to create a PRB. Approximately 10 gallons of EOS[®] concentrate (50 gallons of diluted EOS[®]) were injected into each well followed by approximately 165 gallons of chase water to distribute the EOS[®] throughout the aquifer. The EOS[®] PRB was very effective at degrading perchlorate. Prior to injection, perchlorate concentrations ranged from 3,100 to 20,000 µg/L in the pilot test area. Concentrations in all of the injection wells were non-detect (<4 µg/L) within 5 days of injection. Perchlorate concentrations decreased in the downgradient wells as groundwater migrated through the EOS treatment zone. **Figure 2.5** shows the results from the field test.

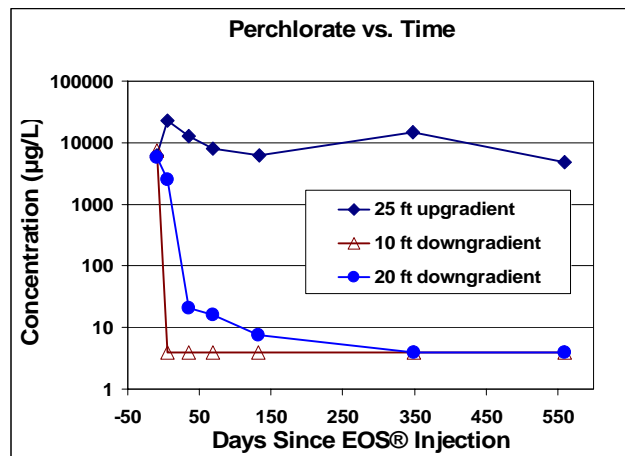


Figure 2.5. Perchlorate Degradation in EOS[®] PRB Field Pilot Test

2.1.3.3 Anaerobic Biodegradation of Explosives

2.1.3.3.1 Microbiology of Explosives Degradation

TNT, RDX, and HMX can be readily biodegraded/transformed under anaerobic conditions. Under anaerobic conditions, TNT is reduced via nitroso and hydroxylamine intermediates to the corresponding amino group analogue (McCormick *et al.*, 1976; Kaplan and Kaplan, 1982). Metabolites containing nitroso groups may then sorb to organic material while metabolites containing aromatic hydroxylamine and amine groups can form irreversible covalent bonds with humics immobilizing these materials (Acht nich *et al.*, 1999; Thorn and Kennedy, 2002). Under anaerobic conditions, the RDX and HMX ring may be cleaved resulting in complete mineralization (Hawari *et al.*, 2000, 2001) or the nitro groups in RDX and HMX may be reduced to nitroso groups (Price *et al.*, 2001; Beller, 2002).

These very promising laboratory results suggest that it may be possible to stimulate *in situ* anaerobic biodegradation of TNT, RDX and HMX by providing organic substrates, and possibly nutrients. However to date, there have only been very limited field tests of this process. In an ongoing ESTCP supported project (CU-0110), Dr. Jeff Davis has demonstrated that acetate addition is effective in stimulating anaerobic biotransformation of RDX. However, cold temperatures and the presence of competing electron acceptors (nitrate and sulfate) can reduce the rate and extent of RDX removal (Davis *et al.*, 2003). These early results are very promising and indicate that enhanced *in situ* bioremediation of RDX and TNT should be feasible.

2.1.3.3.2 Laboratory Studies of Explosives Degradation Using Emulsified Oils

Shaw Environmental evaluated the effectiveness of various cosubstrates for promoting the biodegradation of explosives and perchlorate in microcosms prepared using sediment and groundwater from Indian Head Division, Naval Surface Warfare Center (Schaefer *et al.*, 2006). The cosubstrates tested included: lactate, ethanol, hydrogen gas, crude soybean oil, Wesson oil, and EOS[®] emulsified soybean oil. The groundwater in each bottle was subsampled after 0, 1, 4, 18, 66, 73, and 102 days of incubation and analyzed for nitrate, perchlorate, explosives, and explosive breakdown products, and fatty acids. As shown in **Figure 2.6**, EOS[®] emulsified soybean oil was the most effective substrate for promoting RDX biodegradation. In samples receiving EOS[®] as a cosubstrate, RDX levels declined by an average of 95 % (to 0.20 mg/L) during 102 days of incubation. Besides parent RDX, the three initial nitroso- breakdown products of RDX; hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX), hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX), and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX) were measured in subsamples. Appreciable levels of each of these compounds were observed in the samples receiving EOS[®], confirming that EOS[®] was supporting explosives biodegradation in these samples.

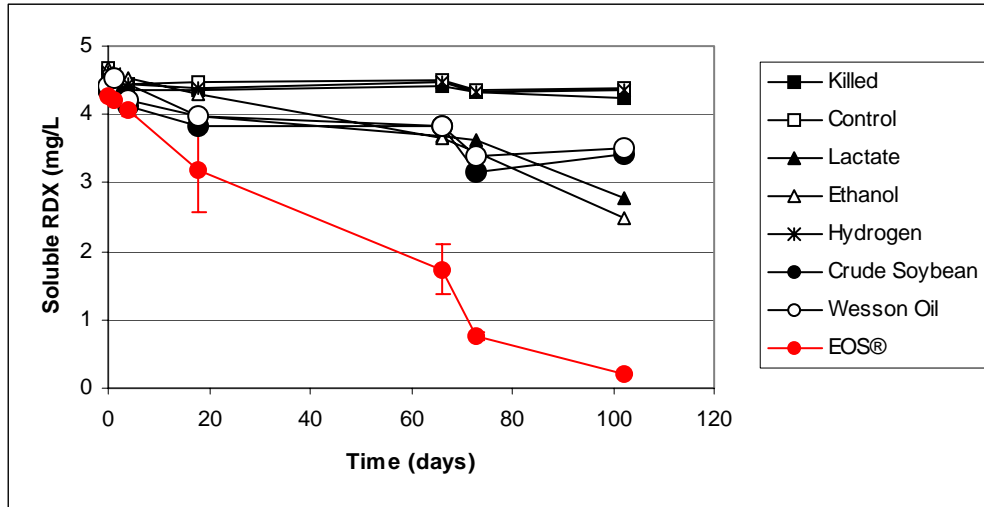


Figure 2.6. Biodegradation of RDX in Microcosms (Schaefer *et al.*, 2006)

2.1.3.4 Microbiology of Other Contaminants

While many other contaminants appear to be amenable to anaerobic biodegradation, much less is known about the microbiology and kinetics of degrading these contaminants. Before proceeding with an enhanced anaerobic bioremediation, literature reviews, microcosm studies, and/or field pilot tests may be useful to identify the applicability of the process for the contaminants of concern.

2.2 IMPACT OF RESIDUAL EDIBLE OIL ON CONTAMINANT SORPTION

The impact of chlorinated solvent partitioning to the edible oil can be evaluated using the using a retardation factor approach (R) where:

$$R \text{ (unitless)} = \frac{\text{Total mass of contaminant}}{\text{Mass of contaminant in aqueous phase}} = \frac{\text{Groundwater velocity}}{\text{Pollutant transport velocity}}$$

The retardation factor can be calculated as:

$$R = 1 + \rho_B f_o K_p / n$$

where: ρ_B is the aquifer bulk density (g/cm^3)
 f_o is the fraction of oil in the sediment (g/g)
 K_p is the oil-water partition coefficient (mL/g)
 n is porosity (ml/cm^3).

This approach assumes that oil-water partitioning is rapid relative to groundwater flow and that partitioning between the oil and water is approximately linear. Long (2004) found that the retardation factor approach provided a reasonably good approximation of chlorinated ethene transport in laboratory columns treated with emulsified soybean oil.

Pfeiffer (2003) examined the partitioning of PCE, TCE, *cis*-DCE, and VC between water and soybean oil at 20 and 10 °C. Oil-water partitioning was approximately linear suggesting that retardation factor may

be appropriate for estimating pollutant transport velocity in edible oil treated aquifers. K_p values were higher for the more hydrophobic compounds (**Table 2.4**). PCE partitioning also appeared to be reduced by the presence of other contaminants, indicating a competitive effect. Lower temperatures also reduced partitioning for PCE and TCE in mixtures. However, temperature effects were not significant for *cis*-DCE and VC. Measured K_p values were similar to literature values of the octanol-water partition coefficient (K_{ow}). The close match between K_p and K_{ow} is not surprising given that sorption to octanol is very similar to sorption to vegetable oil.

Table 2.4. Oil-Water Partition Coefficients (K_p) for Pure PCE, TCE, *cis*-DCE, VC and Mixtures of these Materials Between Water and Soybean Oil

Chlorinated Ethene	Solubility in water (mg/L)	K_p (ml/g)	K_{ow} (ml/g)
PCE	145 @ 24 °C	1240 (pure compound @ 20 °C) 531 (mixture @ 20 °C) 188 (mixture @ 10 °C)	2500
TCE	1100 @ 18 °C	338 (pure compound @ 20 °C) 373 (mixture @ 20 °C) 171 (mixture @ 10 °C)	263
<i>cis</i> -DCE	2100 @ 18 °C	61 (pure compound @ 20 °C) 53 (mixture @ 20 °C) 52 (mixture @ 10 °C)	72
VC	2500 @ 18 °C	22 (pure compound @ 20 °C) 22 (mixture @ 20 °C) 26 (mixture @ 10 °C)	23

Source: Pfeiffer, 2003.

Estimated retardation factors for PCE, TCE, *cis*-DCE and VC in aquifers treated with NAPL edible oil and edible oil emulsions are presented in **Table 2.5**. K_p values were assumed to be the maximum values reported by Pfeiffer (2003). For NAPL oil injections, the sediment oil content was calculated assuming 50% of the aquifer pore space is occupied by oil. For emulsion treated aquifers, the sediment oil content was calculated assuming 2% residual saturation, a typical value reported by Coulibaly and Borden (2004). Estimated retardation factors for PCE and TCE in a NAPL treated aquifer are very high, indicating that NAPL oil injection can be very effective in sequestering the more hydrophobic contaminants in source areas. For example, the fraction of a contaminant in the aqueous phase will be $1/R$, so only 1/570 or 0.2% of the total PCE mass will be in the aqueous phase. Sequestration will be less effective for *cis*-DCE and VC because of the much lower K_p values for these contaminants.

In theory, sorption may substantially delay PCE breakthrough in edible oil emulsion barriers. For example, PCE breakthrough could be delayed by over 2 years in a 10 ft thick emulsion treated barrier with an ambient groundwater velocity of 100 ft/yr. However, in practice, sorption effects are much more limited. Experimental results in laboratory columns have shown that emulsified oil addition results in rapid conversion of PCE and TCE to *cis*-DCE. Because of its much lower partition coefficient, *cis*-DCE breakthrough would only be delayed by a few months (Long, 2004). Similar results have been observed at field sites treated with emulsified oils. Further discussion of the impact of oil injection on the sorption of chlorinated solvents in the aqueous phase is provided in Section 4.5.1.3.

Table 2.5. Estimated Retardation Factors for Different Chlorinated Ethenes

Comment	NAPL	Emulsions
Sediment Bulk Density, ρ_B (g/cm ³)	1.86	1.86
Porosity, n	0.3	0.3
Oil fraction, f_o (g/g)	0.074	0.003
PCE Retardation Factor ($K_p = 1240$)	570	24
TCE Retardation Factor ($K_p = 338$)	156	7
<i>cis</i> -DCE Retardation Factor ($K_p = 61$)	29	2.1
VC Retardation Factor ($K_p = 26$)	12	1.5

2.3 SUMMARY OF KEY POINTS

- Addition of edible oils can be very effective in stimulating complete biodegradation of a variety of compounds amenable to anaerobic biodegradation.
- Partitioning of chlorinated solvents into the adsorbed oil does initially reduce aqueous contaminant concentrations. However, a portion of the contaminants will remain in the aqueous phase. As these contaminants are transformed to more reduced degradation products, additional contaminants will partition out of the oil phase into the water, allowing continued biodegradation. If no additional contaminants are added, this process can continue until all chlorinated compounds are degraded to below analytical detection limits with near stoichiometric production of ethene.
- A one-time addition of soybean oil can support complete biodegradation for over three years.

SECTION 3 INJECTION AND DISTRIBUTION OF EMULSIFIED OILS

Edible oils can be injected into the subsurface as a non-aqueous phase liquid (NAPL) oil or oil-in-water emulsions. NAPL oil injection results in higher residual saturations with a greater reduction in contaminant concentration due to sorption and greater permeability loss. Emulsion injection results in lower residual saturations with much more limited sorption and less permeability loss. The higher residual saturation associated with NAPL oil injection may require injection of more oil through more closely spaced injection points. This protocol focuses on the use of emulsified oils; however, some brief information on NAPL oil injection is provided for comparison purposes.

This section presents background information on the transport and immobilization of NAPL oil and oil-in-water emulsions in the subsurface.

- Section 3.1 presents information on the physical properties of edible oils and edible oil emulsions.
- Section 3.2 describes methods for injecting oil-in-water emulsions into the subsurface and the effect of emulsion and formation properties on the final oil distribution.

Readers that are already knowledgeable about different oil injection approaches may wish to skip directly to Section 4, which provides a step-by-step guide for designing edible oil remediation systems.

3.1 PHYSICAL AND CHEMICAL PROPERTIES OF EDIBLE OILS AND EDIBLE OIL EMULSIONS

The physical properties of the different fats and oils will have a significant influence on their transport and distribution in the subsurface. Triglycerides are classified as ‘fats’ if they are solid at room temperature and ‘oils’ if they are liquid at room temperature.

3.1.1 PROPERTIES OF PURE OILS

3.1.1.1 Water Solubility and Interfacial Tension

Edible oils are commonly described as being ‘insoluble’ in water. However, all materials have at least some limited aqueous solubility. Unfortunately, very little published information is available on the aqueous solubility of common edible oils. In laboratory studies at conducted at 25° C, the aqueous solubility of soybean oil and corn oil were found to be 4.2 mg/L and 2.6 mg/L, respectively (Pfeiffer, 2003). However, biological activity can greatly enhance the rate of carbon release from residual oils. Long (2004) found that live soil columns treated with emulsified soybean oil released between 50 and 100 mg/L dissolved organic and inorganic carbon.

The physical properties of edible oils are directly related to the properties of the fatty acids that they contain. **Table 3.1** lists the aqueous solubility of the long-chain saturated fatty acids. Aqueous solubility increases with increasing temperature and decreases with increasing chain length. While edible oil solubility cannot be directly estimated from the fatty acid solubility, we can expect that oils containing predominantly long-chain fatty acids will be less soluble than oils containing shorter chain length fats and that oils will be somewhat less soluble at lower temperatures.

Table 3.1. Aqueous Solubility of Common Saturated Fatty Acids at Different Temperatures

Common Name	No. of Carbon Atoms	Aqueous Solubility (mg/L)				
		0 °C	20 °C	30 °C	45 °C	60 °C
Caproic	6	8,640	9,680	10,190	10,950	11,710
Caprylic	8	440	680	790	950	1130
Capric	10	95	150	180	230	270
Lauric	12	37	55	63	75	87
Myristic	14	13	20	24	29	34
Palmitic	16	4.6	7.2	8.3	10	12
Stearic	18	1.8	2.9	3.4	4.2	5.0

Source: Ralston and Hoerr, 1942.

Only a few studies have been conducted on the surface tension and interfacial tension (against water) of edible oils and fatty acids. The surface tension of edible oil increases with an increase in fatty acid chain length and decreases with increasing temperature. The surface tension of cottonseed oil at 20 °C is 35.4 dynes per centimeter (dynes/cm). The interfacial tension of soybean oil and cottonseed oil against water at 70 °C are both about 30 dynes/cm. At 20 °C, oleic acid has a surface tension of 32.5 dynes/cm and an interfacial tension against water of 15.6 dynes/cm. Surface tension and interfacial tension of edible oils can be lowered by the addition of different surfactants including lecithin, mono and diglycerides, free fatty acids, and traditional soaps.

3.1.1.2 Density

All edible oils are less dense than water with a density at 15 °C, typically varying between 0.91–0.93 g/mL. Density is temperature dependent and decreases in value when temperature increases (**Figure 3.1**).

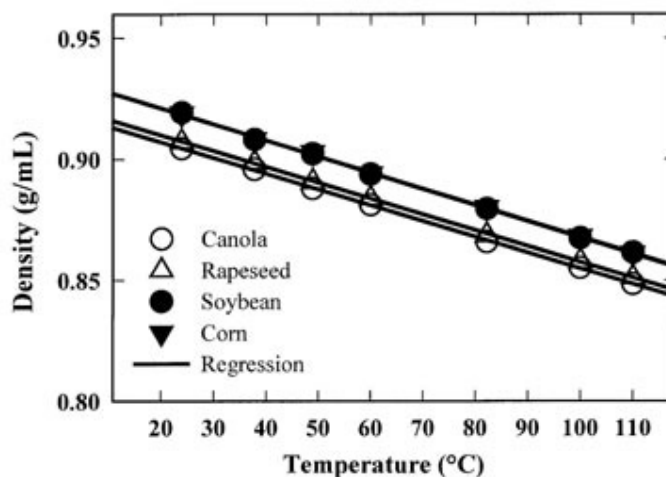


Figure 3.1. Effect of Temperature on Density of Selected Oils.

(Source: Przybylski (2004) adapted from Lang *et al.* (1992) and Nouredini *et al.* (1992a))

3.1.1.3 Viscosity

All edible oils are more viscous than water which increases their resistance to flow. **Figure 3.2** shows the effect of temperature on the viscosity of selected oils. For comparison, the kinematic viscosity of water is 1.3 centistokes (mm^2/s) at 10 °C and 0.85 centistokes at 80 °C.

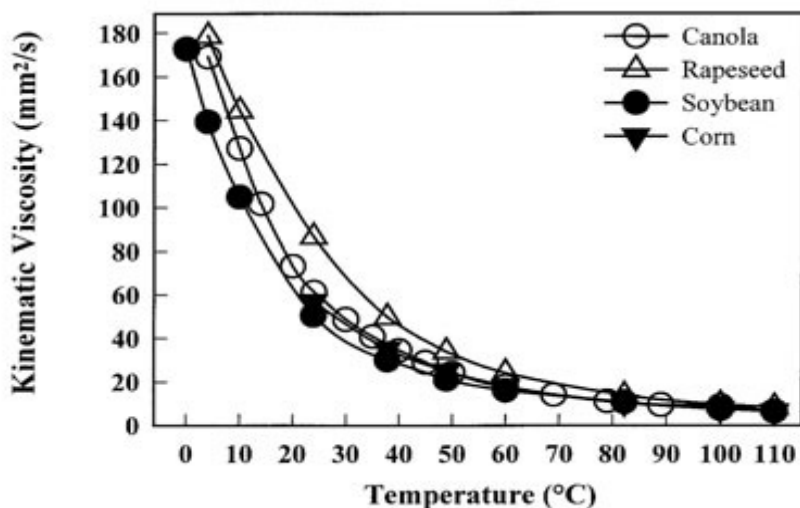


Figure 3.2. Effect of Temperature on Viscosity of Selected Oils.

(Source: Przybylski (2004) adapted from Lang *et al.* (1992) and Nouredini *et al.* (1992a))

3.1.2 PROPERTIES OF OIL-IN-WATER EMULSIONS

3.1.2.1 Emulsion Preparation

The food preparation industry has tremendous experience producing stable oil-in-water emulsions with a uniformly small droplet size (Becher, 2001). The key factors in generating the desired emulsion are: (1) the oil-water interfacial tension and (2) the mixing energy. Coulibaly and Borden (2004) evaluated several different combinations of surfactants and mixers to develop a procedure for generating stable emulsions with small, uniformly-sized oil droplets. Photomicrographs of several of the emulsions are shown in **Figure 3.3**. Most of the oil droplet size distributions are strongly non-symmetric with many small droplets and a few large droplets. However, the few large droplets contain a substantial portion of the total oil since the droplet volume is proportional to the diameter cubed. To provide a more useful presentation of these results, a statistical summary of the Log_{10} transformed droplet size distribution is presented in **Table 3.2**. The cumulative oil volume vs. droplet diameter for the different mixers is presented in **Figure 3.4**.

The modified lecithin (Centrophase C, Central Soya, Inc.) resulted in coarse emulsion with a large average droplet size and wide range of droplets. In contrast, the polysorbate² 85 and polysorbate 80 – glycerol monooleate (GMO) mixtures generated droplet size distributions with smaller, more uniform droplets. A single pass through the Silverson mixer generated a very coarse emulsion that separated rapidly (data not shown). However, over 10 passes through the Silverson laboratory mixer (equivalent to

² Polysorbates are common surfactants used in food preparation.

> 4 passes through a full-size mixer) generated a good emulsion that was stable with small, uniform droplets. The Gaulins homogenizer and the Waring commercial blender at high speed for 5 minutes provided the smallest, most uniform droplets. Emulsions prepared with polysorbate 80 - GMO and both the Silverson high shear mixer and dairy homogenizer were stable for at least one month when stored at 4 °C. Droplet size distributions from both mixers were measured immediately after preparation, after storage for one week and after storage for one month. For both mixers, there was no significant change in the droplet size distribution (data not shown).

Table 3.2. Characteristics of Droplet Size Distributions from Different Surfactant–Mixer Combinations

#	Surfactant	Mixer	Mixing time	Median (µm)	Mean (µm)	Standard Deviation (µm)	Skewness of Log Dia.
1	Centrophase C lecithin	Kitchen blender on high speed	5 min.	2.7	3.9	3.1	0.7
2	Centrophase C lecithin	Silverson high shear mixer	3 passes	2.4	3.0	2.2	1.2
3	Centrophase C lecithin	Silverson high shear mixer	10 passes	3.2	3.6	1.5	0.5
4	Polysorbate 85	Kitchen blender on high speed	5 min.	4.6	4.8	1.7	0.4
5	Polysorbate 85	Lab. Homogenizer	5 min.	3.2	3.4	1.7	0.7
6	Polysorbate 85	Lab. Sonicator	5 min.	1.4	1.5	1.6	0.6
7	Polysorbate 80-GMO	Waring blender on low speed	3 min.	7.4	7.2	1.6	-0.3
8	Polysorbate 80-GMO	Waring blender on high speed	5 min.	1.2	1.2	1.3	0.2
9	Polysorbate 80-GMO	Gaulins homogenizer	1 pass	0.7	0.7	1.3	-0.3

Note: µm = Droplet diameter in micrometers
 Statistics are for Log₁₀ transformed distribution of the oil droplet diameter.

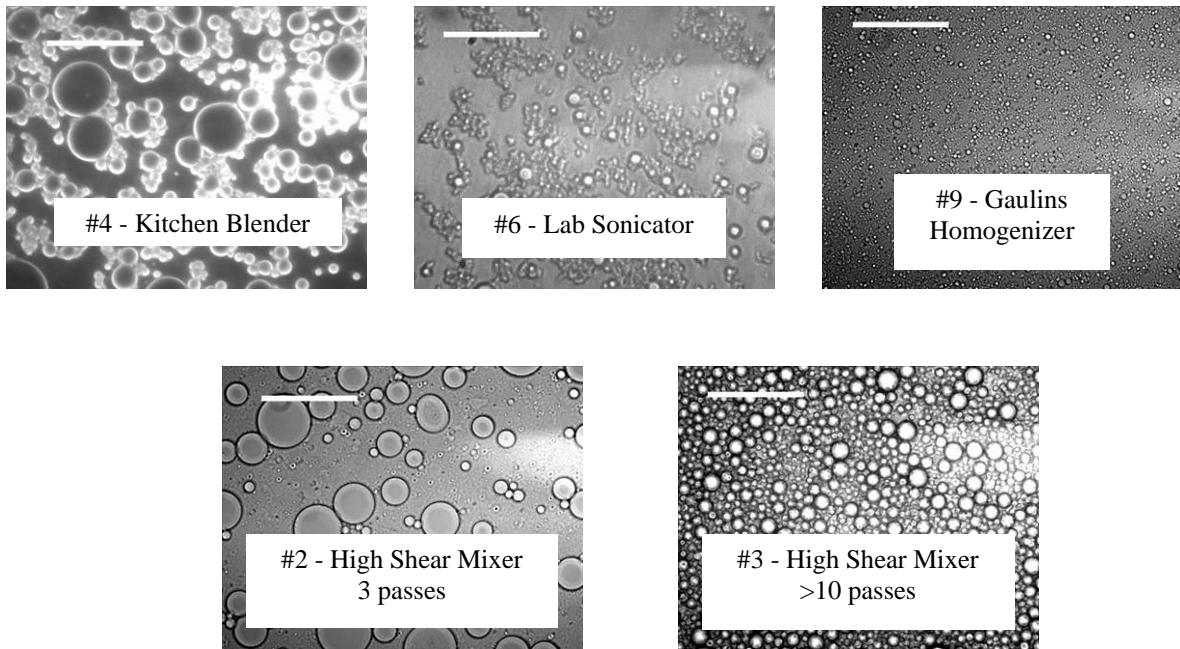


Figure 3.3. Emulsion droplets produced with different surfactants and mixing devices as described in Table 3.2. (White scale bar is 25 μm .)

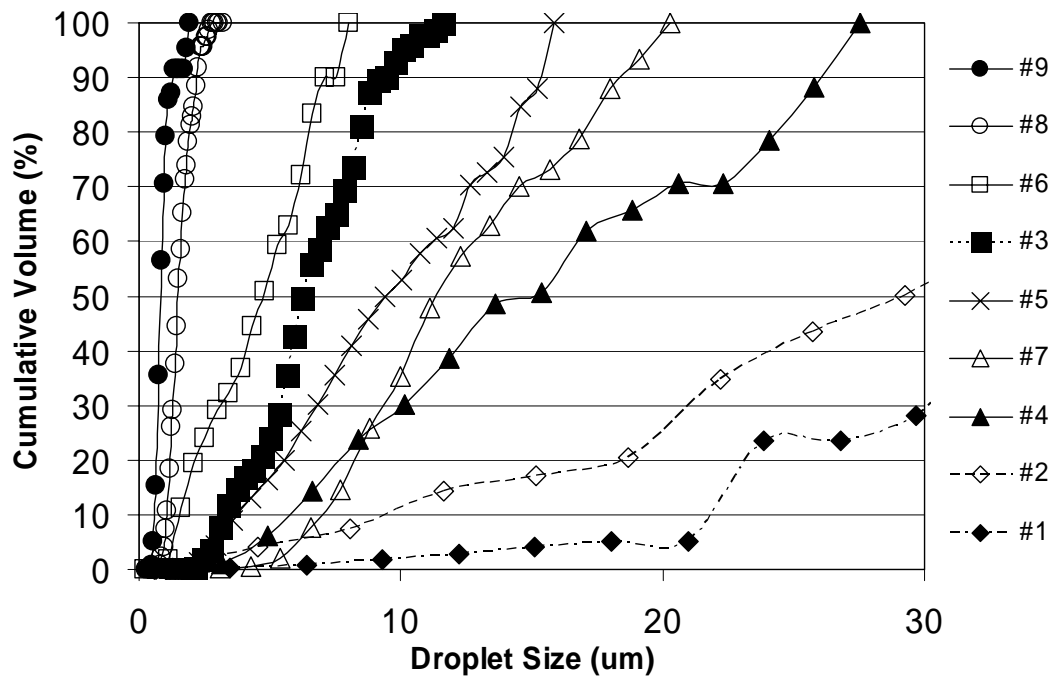


Figure 3.4. Cumulative droplet volume distributions for different emulsion preparation methods. (Emulsion numbers and preparation methods are listed in Table 3.2.)

The primary objective in developing an emulsion formulation is to generate an emulsion with small, uniform droplets that do not flocculate. Ideally, the emulsion mixture would be designed to match the site-specific conditions of the aquifer. However, this is beyond our current capabilities. There are several different formulations currently being used, with the final selection based on the personal preferences and experience of individual practitioners.

Emulsions can be prepared in the field through a four-step process: (1) dissolve all water soluble reagents in water; (2) dissolve all oil soluble reagents in oil; (3) blend oil and water together using an appropriate mixer; and (4) inject emulsion into the subsurface. Approaches used to mix the oil and water in the field include: (1) a single pass through a static in-line mixer; (2) repeated pumping through a high-speed centrifugal pump; (3) a single pass through a 3-phase, 3-Hp high shear mixer (e.g., Silverson Model 150/250 MS, East Longmeadow, MA); and (4) multiple passes through a high shear mixer. Mixing with a static in-line mixer or a centrifugal pump is much simpler to implement in the field, but generates a coarse emulsion with large oil droplets. Use of the high shear mixer generates an emulsion with smaller, more uniformly sized oil droplets. However, the high shear mixers are large pieces of equipment that can be cumbersome to use in the field. Use of emulsions with small oil droplets is preferred, because these emulsions are easier to distribute in most aquifers with less permeability loss and associated pressure build up.

An alternative to on-site emulsion preparation is to use pre-mixed emulsion. Typically, a pre-mixed emulsion is provided as a concentrate and then diluted in the field using an on-site source of water (preferably groundwater). Pre-mixed emulsions are prepared under higher quality control conditions resulting in a more precise mix of the emulsion ingredients and a more controlled droplet size. **Figure 3.5** shows the difference in droplet size between an emulsion prepared in the field and a pre-mixed emulsion. Pre-mixed emulsions are easier to handle in the field, require less equipment, and the amount of labor associated with preparation and injection is reduced. Some emulsion suppliers also include more easily degradable soluble substrates and nutrients (e.g., lactate and yeast extract) to stimulate rapid initial growth of dehalogenating microorganisms. However, pound for pound the materials cost for purchase of the pre-mixed emulsions is typically higher than the cost to purchase the raw materials used to prepare the emulsions.

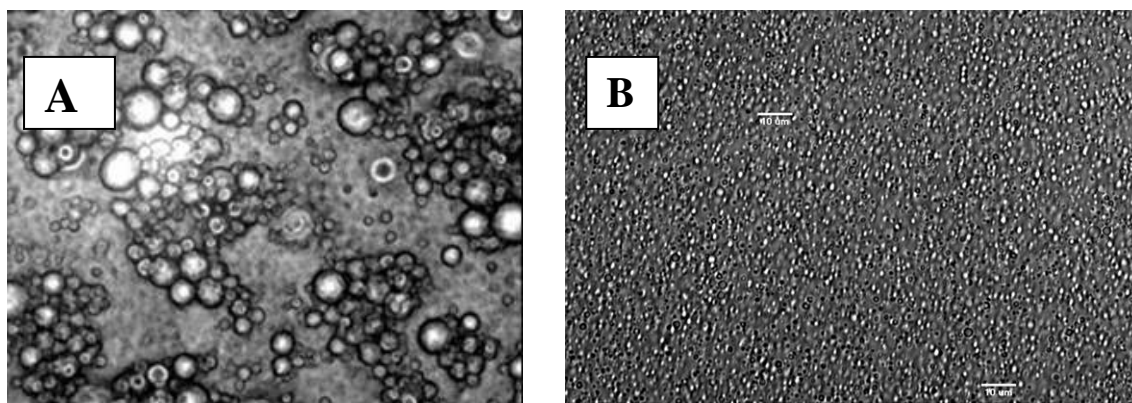


Figure 3.5. Photomicrographs of emulsions: (a) produced in the field with a high shear mixer and (b) a pre-mixed emulsion. (White scale bar is 10 µm.)

3.1.2.2 Emulsion Solubility and Interfacial Tension

Oil-in-water emulsions are very easy to disperse in water, because the individual emulsion droplets are already suspended in the water phase. For example, cream (an oil-in-water emulsion) is very easy to disperse in coffee. However, technically, the emulsion does not ‘dissolve’ since the individual oil droplets remain suspended in the aqueous phase. Similarly, the interfacial tension between water and an oil-in-water emulsion is zero since water is the continuous phase for both materials.

Emulsifying an edible oil does not change the inherent water solubility of the oil used to prepare the emulsions. However, breaking the oil up into many small droplets does increase the oil-water interfacial area for dissolution and access by microorganisms.

3.1.2.3 Density

The density of concentrated oil emulsions is between 0.96 and 1.00 g/mL and varies as a function of oil content. **Figure 3.6** shows the specific gravity of a commercially available emulsion (60% by weight soybean oil) when diluted in varying amounts of water. The manufacturer typically recommends that this material be diluted 19:1 to 4:1 with water prior to injection (3 to 12% final oil concentration), so the injected emulsion will have a specific gravity (ratio of emulsion density to water) between 0.994 and 0.999. Given the small difference in density between the diluted emulsion and water, buoyancy effects are not expected to be significant. These density effects can be further reduced by adding dissolved solutes (salts or sodium lactate) to increase the emulsion density.

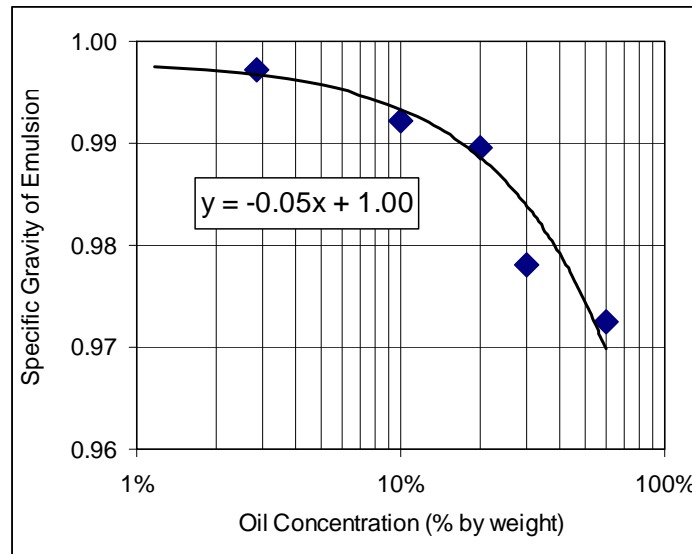


Figure 3.6. Specific gravity of EOS 598B emulsion diluted with varying amounts of water.
(Data provided courtesy of EOS Remediation, Inc., Raleigh, NC.)

3.1.2.4 Viscosity

The viscosity of oil-in-water emulsions varies as a function of droplet size and oil content. **Table 3.3** shows the effect of median oil droplet size on viscosity (Roland *et al.*, 2003). All of these emulsions were prepared with 30% (w/w) soybean oil as oily phase and polysorbate 60 and sorbitan monostearate (53:47

ratio) as surfactants. Increasing surfactant concentration resulted in a smaller droplet size and somewhat higher emulsion viscosity.

Table 3.3. Representative Oil Droplet Sizes and Dynamic Viscosities of Soybean Oil Emulsion Preparations

Emulsion	Mixer	Surfactant Content (%)	Median Droplet Size (µm)	Viscosity (mPa s) ^c
H	Hand		69	122
S	Silverson ^a		7	20
D10	Homogenizer ^b	10%	0.3	22
D5	Homogenizer	5%	0.7	12
D2	Homogenizer	2%	2.3	9

Source: Roland *et al.*, 2003.

^a Silverson L4R mixer (E.J. Payne Ltd., England) (emulsion S)

^b MiniDeBEE high-pressure homogenizer (BEEI International Ltd., Israel)

^c milliPascal seconds = the SI derived unit of dynamic viscosity. The pascal second or kg m⁻¹ s⁻¹ is equivalent to 10 poise.

Concentrated emulsions can be highly viscous (e.g., mayonnaise). However, oil-in-water emulsions commonly used for groundwater remediation are typically much less viscous than NAPL oils and do not require any special equipment for handling. **Figure 3.7** shows the viscosity of a commercially available emulsion (60% by weight soybean oil) when diluted in varying amounts of water. Viscosity in **Figure 3.7** is presented as the ratio of emulsion viscosity to water viscosity at 20 °C. The manufacturer typically recommends that this material be diluted 19:1 to 4:1 with water prior to injection (3 to 12% final oil concentration), so the injected emulsion will be between 1.3 and 2.1 times as viscous as water. The somewhat higher viscosity of the emulsion can result in a slight increase in back pressure during the emulsion injection phase, but may also result in somewhat reduced fingering of the injection front.

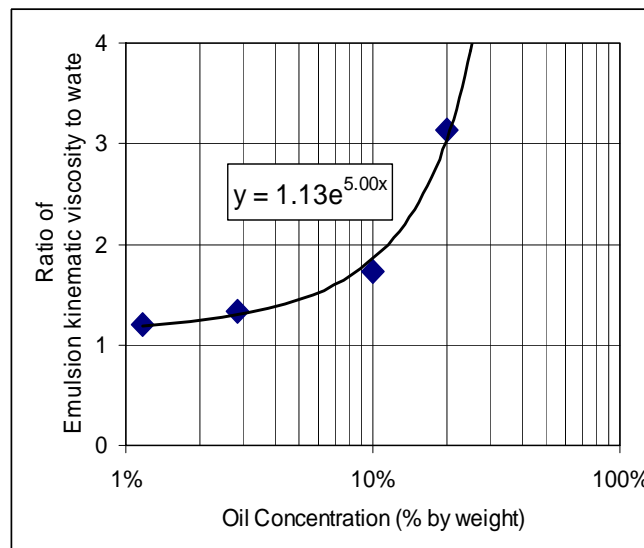


Figure 3.7. Ratio of emulsion kinematic viscosity to water for EOS 598B emulsion diluted with varying amounts of water. (Data provided courtesy of EOS Remediation, Inc., Raleigh, NC.).

3.1.3 IMPACT OF OIL AND EMULSION PROPERTIES ON MATERIAL HANDLING AND INJECTION

Table 3.4 provides information on the viscosity and specific gravity of some typical liquids and common emulsions used for aquifer remediation. At the concentrations typically used for aquifer remediation (1 to 10% oil per volume water), the emulsions have properties similar to milk or cream. Because of their ability to mix evenly in water without outside energy, emulsions can be injected easily using low pressure equipment. Commercial emulsion preparations do not require heating prior to use, even when used at temperatures below 10 °C. However, the emulsions should be prevented from freezing as this may damage the emulsion.

Table 3.4. Viscosity Values and Specific Gravity of Some Typical Liquids

Typical Liquid	centiPoise ^a (cP)	centiStokes (cSt)	Specific Gravity
Water	1	1	1.00
1% Oil-in-water emulsion	1.2	1.2	1.00
Milk	3.2	4	-
5% Oil-in-water emulsion	1.5	1.5	1.00
15% Oil-in-water emulsion	2.4	2.4	0.99
Cream	16.5	20.6	-
Vegetable oil	34.6	43.2	0.91 - 0.95
SAE 30 oil	352	440	0.88 - 0.94
Glycerine	820	650	1.26
Honey	1760	2200	-
Mayonnaise	5000	6250	-

Source: http://www.engineeringtoolbox.com/dynamic-absolute-kinematic-viscosity-21_412.html

^acentiPoise = centiStokes x specific gravity - where specific gravity is assumed to be 0.8 (except for water).

The exact Centipoise can be calculated: centiPois (cP) = centiStokes (cSt) x Density

Note: The pascal second (η) (Pa s) is the SI derived unit of dynamic viscosity. The pascal second or $\text{kg m}^{-1}\text{s}^{-1}$ is equivalent to 10 poise. Stokes is a CGS unit of kinematic viscosity. The stokes is defined to be $1 \text{ cm}^2 \text{ s}^{-1}$, equivalent to $10^{-4} \text{ m}^2 \text{ s}^{-1}$. Kinematic viscosity is defined to be dynamic viscosity (see poise) divided by the density of the liquid.

3.2 INJECTION AND DISTRIBUTION OF EDIBLE OIL EMULSIONS

Edible oils can be distributed in aquifers as oil-in-water emulsions followed by a chase water flush or as a dilute solution to distribute and immobilize the oil droplets. Oil-in-water emulsions are completely miscible with water so the emulsions easily disperse with groundwater after injection. As the oil droplets are transported through the aquifer pore spaces by flowing groundwater, they collide with sediment surfaces and stick. The sediment surfaces gradually become coated with a layer of oil droplets that provides a carbon source for long-term reductive dechlorination. For the best transport, the emulsion should be stable (e.g., non-coalescing); have small, uniform droplets to allow transport in most aquifers; and have a negative surface charge to reduce droplet capture by the solid surfaces.

Experimental and mathematical modeling studies by Soo and Radke (1984; 1986a; 1986b) have shown that oil droplets larger than the sediment pores are rapidly removed by straining with a large, permanent permeability loss. However, oil droplets smaller than the sediment pores can be transported significant distances through porous media with low interception by solid surfaces and low permeability loss. Recently, Coulibaly (2003) demonstrated that transport and retention of emulsified soybean oil droplets can be described by deep-bed filtration theory (Ryan and Elimelech, 1996; Logan, 1999).

Coulibaly and Borden (2004) conducted column experiments to evaluate emulsion transport and associated permeability loss in sands with varying clay contents. **Figure 3.8** shows the variation in emulsion concentration in the column effluent and effective hydraulic conductivity of field sand treated with a fine emulsion. The emulsion concentration is presented as the measured volatile solids (VS) concentration of the column effluent divided by the VS of the injected emulsion (C/C_0). During injection, the emulsion rapidly breaks through in the column effluent demonstrating effective transport in sand with over 5% clay. Then, during the post-injection water flush, the emulsion rapidly declines to background levels with little evidence of tailing or flushout of trapped emulsion. The effective hydraulic conductivity declines to ~ 66% of the pre-injection value and then returns to background levels during the water flushing. Most of the observed reduction in hydraulic conductivity is due to the higher viscosity of the emulsion (1.44 centipoise) compared to water (0.95 centipoise) at the ambient temperature (23 °C). However, when an emulsion with larger droplets is injected, the large oil droplets are filtered out, clogging the soil pores causing a permanent hydraulic conductivity loss (Coulibaly and Borden, 2004; Ullmann, 2004).

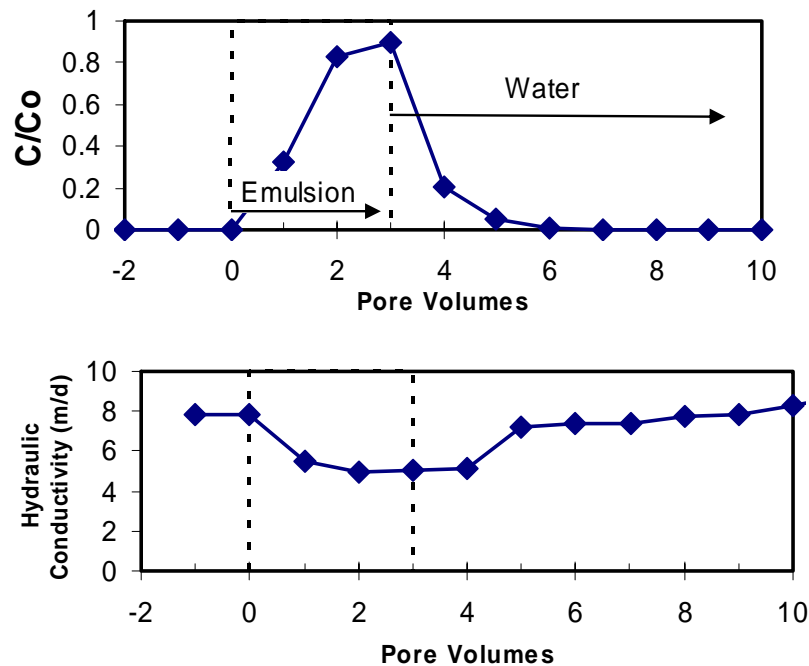


Figure 3.8 Variation in emulsion concentration (C/C_0) in column effluent and effective hydraulic conductivity during injection of field sand with 3 pore volumes of fine emulsion followed by plain water (from Coulibaly and Borden, 2004).

Sandbox studies conducted by Jung (2003) demonstrated that appropriately prepared oil-in-water emulsions can be effectively transported through sands with varying clay content. Oil droplet retention

on the sediment surfaces is proportional to the clay content with larger amounts of clay resulting in higher oil retention (Coulibaly and Borden, 2004). Upward migration of the oil droplets does not appear to be a significant issue. When a homogeneous sandbox was treated with emulsified oil and allowed to sit for almost two months, there was no evidence of upward migration of the oil droplets (Jung, 2003).

Work by Jain and Demond (2002) showed that droplet capture and associated permeability loss may also be strongly related to the surface charge characteristics of the oil droplets. Depending on the type of surfactant used to prepare the emulsion and the ionic strength of the groundwater, oil droplets may repel each other or they may stick together (flocculate). If they stick together, they can coat the pore walls forming mats of droplets many layers thick. **Figure 3.9** shows a photomicrograph of a pore clogged with many tiny emulsion droplets. Each droplet is much smaller than the pore throat. However, when they clump together forming mats, they can clog very large pores (30-70 μm). **Figure 3.10** shows how these mats can break off, migrate downgradient and clog other pores. As a consequence, it is very important to use emulsions that do not clump together.

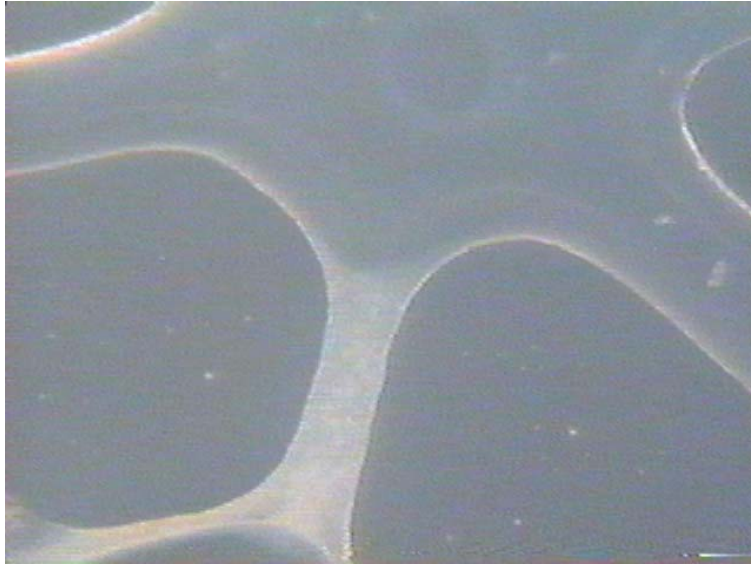


Figure 3.9. Restriction of flow due to emulsion droplet deposition partially plugging a pore throat (from Jain, 2000).

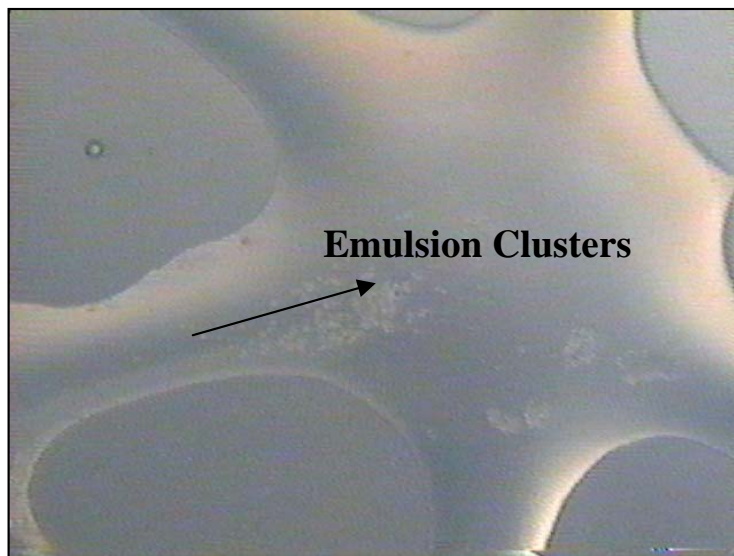


Figure 3.10. Movement of emulsion clusters induced by increasing the flow velocity (from Jain, 2000).

3.2.1 PROCEDURES FOR INJECTION OF EDIBLE OIL EMULSIONS

Projects involving injection of edible oil emulsions typically, but not always, involve the following steps: (1) installation of injection wells and injection system manifold; (2) emulsion preparation; and (3) emulsion and water injection.

3.2.1.1 Injection System Setup

Emulsion injection designs continue to evolve as more sites are treated using this technology. **Figure 3.11** shows the layout of the emulsion injection system employed at Altus AFB. The process used conventionally-drilled injection wells, located 5 ft on-center, that were installed in a linear, barrier configuration. A simple mixing and injection system was set up to control the flow rate into each well. A polyethylene vertical tank stored make-up water. The temporary aluminum mixing tank (i.e., a locally purchased farm trough) was used for blending individual ingredients brought to the site which included soybean oil, emulsifiers, and yeast extract. The mixture was passed through a shear mixer and recirculated into the mix tank so that injection flowrates could be equalized. Three of the six wells in the test barrier were injected simultaneously using this set up.



Figure 3.11 Typical injection system layout showing injection wells, feed lines with flowmeters and control valves, injection pump, emulsion mixing tank, and makeup water tank.

At a perchlorate site in Maryland, a pre-blended emulsion was used. The emulsion was delivered as a concentrate in 55-gallon drums, diluted 4 to 1 (water to emulsion), and injected through 1-inch direct-push wells using a manifold system to inject half of the wells (5 wells) simultaneously. Following injection of the diluted emulsion, chase water was used to further distribute the emulsion throughout the targeted treatment area. A process flow diagram from this project is shown as **Figure 3.12**.

LEGEND

PRESSURE GAGE	⊕
FLOW METER	⊙
VALVE - GATE	⊗
VALVE - BALL	⊗
QUICK-CONNECT UNION	-]-
AIR RELEASE VALVE	⊕

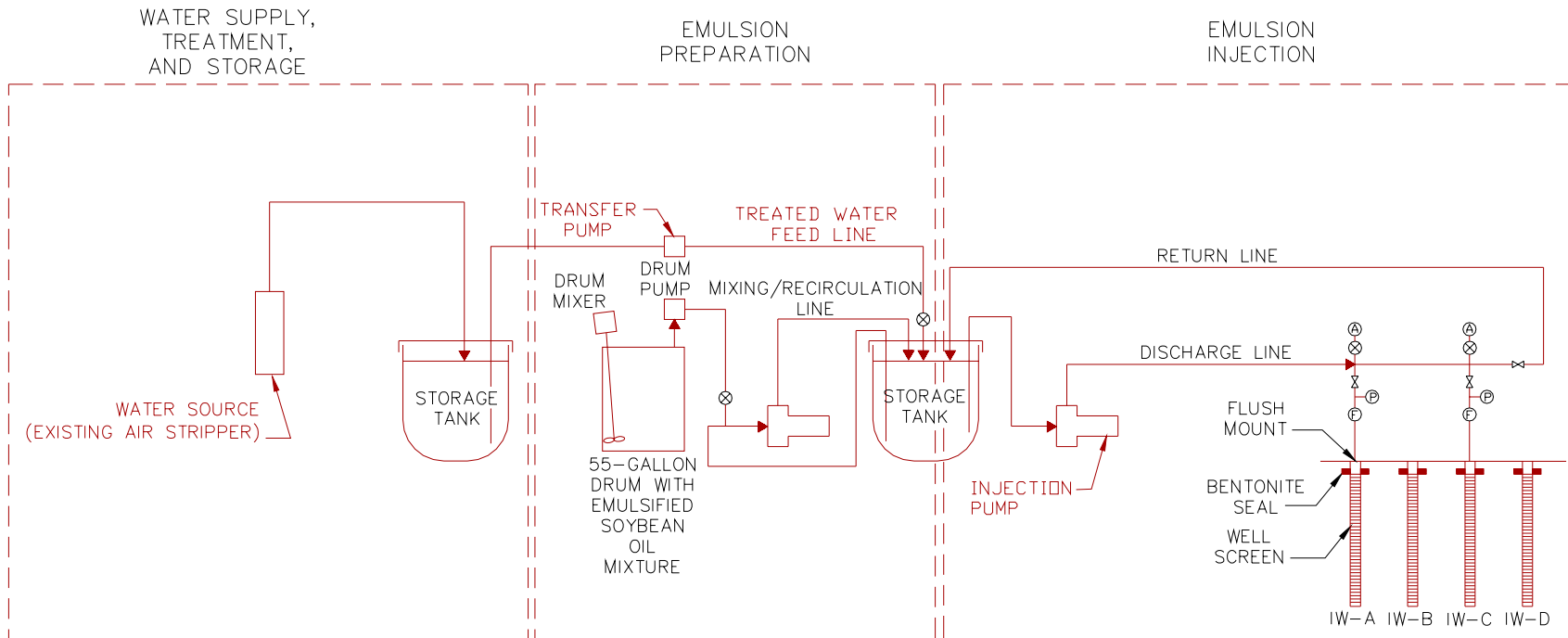


Figure 3.12. System used to prepare and inject pre-blended emulsion at a perchlorate site in Maryland.

To achieve the proper blend of emulsion and water, it is often simpler, but not required, to use a pre-manufactured emulsion concentrate. Automatic dosing systems use water pressure from the water source (e.g., fire hydrant) to mix with the emulsion and dilution water. The desired final concentration of dilute emulsion (e.g., 1:4 to 1:20 dilutions) can be adjusted by simply dialing in the amount of water and emulsion. A typical set up is shown in **Figure 3.13**. These systems install directly to any available water supply line and operate without electricity, using water pressure as the power source. The emulsion concentrate is pulled directly from the supply drum, tote, or tank and is mixed with water at the set dilution rate. The water pressure forces the diluted emulsion downstream to the injection well. The amount of emulsion concentrate is directly proportional to the volume of water entering the system, so variations in water pressure or flow rate have no effect on the dilution.

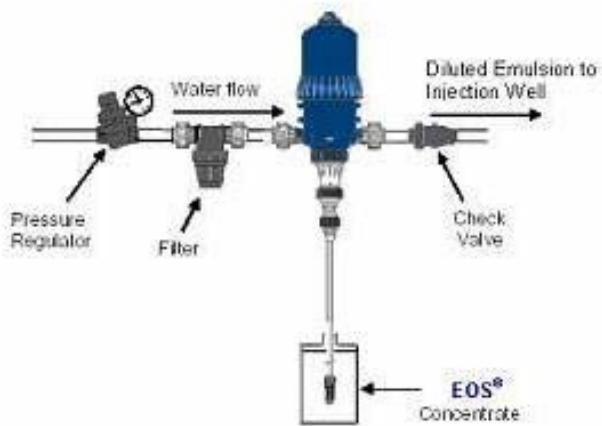


Figure 3.13. Typical setup showing automatic metering system for dilution of concentrated emulsion (courtesy of EOS Remediation, Inc.).

Depending on the injection well layout and formation permeability, emulsion injection can require a few hours to several days per well. As a consequence, several wells are typically injected at one time using a simple injection system manifold (**Figure 3.14**). Each injection line has a dedicated pressure gage, air blow-off valve, flow totalizer and flow control valve.



Figure 3.14 Injection system manifold showing separate control valve and flow totalizer for each injection well.

3.2.1.2 Emulsion Injection Wells

Emulsions can be injected through the end of a direct push rod, through temporary 1-inch direct-push wells, or through permanent 2-inch or 4-inch conventionally-drilled wells. The selection of the most appropriate method for installing injection points depends on site-specific conditions including drilling costs, flow rate per well, and volume of fluid that must be injected. Injection designs are typically optimized to provide the maximum injection flow rate while trying to minimize the drilling cost.

Using properly prepared emulsions, it is possible to move injected emulsions 10, 20 or 50 ft away from the injection point. However, achieving effective distribution of the emulsion often requires injecting large volumes of water. Process economics will depend on the cost for installation of each injection point and the injection flow rate that can be achieved. In many cases, it is desirable to install temporary or permanent wells that can be manifolded together to allow simultaneous injection of multiple points.

Injection wells should be thoroughly developed prior to beginning injection to obtain maximum injection rates and minimize the injection time. In addition, whether injections are performed through conventionally drilled or temporary wells, care should be taken to install a good cement/bentonite well seal directly above the target injection interval. This will reduce surface breakout if the emulsion is injected under pressure. Site-specific injection pressures should be estimated and wells should be constructed to withstand injection pressures (e.g., >10 psig). Wells are typically installed with 6 to 24 inches of PVC casing projecting above grade and a glue-on threaded PVC coupling. The injection hose can then be connected directly to the injection well. Once injection is complete, the well can be removed or the casing can be cut off below grade and the well completed with a conventional flush mount protective cover.

When the contamination extends over a significant vertical extent, it may be desirable to install several shorter screened wells to target specific intervals. This allows a known quantity of emulsion to be injected in each interval. However, this also increases injection system cost and complexity.

3.2.1.3 Emulsion and Water Injection

When injecting into multiple wells, a common approach is to inject every other well at one at one time. The aquifer is allowed to rest over night and then the system is reversed to inject the remaining wells. This approach reduces the potential for excessive head buildup in the aquifer and provides better distribution of emulsion between the injection wells.

During the injection, field personnel should regularly record the time, injection pressure, volume injected into each well, and other relevant information. Often, some injection wells will accept flow more rapidly than others. When the flow totalizer indicates that a well has received the required volume of emulsion and/or water, the control valve is closed and flow is diverted to the remaining wells.

SECTION 4

APPROACHES FOR FULL-SCALE APPLICATION OF EMULSIFIED OILS

This section presents a standardized procedure for the planning and design of anaerobic bioremediation projects using emulsified oils. The first step is for users to complete an initial screening to evaluate whether emulsified oils are potentially applicable to remediation at their site. After the initial screening is complete, remediation objectives should be defined, a conceptual site model should be established, and a preliminary conceptual design for remediation of the site should be developed following the procedures described in this section. The cost and performance of this approach can then be compared against other alternatives. If application of the emulsified oils process appears to be the most reasonable approach, then a pilot test of this process can be implemented as described in Section 5. Methods and procedures for evaluating field test results are discussed in Section 6. Lessons learned in the pilot test can then be used to revise the preliminary conceptual design to improve performance and reduce costs. Before proceeding with a pilot test or full-scale project, users should review the detailed description of the emulsified oils process provided in Sections 2 and 3.

4.1 INITIAL SITE SCREENING

When anaerobic bioremediation using emulsified oils is first considered for a site, three critical questions need to be answered.

- Can all target contaminants be anaerobically biodegraded?
- Have risks to critical receptors already been controlled?
- Can the emulsified oil be cost-effectively injected into the subsurface?

4.1.1 CONTAMINANTS

There are a wide variety of compounds that can be anaerobically biodegraded including chlorinated ethenes, chlorinated ethanes, halomethanes, perchlorate, nitrate, and explosives (e.g., RDX, HMX). For a few of these compounds (e.g., PCE, TCE, perchlorate, and nitrate), the biodegradation pathways and microorganisms that carry out this process are relatively well understood and enhanced anaerobic biodegradation has been demonstrated in the field at multiple sites. However, there are many other compounds (e.g., chlorinated ethanes and methanes, freons, etc.) where the factors controlling contaminant biodegradation are much less well understood. Site managers considering use of emulsified oils should carefully review the available information to determine if all of the target contaminants at their site are anaerobically biodegradable and the level of experience in treating these contaminants (see Principles and Practices, AFCEE 2004). For example, the microbiology of PCE biodegradation is relatively well understood and there is considerable practical experience with *in situ* anaerobic biodegradation of PCE. In contrast, 1,1,1-TCA has been shown to be anaerobically biodegradable in the laboratory. However, field experience with *in situ* anaerobic biodegradation of 1,1,1-TCA is much more limited and the environmental conditions and microorganisms that are required for 1,1,1-TCA biodegradation are less well understood. In addition, 1,1,1-TCA is susceptible to hydrolysis and its breakdown products have been shown to be aerobically biodegradable which has complicated the understanding of degradation mechanisms in the field. Site managers should be cautious about extrapolating results from laboratory biodegradation studies to the field.

Many sites contain mixtures of contaminants or co-contaminants (e.g., chlorinated solvents and petroleum hydrocarbons). Anaerobic biodegradation of many chlorinated solvents can be enhanced through

substrate addition. However, substrate addition could potentially inhibit biodegradation of petroleum hydrocarbons and related contaminants. If mixtures of chlorinated solvents, petroleum hydrocarbon, and/or solvent stabilizers (e.g., 1,4-dioxane) are present, other alternatives may need to be considered. Emulsified oil addition should be reserved for those sites where a source of dissolved organic carbon is thought to be limiting and target contaminants are preferentially degraded under anaerobic conditions.

Enhanced bioremediation is necessarily limited in its ability to treat DNAPL source zone areas due to many of the same factors (e.g., mass transfer limitations or heterogeneity) that affect conventional technologies. Sites that contain large amounts of DNAPL may not be appropriate for this process.

4.1.2 RISKS TO CRITICAL RECEPTORS

If a critical receptor such as a water supply well is located a short distance downgradient, then potential risks need to be controlled before implementation of the process. Potential alternatives include relocation of the water supply well or providing an alternative water source. Once these risks are controlled, use of emulsified oils can be reconsidered.

4.1.3 AQUIFER PERMEABILITY

Use of emulsified oils requires injection of the substrate into the subsurface. It is generally not cost-effective to distribute substrates in zones having a hydraulic conductivity less than 1 ft/day (4×10^{-4} cm/sec). Alternate injection techniques such as pneumatic fracturing have been used to inject emulsified oil away from the injection points. However, fracturing techniques will result in a much less uniform oil distribution and may not bring the oil into direct contact with the contaminant, reducing the effectiveness of this treatment. In aquifers with low hydraulic conductivities, the timeframe for remediation may be many years longer as remediation of the entire aquifer volume will likely be diffusion-limited.

4.2 REMEDIATION OBJECTIVES

The emulsified oils process is a very flexible technology that can be used in a variety of different configurations to treat contaminated aquifers including source area treatments and barriers. Potential benefits of this process include reduced source longevity, reduced contaminant mass flux, enhancement of ongoing natural attenuation, and/or control of dissolved plume migration. However, the benefits achieved will depend on the injection system layout and the method used to distribute the emulsion. Before planning an emulsified oil project, site managers should carefully define the remediation system objectives including compliance standards and remedial endpoints. The ability of enhanced anaerobic bioremediation to achieve drinking water MCLs has been demonstrated in some settings, but may not be possible at all sites. The use of less stringent, risk-based remedial goals may be more appropriate and achievable than default drinking water standards. Enhanced bioremediation is necessarily limited in its ability to treat DNAPL source zone areas due to many of the same factors (e.g., mass transfer limitations or heterogeneity) that affect conventional technologies.

Typical remediation objectives that the emulsified oils process can be used to address include the following:

- Destruction of contaminant mass in source zones.
- Reduction of contaminant concentrations in a dissolved plume.
- Reduction of mass flux from a source zone or across some containment boundary.
- Enhancement of already occurring natural attenuation.
- Cost-effective and continuous treatment over relatively long remediation timeframes.

Performance objectives based on dissolved contaminant concentrations alone should be used with caution. A significant amount (usually the majority) of contaminant mass in an aquifer system may be present as DNAPL or sorbed to the aquifer matrix. Due to the effects of dissolution and desorption of this contaminant mass, aqueous-phase concentrations alone may not accurately reflect the amount of mass being destroyed if there is continued mass transfer from DNAPL or sorbed mass to the aqueous phase.

4.3 CONCEPTUAL SITE MODEL

Once remediation objectives are defined, a conceptual site model (CSM) should be developed to determine if the emulsified oils process is suitable for the site. Most sites being evaluated for enhanced anaerobic bioremediation generally have been investigated and characterized to some extent, and a limited assessment of remedial alternatives has been conducted.

An assessment of the potential to stimulate anaerobic reductive dechlorination is based upon a review of site-specific data including hydrogeology, contaminant distribution and trends, and biogeochemical conditions (electron donors, electron acceptors, metabolic byproducts, and general geochemical indicators). A CSM summarizes the fate and transport of contaminants, migration pathways, exposure mechanisms, and potential receptors. Site characterization considerations for selection, development, and evaluation of an emulsified oil field test are described in the following subsections.

4.3.1 HYDROGEOLOGY

The subsurface hydrogeology must be considered in the site selection and design process, as inadequate characterization of the site hydrogeology can lead to system failure. In many cases, the system can be designed to mitigate difficult hydrogeologic conditions. Depth to water and the depth of the contaminant plume primarily impact the capital cost of drilling and delivering the emulsion to the intended treatment zone. In addition, there are practical limits to the maximum length of well screen across which an edible oil emulsion can be uniformly injected. Therefore, practitioners designing treatment of contaminated aquifers with a large saturated thickness should consider using multiple vertical injection points to achieve the desired vertical coverage.

4.3.1.1 Depth to Groundwater

Depth to water and the vertical thickness of the plume primarily impact the capital cost of drilling and delivering the substrate to the intended treatment zone. Where possible, installation of injection wells using direct push equipment will result in a less costly installation. Direct push equipment may also be used to inject the emulsion directly, which may further reduce cost for materials, but may increase time to perform. The capital expense of installing multiple injection wells in deep settings (e.g., greater than 100 feet below ground surface [bgs]), or across thick formations may inflate the cost of the injection process to a level not competitive with other remedial technologies. For example, pump-and-treat methods may provide hydraulic control and remediation of a deep plume using only a few large-diameter recovery wells spaced at distances determined by appropriate groundwater models. Emulsion injection to form a barrier across a similar hydraulic front would likely require more wells on closer spacings than a pump-and-treat design. Although the emulsified oil process may require more wells to implement, it should not be ruled out for this reason alone because O&M costs may be significantly lower than other technologies.

4.3.1.2 Hydraulic Conductivity

Hydraulic conductivity is a primary factor in effective distribution of substrate in the subsurface. In general, hydraulic conductivities greater than 10 feet per day (ft/day), or approximately 4×10^{-3} centimeters per second (cm/sec), are best for effective distribution of emulsified oils out away from the injection points. As discussed above, it is generally not cost effective to distribute substrates in zones having a hydraulic conductivity less than 1 ft/day. Although alternate injection techniques such as pneumatic fracturing have been used to inject emulsified oil, these techniques often result in a much less uniform oil distribution and may not bring the oil into direct contact with the contaminant, reducing treatment effectiveness. Strongly heterogeneous sites present special challenges for achieving uniform substrate distribution. Any injected fluid will preferentially flow into more permeable materials. Thus, attention should be applied to understanding whether contaminants are localized in more or less permeable layers. Distribution of emulsified oil in more permeable materials may be very effective in reducing the mass flux of contaminants out of a source area since the contaminants will be treated as they pass through these higher permeability, emulsion treated zones. However, if the majority of the contaminant mass has partitioned into less permeable clays, silts, or bedrock, then overall biodegradation rates will be slow and will be controlled by slow diffusion of the contaminants out of these lower permeability layers.

4.3.1.3 Groundwater Flow

Groundwater velocity, flow direction, and horizontal and vertical gradients will impact the effectiveness of emulsified oil addition. Excessively high groundwater flow rates (greater than 5 ft/day) may require large amounts of substrate to overcome a large influx of competing electron acceptors migrating into the reactive zone. A substantially larger treatment zone may also be required to maintain sufficiently reducing conditions in high-flow aquifers. Where groundwater flow rates are very low (less than 1 to 20 ft/yr), the timeframe for remediation may be extended due to reduced mixing of substrate and contaminant mass.

4.3.2 CONTAMINANT DISTRIBUTION

4.3.2.1 Source Area Size

Emulsified oils can provide a long-lasting substrate to support anaerobic biotransformation processes. Emulsified oils will be most cost-effective for small to mid-size source areas. For very large sources, it may be more cost-effective to contain the source using either an impermeable barrier or possibly an emulsified oil biologically active barrier surrounding the source.

4.3.2.2 Plume Size

For large plumes, it may not be economically feasible to remediate the entire plume at one time due to the relatively high cost of installing injection wells. As in treating the source area, oil emulsions can be used to generate a larger radius of influence around each injection point. However, a much more cost-effective approach is to install barriers at several different points along the plume. For example, if the barriers are spaced 1 to 2 years travel time apart, the entire plume should be treated by passage of contaminated groundwater through one or more barriers within five years.

4.3.3 GEOCHEMISTRY

Geochemical evaluations are focused on determining the prevailing oxidation-reduction (redox) conditions and demonstrating that the “footprints” of the expected degradation processes are present. Characterizing the initial geochemical and redox conditions is useful to determine the prevailing terminal electron acceptor processes to evaluate the changes in redox conditions required for optimal contaminant biodegradation. High levels of alternate electron acceptors (e.g., DO, nitrate, or sulfate) should be considered when determining substrate demand. Electron donor supply is often measured and tracked by measuring parameters such as TOC or metabolic volatile fatty acids (VFAs).

4.3.3.1 Sulfate/Sulfides

Existing guidance documents (Principles and Practices, AFCEE 2004) suggest that while CAH dechlorination under sulfate reducing conditions is feasible, high sulfate levels can be problematic for CAH bioremediation. The anaerobic dechlorination scoring matrix in the USEPA (1998) protocol results in a lower score (lower potential for anaerobic dechlorination) if sulfate exceeds 20 mg/L; similar cautions are provided by Morse *et al.* (1998). Sulfate must be reduced in order to reach methanogenic conditions, and high sulfate levels may lower the efficiency at which substrate is utilized for anaerobic dechlorination.

However, there is ample evidence in the literature for dechlorination of a wide variety of CAHs at sites containing elevated dissolved sulfate levels (ITRC, 1998; Devlin and Muller, 1999; and Suthersan *et al.* 2002 reported successful application of enhanced anaerobic bioremediation at sites containing up to 500 to 700 mg/L of sulfate). Complete anaerobic dechlorination has been stimulated at several high-sulfate Air Force sites including Altus AFB, Oklahoma (sulfate up to 2,600 mg/L) and Travis AFB, California (sulfate up to 5,400 mg/L). However, users of this technology should be cautious about application of this technology at sites with both high sulfate levels and very low iron concentrations in soil, since excessive levels of sulfides produced by reduction of sulfate can be inhibitory to anaerobic dechlorination. This is not an issue at most sites (e.g., those with appreciable amounts of iron in the soil), since sulfide rapidly reacts with iron and is removed from solution as an insoluble precipitate (FeS).

4.3.3.2 Dissolved Oxygen, Nitrate, Iron, Manganese and Oxidation-Reduction Potential

Anaerobic bacteria generally cannot function at DO concentrations greater than about 0.5 mg/L, and hence anaerobic biodegradation will not occur. Consequently, some users have been concerned about the ability to stimulate anaerobic biodegradation in naturally aerobic aquifers. In practice, this is not a significant issue. At every site tested to date, injection of edible oil emulsions has resulted in rapid depletion of dissolved oxygen and strongly reducing conditions.

High levels of oxidized iron and manganese in soils also have the potential to inhibit anaerobic biodegradation processes by increasing the rate of substrate consumption and reducing the amount of available hydrogen (Evans and Koenigsberg, 2001; Wilson *et al.*, 2003). However in practice, this has not been a problem when emulsified oils are effectively distributed throughout the treatment zone.

In summary, elevated levels of competing electron acceptors (O₂, NO₃, Mn, Fe) will increase the substrate demand and require additional contact time between the oil and the groundwater to generate strongly reducing conditions.

4.3.3.3 pH and Alkalinity

As with most biological processes, a pH close to neutral is optimum for microbial growth and contaminant biodegradation. However, this may be especially important when stimulating reductive dechlorination since dechlorinating bacteria appear to be more sensitive to pH than other common microorganisms. In one series of experiments with an enrichment culture known to contain *Dehalococcoides*, dechlorination of PCE was four-fold slower at pH 6 than at pH 7 (Young and Gossett, 1997). Similarly, the KB-1™ bioaugmentation culture exhibits no dechlorination below pH 5 (Rowlands, 2004).

Low pH conditions in an aquifer can result from several different factors. In many areas, aquifers have a naturally low pH (less than 6) and low buffering capacity. When stimulating anaerobic biodegradation processes, hydrogen (H₂) and acetic acid are produced by fermentation of the added organic substrate (sugars, fatty acids, and edible oils). If the aquifer buffering capacity is too low, the acetic acid can result in a further decline in pH. Since the microorganisms that produce acetic acid (fermenters) are generally less sensitive to pH than the organisms that consume acetic acid (dechlorinators and methanogens), acetic acid can gradually accumulate resulting in a progressive drop in pH and a ‘sour’ aquifer.

A decline in pH is generally not a problem in aquifers with significant carbonate alkalinity (> 1 g/L as CaCO₃) or significant levels of iron hydroxides (e.g., Fe(OH)₃ and FeOOH) since H⁺ is consumed during iron reduction. If the pH buffering capacity of the aquifer is too low, basic salts such as sodium bicarbonate or magnesium hydroxide may be used to increase the pH.

4.3.4 MICROBIOLOGY

The emulsified oil process may be suitable for *in situ* biological treatment of a wide variety of contaminants including chlorinated solvents, perchlorate, nitrate, TNT, RDX, HMX and some metals (e.g., chromate). However, our understanding of the underlying microbiology of these processes varies widely. For the chloroethenes (PCE, TCE, DCE and VC) and perchlorate, we have a reasonably good understanding of the different organisms that degrade the contaminants, their distribution in the environment, and required growth conditions. In contrast, our understanding of the microbiology of other degradation processes is much more limited.

4.3.4.1 Reductive Dechlorination of Chloroethenes

There are a variety of different bacteria capable of reducing PCE and TCE to DCE. However, only *Dehalococcoides* like organisms have been shown to be capable of complete dechlorination of PCE and TCE to ethene in a pure culture (e.g., Maymo-Gatell *et al.* 1997). Recent results indicate that biological reduction of *cis*-DCE to ethene may require certain strains of the *Dehalococcoides spp.* and that some strains do not gain energy from the reduction of VC to ethene (Löffler *et al.* 2003, Maymo-Gatell *et al.* 1997). Hendricksen *et al.* (2002) found that *Dehalococcoides* like organisms were present at every site examined (21) where *cis*-DCE was dechlorinated to ethene, but were not found at three sites where dechlorination stopped at *cis*-DCE. These results indicate that while *Dehalococcoides* like organisms are relatively common, if the required organisms are absent, dechlorination may stall at *cis*-DCE or VC.

There are several different approaches that can be used to determine if the microbial community at a site is capable of complete dechlorination of PCE, TCE and *cis*-DCE to ethene. If monitoring data indicate that ethene is being produced at the site, even at low levels, this is a strong indication that the indigenous microbial community is capable of complete dechlorination. A wide range of molecular techniques are also available to characterize subsurface microbial communities and can be used to determine if

Dehalococcoides like organisms are present at a site and if they have the required genes to reduce VC to ethene. However, the inability to detect *Dehalococcoides* like organisms at a site does not necessarily mean that complete dechlorination will not occur. Their numbers may initially be very low or patchy due to the absence of a suitable electron donor. Once a biodegradable organic substrate is added (e.g., emulsified edible oil), the number of dechlorinators may increase rapidly resulting in rapid and complete dechlorination of PCE and TCE to ethene.

4.3.4.2 Bioaugmentation

Bioaugmentation may be utilized at a site when an appropriate microbial population is not present or is present in low population numbers and not sufficiently active to achieve remediation goals. Bioaugmentation is the application of a microbial inoculant comprised of enriched microorganisms developed from the site or of non-native origin to accelerate anaerobic biodegradation processes in the aquifer. At several sites where reductive dechlorination had stopped at *cis*-DCE, researchers were able to simulate complete dechlorination to ethene in the subsurface by introducing mixed cultures containing *Dehalococcoides* like organisms (e.g., Ellis *et al.* 2000, Henssen *et al.*, 2001, Major *et al.* 2002). These promising results have led to the introduction of several commercially available bioaugmentation cultures.

Bioaugmentation can be performed at the start of treatment (soon after emulsified oil addition has generated strongly reducing conditions) or after monitoring for some period of time to determine whether complete dechlorination will occur in the absence of bioaugmentation. The economics of bioaugmentation will depend on the amount and cost of the bioaugmentation culture and method used to distribute the inoculum. In most of the demonstrations, active recirculation has been used to distribute the inoculum. However, active recirculation can be more expensive. There is increasing evidence that relatively low-cost, “passive” bioaugmentation (direct injection of culture solutions, without recirculation) can be effective. A passive application approach is expected to be more compatible with the emulsified oil process.

The ESTCP white paper “Bioaugmentation for Remediation of Chlorinated Solvents: Technology Development, Status, and Research Needs” provides a detailed evaluation of this practice and some preliminary guidance on application of this technology (ESTCP, 2005). Conclusions from the ESTCP (2005) review are presented below:

1. Several bioaugmentation cultures are commercially available for stimulating reductive dechlorination of chloroethenes, and their value has been demonstrated under field conditions.
2. Cultures can be grown efficiently, transported to field sites effectively, successfully injected, and in most cases they will survive and grow in aquifers given proper environmental conditions.
3. Project managers should address the question of bioaugmentation as early in the design stage as possible, and perform an explicit cost-benefit assessment, including a life-cycle cost analysis, to determine whether bioaugmentation has the potential to reduce the time and costs for bioremediation.
4. The costs for bioaugmentation generally represent a low fraction of the total remediation costs (typically 1-3%). In many cases, passive bioaugmentation can pay for itself if it reduces the time needed for complete dechlorination by even a couple months.
5. The key practical issues appear to be determining *a priori* whether bioaugmentation will be beneficial, ensuring adequate distribution of added cultures throughout a target zone, overcoming potential inhibitory conditions, and ensuring adequate quality controls.

6. The roles of the other organisms present within the mixed cultures used for bioaugmentation are not clear. Other organisms appear to be needed for complete dechlorination, at least at some sites.
7. Site-specific tests to characterize the indigenous microbial population have been developed and can be useful in deciding whether to bioaugment. These tests include targeted microbiological and molecular biological analyses that can rapidly assess the potential for complete dechlorination.
8. Project managers should work closely with the culture vendors to ensure that the cultures are added in a manner that maximizes the potential for success. The timing and locations of injections and the numbers of organisms added should all be carefully designed in a cooperative manner.
9. The methods used to add and distribute the augmentation cultures are key economic considerations. Most vendors report considerable success with much less expensive “passive” injection techniques, although definitive demonstrations of this approach are not yet available.

4.4 CONCEPTUAL DESIGN

A general conceptual design for the distribution of the edible oil emulsion should be developed after defining the remediation objectives and conceptual site model. This design will consist of determining the general layout of the emulsified oil treatment and will take into account additional planning considerations, such as secondary water quality issues and soil gas emissions.

4.4.1 TREATMENT ZONE LAYOUT

Several treatment approaches are commonly considered for application of edible oils. The most common approaches are source area treatment and use of emulsions in a permeable reactive barrier. In choosing a treatment approach for a given site, it is important to understand the overall objectives of the project. The objectives may be to reduce contaminant concentrations below the maximum contaminant levels (MCLs), to reduce mass flux as part of an overall risk reduction approach, or to limit plume migration.

4.4.1.1 Source Area

Emulsified oils can be distributed throughout a source area to reduce the contaminant mass flux out of the source area and to eventually treat the contaminants. Oil injection will stimulate microbial activity, generating strongly reducing conditions and promoting anaerobic biodegradation of the target contaminants. Biodegradation of the aqueous phase contaminants is enhanced by dissolved organic carbon released during the biodegradation of the edible oils. For chlorinated solvents, a portion of the contaminant mass will partition into the edible oil, initially reducing aqueous phase concentrations. Over time chlorinated solvents that have partitioned into the edible oil will be released back into the groundwater and be degraded. The time for complete contaminant biodegradation should be considered in the design of the oil application. Methods for estimating the volume of injected oil required are included in Section 4.5.

A variety of different injection patterns can be used to treat source areas including uniform grids of injection wells, grids of injection and extraction wells, or a series of barriers to repeatedly treat contaminated water as it flows through the area. Where the thickness of the contaminated zone is

substantially greater than the desired injection well screen, injection wells can be staggered at variable depths. For example, injection into a contaminated aquifer approximately 60 feet in thickness can be accomplished using three 20-foot injection screens at staggered depths. All subsurface injection strategies should consider the distribution of contaminants in relation to different soil layers. The presence of more permeable soil layers next to less permeable layers will lead to preferential flow into more permeable strata and could result in less than optimal substrate-to-contaminant contact.

Injection of edible oil emulsions typically results in low residual saturations (1 – 3%). Consequently, emulsions will be much less useful for sequestering chlorinated solvents and blocking groundwater flow, compared to NAPL oil injection. However, if the objective is to bioremediate the source area, emulsions may be more useful because of their easier distribution in the subsurface and lower residual saturation. Assuming a porosity of 30% and residual saturation of 2%, 4,500 gallons of emulsified oil would be required to treat a 100-ft x 100-ft x 10-ft thick source area.

4.4.1.2 Permeable Reactive Barrier

In many cases, the source of a contaminant plume is poorly defined or a plume is a result of multiple dispersed sources where source containment/reduction is not feasible. In other cases, it may be desirable to intercept a contaminant plume upgradient of a property boundary or a potential receptor. Under these conditions, emulsified oils can be injected in a reactive barrier configuration for plume containment through enhanced biodegradation. As with any permeable barrier configuration, the reaction zone must be uniformly distributed and an effort made to maintain the permeability of the reaction barrier. Edible oil emulsions can be effectively used to create permeable reactive barriers.

Residence time within the barrier reaction zone will be controlled by the groundwater flow velocity and length of the oil treated zone along the direction of groundwater flow. At present, there is no reliable, all-inclusive method for determining the required contact time for effective treatment. Laboratory column experiments and limited field studies suggest a 1 to 3-month contact time should be sufficient in many cases. Assuming a groundwater flow velocity of 100 ft/yr, a 1 to 3-month contact time results in a required barrier length of 8 to 24 ft. However, the 1 to 3-month contact time estimate should be used for preliminary planning purposes only; field pilot studies are needed to determine the required contact time / barrier length for a specific site.

Barriers are typically installed across the plume, perpendicular to groundwater flow. The barrier width (perpendicular to groundwater flow) should be wider than the contaminant plume to allow for uncertainties in the actual plume dimensions, variations in groundwater flow direction, and some permeability loss. When using edible oil emulsions, the permeability loss associated with the actual emulsion injection is expected to be minor. However, biomass growth and gas production may result in up to an order-of-magnitude reduction in permeability (Long and Borden, accepted). Common groundwater flow and transport models (MODFLOW and MT3D) can be used to assess the impacts of permeability loss on barrier performance and determine the required barrier width to prevent contamination from bypassing the barrier. In most cases, up to a factor of ten reduction in permeability in a 20 to 40-ft thick barrier is not a significant issue. However, the barrier width must be increased somewhat (typically 10 to 30%) to prevent a portion of the flow from bypassing around the edges of the barrier.

4.4.2 ADDITIONAL PLANNING CONSIDERATIONS

While emulsified oil injection can enhance biodegradation of contaminants, there are some secondary effects of the injection that need to be considered including secondary water quality issues and soil gas emissions.

4.4.2.1 Secondary Water Quality Issues

The term “secondary water quality” is used in this document to refer to water quality issues or concerns, apart from the primary contaminants being treated, that result from substrate addition. Degradation of secondary water quality can occur as a result of mobilization of formerly insoluble forms of metals that occur naturally in the aquifer matrix. Other secondary water quality parameters that may be degraded include chemical oxygen demand (COD), biochemical oxygen demand (BOD), total dissolved solids (TDS), and sulfides that affect taste and odor. These parameters should be monitored if regulated at the site.

When planning an edible oils project, an *in situ* bioreactor approach should be adopted. In this approach, organic substrate is added to a specific reactive zone generating strongly reducing conditions and stimulating biodegradation of the target contaminants. Within the anaerobic reactive zone, intermediate degradation products (e.g., *cis*-DCE) may temporarily accumulate before subsequent treatment in downgradient anaerobic or aerobic zones. Within the reactive zone, the taste and odor of groundwater will be impacted due to elevated levels of COD, BOD, TDS, sulfides and/or fatty acids. In addition, the reduced groundwater environment in the reactive zone may increase the mobility of some naturally occurring, but regulated metals (e.g., iron, manganese, and arsenic). While these metals are more soluble under reducing conditions, migration of metals out of the reactive zone is often substantially retarded by adsorption to the aquifer matrix and/or precipitation as insoluble metal sulfides (Butler and Hayes, 1999).

In naturally aerobic aquifers, groundwater typically returns to near background conditions within a relatively short distance downgradient of the reactive zone. As groundwater migrates downgradient, the excess substrate will be consumed by biological processes, and the anaerobic groundwater will mix with background aerobic groundwater resulting in precipitation / immobilization of dissolved metals. In naturally anaerobic aquifers, secondary water quality impacts may extend farther downgradient. However, the natural groundwater quality in naturally anaerobic aquifers is generally not of drinking water quality or beneficial use.

The potential for degradation of secondary water quality should be considered when working in close proximity to drinking water supplies. It also should be noted that these changes in water quality, and those discussed under generation of noxious gases, are not easily reversed and, in the case of a slow release carbon source, may take many years for the effects of the substrate addition to diminish. These secondary water quality issues should be carefully considered before proceeding with an enhanced anaerobic bioremediation project. Specific groundwater quality goals should be established for wells upgradient of sensitive areas, but allow for temporal increases in breakdown or byproducts within the reactive zones.

Based on petroleum hydrocarbon plume studies, dissolved organic reactive zones are not likely to exceed 200 to 300 feet downgradient of the oil injection zone. Impacted zones downgradient of emulsified oil injections are expected to be much more limited because emulsified oils are much more biodegradable than petroleum hydrocarbons. Monitoring data from existing emulsified oil sites indicate that DOC and secondary groundwater quality parameters are not affected more than 50 feet downgradient of emulsified oil injection zones. To provide a substantial factor of safety, a minimum distance of 250 feet should be maintained between injection locations and critical downgradient receptors.

4.4.2.2 Soil Gas Emissions

There is a potential for methane production as a result of emulsified oil injection. Highly elevated methane concentrations could potentially pose a problem when found near buildings. Therefore, soil gas

monitoring should be conducted when emulsified oils are applied near the water table surface and in close proximity to buildings. Biodegradation of the methane will occur rapidly in the presence of oxygen, and soil gas oxygen concentrations should be measured to determine if methane is likely to be biodegraded *in situ*. Soil gas carbon dioxide concentrations should also be measured, because elevated carbon dioxide levels often correlate with methane generation.

4.5 DETAILED DESIGN OF AN EMULSIFIED OIL PROJECT

The primary factors to consider when designing an emulsified oil source area treatment or permeable reactive barrier are: (1) amount of oil required for effective treatment; (2) the amount of water required to distribute the oil; and (3) injection well spacing.

4.5.1 AMOUNT OF OIL REQUIRED

There are two main issues to consider in determining how much emulsion to inject into the subsurface:

- Consumption of oil during biodegradation of the contaminants including biodegradation of competing electron acceptors (e.g., oxygen, nitrate, sulfate) and downgradient release of dissolved organic carbon and methane; and
- Entrapment of emulsified oil by aquifer material.

4.5.1.1 Oil Consumption During Contaminant Biodegradation

The amount of oil required to support contaminant biodegradation will be a function of: (a) treatment zone dimensions; (b) site hydrogeology; (c) system design life; (d) amount of electron acceptors entering the treatment zone (both contaminants and naturally occurring electron acceptors); and (e) additional hydrogen demands and release of dissolved organic carbon to the downgradient aquifer. The following subsections outline the various calculations and potential safety factors that should be considered to estimate the amount of substrate required using site-specific data and design criteria. For ease of understanding and calculation, the factors that are considered are presented in a spreadsheet in **Appendix A**. Based on these calculations the practitioner can determine the amount of substrate needed for a given site. Other approaches may be available, and as the science and engineering behind the edible oils technology evolves, new and improved tools will likely become available. ESTCP is funding a new project, “Development of a Design Tool for Planning Aqueous Amendment Injection Systems” (ER-0626), which will provide an improved tool for designing emulsified oil (and other amendment) injection systems.

Treatment Zone Dimensions

A typical source area treatment can be designed by first determining the width perpendicular to flow, length parallel to flow and effective vertical height targeted for treatment. Then, the amount of substrate required should be determined based on the treatment volume, contaminant concentrations, and competing electron acceptor concentrations. The potential mass flux of contaminants and competing electron acceptors into the treatment area should also be calculated using the upgradient concentrations, width and effective height of the treatment area, and the groundwater flow velocity. The goal of the treatment is to provide sufficient substrate to destroy the contaminant mass within the treatment area and reduce any potential mass flux of contaminants into the area during the treatment time period.

Figure 4.1 shows the dimensions that must be considered in planning a permeable reactive barrier design. The width of the barrier perpendicular to groundwater flow (y) and height (z) of the barrier to impact the contaminated zone must first be determined based on plume dimensions. The length (x) of the barrier along the direction of groundwater flow should be sufficient to provide a 1 to 3-month contact time between the contaminant and the oil treated aquifer material. Shorter contact times may be acceptable if high treatment efficiencies are not required. Longer contact times are needed for high contaminant or electron acceptor (e.g., sulfate) concentrations. The area of the barrier is then used along with the groundwater flow velocity, contaminant concentrations, and competing electron acceptor concentrations to calculate the anticipated mass flux of contaminants and competing electron acceptors through the barrier. The barrier should be designed to provide sufficient substrate for a given time period (e.g., 5 to 10 years) taking into consideration the mass flux of contaminants and competing electron acceptors and accounting for losses from the barrier due to methane production and release of organic and inorganic carbon.

Figure 4.1. Treatment Zone Dimensions
(Courtesy of EOS Remediation, Inc., Raleigh, NC.)

Site Hydrogeology

For a source area treatment, the volume of water to be treated is determined based on the volume within the treatment zone and the flow into the treatment zone during the treatment period. The treatment area dimensions are entered into the spreadsheet (**Appendix A**) along with the effective porosity. The volume of water within the treatment zone is simply obtained by multiplying the width perpendicular to flow (y), vertical height of the treated zone (z), length along the direction of groundwater flow (x), and effective porosity (n) of the treatment area. The flow into the treatment zone is determined using the same procedure as for the barrier to calculate the groundwater flux through the upgradient cross-sectional area of the treatment cell based on site-specific groundwater flow inputs. The groundwater flux (gallons/year or L/year) is then multiplied by the design life (years) and this value is added to the volume within the treatment cell to obtain the total treatment volume (gallons or liters).

For a barrier design, the volume of water to be treated is calculated by multiplying the width of the barrier perpendicular to flow (y), effective vertical height of the treated zone (z), effective porosity of the treatment area (n), groundwater flow velocity, and the design life. Barriers are typically placed across a plume perpendicular to the direction of groundwater flow with a width (y) that is somewhat greater than the plume to minimize the potential for contaminated groundwater to flow around the barrier without passing through the treatment zone.

When determining the effective vertical height (z), designers should consult boring logs from the site to estimate the vertical thickness of the aquifer that transmits most of the groundwater. For example, at a typical site, the chlorinated solvent plume may extend from 20 to 40 ft below grade. However, this contaminated interval consists of sand and clay layers. Essentially all of the groundwater flow will be through the sand layers, so these layers should be targeted for treatment. While it might be desirable to treat the entire vertical extent of contamination, experience has shown that most of the emulsion is distributed in the higher permeability layers.

The width of the proposed barrier can be entered into the barrier design spreadsheet in **Appendix A** (Section A) along with the minimum and maximum depths of the contaminated zone. These inputs are used to calculate the cross-sectional area of the barrier. Site-specific hydrogeologic properties (effective porosity, hydraulic conductivity, and hydraulic gradient) are then entered in Section B of the spreadsheet and are used to calculate the groundwater seepage velocity through the barrier by applying Darcy's Law. The spreadsheet uses the cross-sectional area of the barrier and the groundwater seepage velocity to determine the groundwater flux through the barrier (gallons/year). The treatment volume is then calculated using the design life (e.g., 10 years) entered in Section C of the spreadsheet.

System Design Life

When selecting a design life, users should be aware that the design spreadsheet assumes the barrier or source area treatment will operate at 100% efficiency until the day when the organic substrate runs out. On that day, the treatment efficiency is assumed to drop to zero. However, in practice, treatment efficiency will begin to decline as substrate is depleted from the more permeable/contaminated zones. Consequently, users should include an appropriate factor of safety when selecting the design life. In addition, users should take into account project cost, contaminant source(s) and concentrations, and remedial objectives when selecting a design life. In some barrier projects, a 10-year design life has been used with the assumption that additional edible oil may need to be injected after five years.

Estimating the required design life for a source area treatment is more difficult. Laboratory studies and field pilot tests have demonstrated that edible oil addition can stimulate rapid biodegradation of contaminants in the more mobile zones with contaminants degraded to low levels in 6 to 12 months. However, mass transfer limitations may greatly reduce the rate that DNAPLs and contaminants in low permeability zones are degraded. If residual edible oils are present, aqueous phase contaminants will be degraded as they diffuse out into the more mobile portions of the aquifer. However, once the edible oil is depleted, aqueous phase contaminants may be released to the downgradient aquifer. For heavily contaminated source areas, a five-year substrate supply should be provided as a minimum with the expectation that additional edible oil will need to be injected at some time in the future.

Hydrogen Demand

As previously discussed, edible oils ferment in the subsurface generating hydrogen and acetate. The hydrogen and acetate is then used to support reductive dechlorination. However, hydrogen and acetate may also be consumed during biodegradation of naturally occurring electron acceptors including oxygen, nitrate, sulfate, ferric iron, and manganese. As a consequence, designers must consider both the amount of contaminant to be degraded and the background electron acceptor load.

The amount of substrate required to reduce the mass of dissolved contaminants and/or electron acceptors can be determined by calculating the stoichiometric hydrogen demand of the dissolved contaminants and electron acceptors. First, the contaminant and electron acceptor mass to be degraded is calculated by multiplying the average concentrations by the total groundwater treatment volume. The stoichiometric hydrogen demand required to reduce the contaminant mass can then be calculated by determining the amount of molecular hydrogen (H₂) required for complete reduction of each contaminant or background electron acceptor. The stoichiometric demand is the mass ratio of the contaminant to hydrogen (weight contaminant/weight H₂) and is based upon balanced chemical reduction equations. For example, TCE is completely reduced to ethene according to the following equation:



Since it takes 3 moles of hydrogen (molecular weight = 2.0158) to reduce 1 mole of TCE (molecular weight = 131.389) to ethene, the stoichiometric hydrogen demand is 131.389 divided by 6.047 (3 x 2.0158) or 21.73 (wt/wt H₂). Therefore, 21.73 grams of TCE is degraded per gram of hydrogen. Similar calculations can be done for each contaminant and electron acceptor to determine the stoichiometric hydrogen demand. For each contaminant or electron acceptor, the mass is divided by the stoichiometric hydrogen demand to determine the mass of hydrogen required to reduce the contaminant mass. **Table 4.1** provides the chemical reduction equations and stoichiometric hydrogen demand for typical chlorinated solvents and electron acceptors.

Table 4.1. Stoichiometric Hydrogen Demand for Different Contaminants and Electron Acceptors

Chlorinated Solvents and Electron Acceptors	Chemical Reduction Equation	Stoichiometric Hydrogen Demand (wt/wt H ₂)
PCE	$\text{C}_2\text{Cl}_4 + 4\text{H}_2 \rightarrow \text{C}_2\text{H}_4 + 4\text{H}^+ + 4\text{Cl}^-$	20.57
TCE	$\text{C}_2\text{HCl}_3 + 3\text{H}_2 \rightarrow \text{C}_2\text{H}_4 + 3\text{H}^+ + 3\text{Cl}^-$	21.73
<i>cis</i> -DCE	$\text{C}_2\text{H}_2\text{Cl}_2 + 2\text{H}_2 \rightarrow \text{C}_2\text{H}_4 + 2\text{H}^+ + 2\text{Cl}^-$	24.05
Vinyl Chloride	$\text{C}_2\text{H}_3\text{Cl} + \text{H}_2 \rightarrow \text{C}_2\text{H}_4 + \text{H}^+ + \text{Cl}^-$	31.00
Carbon Tetrachloride	$\text{CCl}_4 + 4\text{H}_2 \rightarrow \text{CH}_4 + 4\text{H}^+ + 4\text{Cl}^-$	19.08
Chloroform	$\text{CHCl}_3 + 3\text{H}_2 \rightarrow \text{CH}_4 + 3\text{H}^+ + 3\text{Cl}^-$	19.74
1,1,1-TCA	$\text{C}_2\text{H}_3\text{Cl}_3 + 3\text{H}_2 \rightarrow \text{C}_2\text{H}_6 + 3\text{H}^+ + 3\text{Cl}^-$	22.06
1,1-DCA	$\text{C}_2\text{H}_4\text{Cl}_2 + 2\text{H}_2 \rightarrow \text{C}_2\text{H}_6 + 2\text{H}^+ + 2\text{Cl}^-$	24.55
Chloroethane	$\text{C}_2\text{H}_5\text{Cl} + \text{H}_2 \rightarrow \text{C}_2\text{H}_6 + \text{H}^+ + \text{Cl}^-$	32.18
Oxygen	$\text{O}_2 + 2\text{H}_2 \rightarrow 2\text{H}_2\text{O}$	7.94
Nitrate	$2\text{NO}_3^- + 2\text{H}^+ + 5\text{H}_2 \rightarrow \text{N}_2 + 6\text{H}_2\text{O}$	12.30
Sulfate	$2\text{SO}_4^{-2} + 3\text{H}^+ + 8\text{H}_2 \rightarrow \text{H}_2\text{S} + \text{HS}^- + 8\text{H}_2\text{O}$	11.91
Ferric Iron	$2\text{Fe}^{+3} + \text{H}_2 \rightarrow 2\text{Fe}^{+2} + 2\text{H}^+$	55.41

Chlorinated Solvents and Electron Acceptors	Chemical Reduction Equation	Stoichiometric Hydrogen Demand (wt/wt H ₂)
Manganese	$\text{MnO}_2 + 2\text{H}^+ + \text{H}_2 \rightarrow \text{Mn}^{+2} + 2\text{H}_2\text{O}$	27.25

The hydrogen released from different edible oils was shown in **Table 2.3** and varies from 0.178 to 0.181 moles of H₂ per gram of oil (0.36 to 0.365 g H₂/g oil) depending on the oil composition. The substrate demand is determined by dividing the calculated hydrogen demand for degradation of contaminants and electron acceptors by the amount of hydrogen produced from oil. Section D of the barrier and source treatment design spreadsheet in **Appendix A** calculates the hydrogen demand of each contaminant and electron acceptor based on the entered concentrations.

Additional Hydrogen Demands and Organic Carbon Released Downgradient

In addition to the contaminants and electron acceptors entering the treatment zone, hydrogen can be consumed during reduction of iron oxides and manganese oxides present in the sediment, production of methane, and release of dissolved organic carbon (DOC). The best approach for estimating the iron and manganese demand is to directly measure the amount of iron and manganese oxides in the aquifer material. Unfortunately, these data are not commonly available. An alternative approach is to calculate the iron and manganese demand based on the amount of dissolved iron and manganese released to the downgradient aquifer. This approach may somewhat under estimate the iron and manganese demand, but should be a reasonable approximation in most cases. In previous field studies, dissolved iron concentrations released from emulsified oil barriers typical varied between 10 and 100 mg/L with somewhat lower levels of dissolved manganese.

Hydrogen and acetate that is not consumed by reductive dechlorination or electron acceptor reduction will be fermented to methane or released to the downgradient aquifer. As a consequence, additional substrate must be injected to account for any methane production and dissolved organic carbon (DOC) released. In previous emulsified oil projects, methane concentrations downgradient from the treatment zone have varied between 5 and 20 mg/L. Immediately after oil injection, DOC concentrations released from edible oil barriers may exceed 500 mg/L. However, DOC concentrations decline with time reaching quasi-steady-state levels of 20 to 50 mg/L. Consequently, 60 to 100 mg/L DOC appears to be a reasonable range for the long-term average concentration released.

The barrier and source treatment design spreadsheets estimate the amount of substrate used for methane production and the amount of carbon lost from the barrier over time. These values are estimated by entering estimated methane concentrations and DOC concentrations in Section E of the spreadsheet. The total amount of oil required to support contaminant biodegradation is then calculated in Section F of the spreadsheet. This value is only the amount of oil required. Other materials including easily biodegradable soluble substrates, bacterial nutrients and vitamins, and surfactants may be added to aid in emulsion preparation and to stimulate rapid growth of desired microbial populations. However, these materials are rapidly depleted and are not expected to support long-term anaerobic biodegradation.

4.5.1.2 Oil Entrapment by Aquifer Material

For effective treatment, edible oil emulsions must be distributed throughout the treatment zone. However, as emulsions migrate through the aquifer pore spaces, a significant amount is retained. The small oil droplets coat the sediment surfaces, typically retaining between 0.1 to 0.10 lb of oil per cubic foot of treated material. **Table 4.2** illustrates the range of emulsion retained in a variety of sediments.

Table 4.2. Observed Emulsion Retention by Sediment

Site-Specific Aquifer Material	Maximum Retention (g/g)	Effective Retention (g/g)
Blended sand (7% silt + clay) (Coulibaly and Borden, 2004)	0.0054 (Lab Column)	.0066 (Sandbox)
Blended sand (9% silt + clay) (Coulibaly and Borden, 2004)	0.0061 (Lab Column)	0.0035 (Sandbox)
Blended sand (12% silt + clay) (Coulibaly and Borden, 2004)	0.0095 (Lab Column)	0.0037 (Sandbox)
Aluvium (clayey sand) (Maryland Perchlorate Site, EOS [®])	0.0037 (Lab Column)	0.0013 (Field)
Low K, weathered rock (sandy clay with remnant fractures) (Burlington, NC)		0.0017
High K, gravelly sand (Indiana, EOS [®])		0.0002

The amount of oil required to treat an aquifer is determined by multiplying the width perpendicular to flow (y), vertical height of the treated zone (z), thickness along the direction of groundwater flow (x), and oil retention by the aquifer material in lb/ft^3 . In many aquifers, the amount of oil required to coat the aquifer material is much greater than the amount of oil required for biodegradation and will control the total amount of oil that must be injected.

4.5.1.3 Chlorinated Solvent Sorption to Oil

As discussed previously in Section 2.2, the retardation factor can be used to express pollutant transport velocity. As shown below, it also can be used to estimate the effect of oil injection on the concentration of chlorinated solvents in the aqueous phase as follows:

$$R \text{ (unitless)} = \text{total mass of contaminant} / \text{mass of contaminant in aqueous phase}$$

If all the contaminant is initially dissolved, oil injection should reduce the aqueous phase concentration by a factor equal to R . However, if a significant fraction of the chlorinated solvent is already sorbed to the aquifer sediment or is present as a dense non-aqueous phase liquid (DNAPL), the reduction in aqueous phase concentration will not be as dramatic. From Section 2.2, R can be calculated by the equation:

$$R = 1 + \rho_B f_o K_p / n$$

where: ρ_B is the aquifer bulk density (g/cm^3)
 f_o is the fraction of oil in the sediment (g/g)

K_p is the oil-water partition coefficient (ml/g)
 n is porosity (ml/cm³).

K_p values measured by Pfeiffer (2003) for PCE, TCE, *cis*-DCE and VC are presented in **Table 2.4**. For other compounds, the octanol-water partition coefficient (K_{ow}) can be used as a reasonable estimate of K_p . f_o is the grams of oil injected per gram of aquifer material and can be calculated as:

$$f_o = \frac{\text{pounds oil injected} / \text{ft}^3 \text{ aquifer}}{\text{aquifer bulk density (lb/ft}^3\text{)}}$$

Representative retardation factors for PCE, TCE, *cis*-DCE and VC in aquifers treated with NAPL edible oil and edible oil emulsions are presented in **Table 2.5**. While sorption of PCE and TCE can be significant in emulsion treated zones, edible oil emulsions typically stimulate rapid conversion of PCE and TCE to *cis*-DCE. Since *cis*-DCE is much more soluble, sorption to trapped emulsion is not expected to dramatically reduce the total amount of chlorinated solvents released from a treated area. Consequently, the effect of emulsion addition in enhancing sorption is not normally used to determine the amount of oil that must be injected. If enhanced sorption is an important remediation objective, users should consider use of NAPL oils.

4.5.1.4 Summary – How much oil do you need?

To determine the amount of oil required, calculate the oil requirement for biodegradation and entrapment by the aquifer material. The oil required will be the larger of the two values. When designing barriers using emulsions, oil entrapment by sediment controls in lower velocity environments, while substrate demand for biodegradation commonly controls in very high flow rate systems with large amounts of competing electron acceptors (e.g., sulfate).

4.5.2 AMOUNT OF WATER REQUIRED DURING EMULSION INJECTION

Edible oil emulsions are transported in the subsurface by flowing groundwater. Consequently, water must be injected to transport the oil droplets throughout the target treatment zone. Common procedures used include: (a) injecting a concentrated emulsion followed by chase water to distribute the oil; (b) continuous injection of a more dilute emulsion; and (c) recirculation of emulsion through the treatment zone.

Modeling studies (Borden, submitted) indicate that injection flow rate and concentration have essentially no effect on the final oil distribution in the sediment. The only factors that significantly influence the final oil distribution are: (1) the total amount of oil injected and (2) the total amount of water injected.

Procedures for determining the amount of oil to inject are described in Section 4.5.1, above. The total water volume to inject should be equal to the effective pore volume of the target treatment zone. When installing an edible oil barrier using injection wells, the water volume injected per well can be calculated as:

$$\text{Injection water volume per well (V)} = (\pi D^2/4) (Z) n_e$$

Where: D is the injection well spacing
 Z is the effective vertical height of the treatment zone
 n_e is the effective porosity.

Typical values of effective porosity are presented in **Table 4.3**.

Table 4.3. Typical Values for Dry Bulk Density, Total Porosity and Effective Porosity of Aquifer Materials

Aquifer Matrix	Dry Bulk Density (g/cm³)	Total Porosity	Effective Porosity
Clay	1.00-2.40	0.34-0.60	0.01-0.2
Peat	--	--	0.3-0.5
Glacial Sediments	1.15-2.10	--	0.05-0.2
Sandy Clay	--	--	0.03-0.2
Silt	--	0.34-0.61	0.01-0.3
Loess	0.75-1.60	--	0.15-0.35
Fine Sand	1.37-1.81	0.26-0.53	0.1-0.3
Medium Sand	1.37-1.81	--	0.15-0.3
Coarse Sand	1.37-1.81	0.31-0.46	0.2-0.35
Gravelly Sand	1.37-1.81	--	0.2-0.35
Fine Gravel	1.36-2.19	0.25-0.38	0.2-0.35
Medium Gravel	1.36-2.19	--	0.15-0.25
Coarse Gravel	1.36-2.19	0.24-0.36	0.1-0.25
Sandstone	1.60-2.68	0.05-0.30	0.1-0.4
Siltstone	--	0.21-0.41	0.01-0.35
Shale	1.54-3.17	0.0-0.10	--
Limestone	1.74-2.79	0.0-50.0	0.01-0.24
Granite	2.24-2.46	--	--
Basalt	2.00-2.70	0.03-0.35	--
Volcanic Tuff	--	--	0.02-0.35

Example calculations illustrating the amount of emulsified oil and water required to treat a 100-ft x 100-ft area that is 10 ft thick are provided in **Table 4.4**. Edible oils, when purchased in bulk, are relatively inexpensive. However, potential treatment volumes at some contaminated sites are considerable. Thus, the thoughtful selection of a specific source treatment zone and/or barrier location represents an effective means to achieve plume containment or remediation without unnecessary expense.

Table 4.4. Estimated Volumes of Oil and Water Required for Treatment of 100 ft x 100 ft Area

Injection Well Spacing (ft)	5	10	15	20	25
Vertical Injection Interval (ft)	10	10	10	10	10
Porosity (n)	0.25	0.25	0.25	0.25	0.25
Total Volume of 100 ft x 100 ft Treatment Zone (gallons)	748,000	748,000	748,000	748,000	748,000
Pore Volume (PV) of 100 ft x 100 ft Treatment Zone (gallons)	187,000	187,000	187,000	187,000	187,000
Number of Injection Wells to Treat 100 ft x 100 ft Area	400	100	49	25	16
Injection Volume per Well (gallons)	468	1,870	3,816	7,480	11,688
Time to Inject One PV at 1 gpm per well (hr)	8	31	64	125	195
Emulsified Oil required for Emulsion Treatment with 2% Residual Saturation (lb)	29,920	29,920	29,920	29,920	29,920

4.5.3 INJECTION WELL SPACING

The injection point spacing is primarily a trade off between well installation costs and labor costs. Wider spacing of the injection points reduces injection well installation costs, but increases the time/labor required for injection. The well installation costs are affected by the geology and depth to groundwater, while the labor costs are determined by the time required to inject the oil which is largely a function of the aquifer permeability. If the aquifer has a high permeability, the oil will be easier to inject and the injections will take less time. Often, multiple wells can be injected simultaneously to reduce the amount of time required to complete the injections. Injection tests are often done to help determine the anticipated injection flow rates and pressures and the approximate time it will take to complete the injections. Well installation and labor costs associated with injection of oil should be evaluated on a site-specific basis to determine the appropriate injection point spacing. **Figure 4.2** demonstrates how the well installation, injection labor, and substrate costs vary with different well spacings. ESTCP is funding a new project (ER-0626) to develop design tools for planning aqueous amendment injection systems which will aid users in conducting this evaluation and designing more cost-effective, successful designs for *in situ* application of emulsified oil and other substrates.

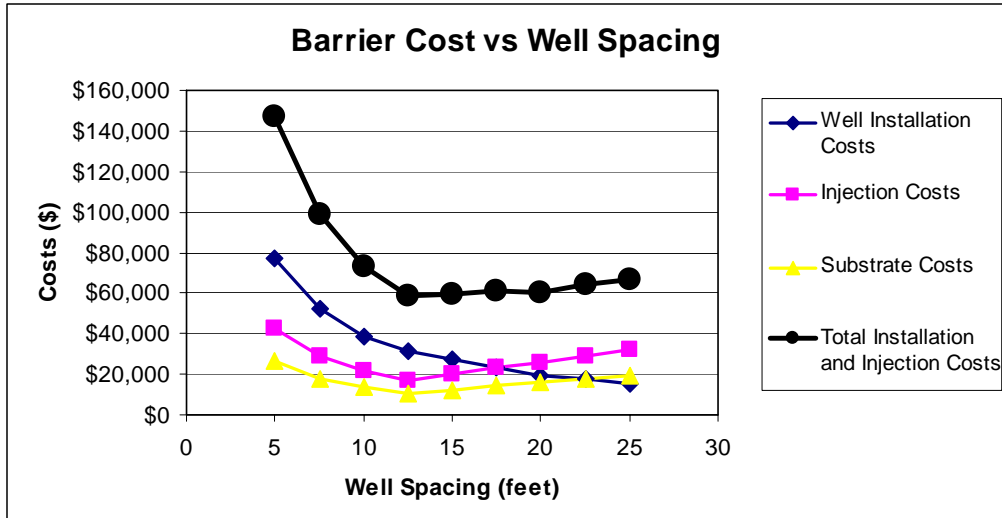


Figure 4.2. Example cost analysis for a PRB with various injection well spacings.

Injections are typically designed to provide 100% coverage of a desired treatment zone. However, subsurface heterogeneities will affect the distribution of the oil in the subsurface. Permeability differences will cause some zones to be over-treated and some zones to remain untreated. Groundwater flow and dispersion will provide some spreading of aqueous organic carbon increasing the reactive zone. However, safety factors are often used to provide overlap between the injections and minimize the potential for untreated zones. The need for a safety factor will depend on hydrogeologic complexities, the amount of available site characterization data, and site-specific concerns such as sensitive downgradient receptors.

SECTION 5 PILOT TEST PLANNING AND IMPLEMENTATION

Implementing a field pilot test of enhanced anaerobic bioremediation using emulsified oils requires careful consideration of site conditions, remedial objectives, design alternatives, and field methods. This section describes the procedures and protocols required for planning and implementing a field test, including baseline characterization, types of pilot tests, and development of site-specific test plans. Methods and procedures for monitoring and evaluating, field test results are discussed in Section 6.

Prior to developing the pilot test plan, a general conceptual design for the potential full-scale remediation system should be developed as discussed in Section 4. Development of the conceptual design is critical in defining the pilot test objectives. Additional technical information on the emulsified oil process is presented in Sections 2 and 3.

5.1 DEFINING PILOT TEST OBJECTIVES

The primary objectives of an enhanced anaerobic bioremediation pilot test using emulsified oils are to: (1) determine if this technology is suitable to achieve remedial goals for the site, and (2) determine critical design parameters required for a successful full-scale implementation. These objectives are site-specific, and pilot tests designed to achieve both of these objectives allow the site manager to determine whether enhanced bioremediation using emulsified oils is the most reasonable approach relative to other remedial technologies (e.g., MNA, oxidation strategies, groundwater extraction).

In order to define the pilot test objectives, a preliminary conceptual design for a potential full-scale remediation system should be developed first, as described in Section 4. Once a preliminary design has been developed and remediation goals have been established, pilot test objectives can be defined. Test objectives may include degradation of contaminant concentrations to specified compliance levels (e.g., MCLs) or achieving degradation rates that are deemed sufficient to contain or attenuate the contaminant plume within a reasonable timeframe. Test objectives may also include limits to the accumulation of intermediate dechlorination products (e.g., VC) or limits on degradation of secondary water quality (e.g., dissolved metals). For pilot- or small-scale field tests, it is only necessary to achieve objectives within the immediate treatment zone, and sufficient time (perhaps 1 to 3 years) may be required for the treatment system to demonstrate its effectiveness over the typical life-cycle of an enhanced bioremediation application.

A second objective of a field test is to determine critical design criteria and to evaluate potential adverse secondary impacts to groundwater associated with enhanced anaerobic bioremediation using emulsified oils. These include, but are not limited to, the following:

- **Substrate Requirements.** Determine how much substrate is required to deplete alternative electron acceptors and sustain an anaerobic reactive zone conducive to reductive dechlorination of CAHs.
- **Injection Methodology and Radius of Influence.** Determine an appropriate injection method and well spacing that achieves the desired injection rate and radius of influence (ROI).

- **Contaminant Biodegradation Rates and Required Contact Time.** Determine contaminant degradation rates and use this information to estimate the contact time required for contaminant biodegradation in a source area or barrier treatment.
- **Impacts to Hydrogeology.** Determine whether substrate addition results in an undesirable reduction in aquifer permeability due to biological activity or clogging by trapped oil droplets.
- **Secondary Impacts.** While anaerobic biodegradation may be effective in degrading the target contaminants, there is some potential for secondary degradation of groundwater quality (e.g., solubilization of heavy metals) or generation of noxious gases (e.g., methane or hydrogen sulfide) to occur. These changes are not easily reversed, and it may take many years for the effects of the substrate addition to diminish. Therefore, an evaluation of these potential impacts is typically required by regulatory authorities.

Proper planning and field test design are required to optimize system performance in order to achieve the pilot test objectives and to mitigate potential impacts to site hydrogeology and groundwater quality. Adequate site characterization is the first step to determining a suitable approach for demonstrating the effectiveness of enhanced anaerobic bioremediation using edible oils.

For some sites, site managers may feel that pilot testing is not necessary. However, at a minimum, a simple injection test should be conducted to determine injection flow rates and operating conditions. Data from an injection test are useful for determining an appropriate injection method and well spacing. These parameters can greatly impact the costs of a full-scale project.

5.2 DEVELOPING A SITE-SPECIFIC TEST PLAN

The natural variation in lithology, hydrogeology, geochemistry, and microbial ecology of aquifer systems makes each site different and unique. For most sites, it is valuable to conduct some form of field or bench-scale testing. Preliminary screening and small-scale field tests are a cost-effective way to demonstrate the utility of using edible oil emulsions for enhanced anaerobic bioremediation and are strongly recommended. The cost of field testing can be recovered by the optimization and greater efficiency of a full-scale design based on field test performance data. The procedures and protocols required for planning and implementing a field test include baseline characterization, development of site-specific test plans, system design, system construction, substrate injection, and process monitoring.

Conducted in a careful and thorough manner, field testing provides the performance basis required for full-scale implementation of enhanced bioremediation using emulsified oils. Advantages of the technology can be exploited in this process, while avoiding or mitigating potential adverse impacts.

A site-specific test plan is required for successful implementation of enhanced anaerobic bioremediation using edible oil emulsions. The test plan should review and identify site remedial objectives, review and screen site conditions for enhanced anaerobic bioremediation, describe the proposed technical approach, provide detail on system design and construction, and describe the monitoring protocols to be used to evaluate the test. Elements of a site-specific test plan should include, but not be limited to, the following:

- **Introduction:** Problem statement, pilot test objectives, and a brief description of the scope of work and technology being applied.
- **Site-Specific Data Review:** Operational history, regulatory status, groundwater use, hydrogeology, and nature and extent of contamination.

- **Preliminary Screening for Enhanced Anaerobic Bioremediation:** Distribution of parent and dechlorination products, groundwater geochemistry, hydrogeological limitations, and suitability for enhanced bioremediation.
- **Proposed Technical Approach:** System design including configuration, injection strategy, substrate calculations, and monitoring program. Provide contingencies for potential problems. To the extent possible, pilot test injection procedures should be similar to those being considered for the full-scale remediation system (Section 4).
- **Field Program:** Protocols for baseline monitoring, system installation, emulsified oil injection, process monitoring, and disposal of investigation-derived waste.
- **Proposed Project Schedule and Project Contacts:** Schedule for field program and reporting, and a list of pertinent project contacts and personnel.
- **Health and Safety Plan.** Site-specific health and safety plan including contingencies and directions to local emergency care. Health and safety considerations should address traffic in the work areas, utility clearances, spill containment measures, and procedures for working with drilling equipment and injection systems.
- **Access Considerations.** The test plan should identify site access requirements and potential impacts to site operations and infrastructure. For DoD facilities, personnel security passes may be required and should be procured in advance.

5.3 PILOT TEST CONFIGURATIONS

Pilot test configurations using emulsified oils for enhanced bioremediation may range from single well push-pull tests to multiple well injection tests. In some cases, a pilot field test may be configured to achieve an interim remedial objective such as source or “hot spot” reduction. The field test work plan should detail and describe the protocols and procedures to be followed when constructing the injection system, injecting the emulsified oil, and conducting the baseline and performance monitoring. Changes to the field protocol should be noted in order to replicate or modify the field program accordingly for future full-scale operations. The following sections describe the most common pilot test approaches: (1) single well push-pull test; and (2) multiple well injection tests.

5.3.1 SINGLE WELL PUSH-PULL INJECTION TEST

Single well push-pull methods may be used as a simple pilot test to evaluate pre-design parameters for implementing enhanced bioremediation using emulsified oils. In this approach, a known volume of groundwater is extracted from the well, amended with an edible oil emulsion, and re-injected (pushed) into the aquifer. The treated well and a parallel untreated well are monitored periodically for several months to evaluate contaminant biodegradation. Typical test procedures are summarized below.

1. Identify two wells for use in the test. These wells can be existing monitoring wells that are no longer needed for compliance monitoring or new wells installed specifically for the pilot test. Ideally, these two wells should be reasonably close together and have generally similar geochemical characteristics. If one well is upgradient of the other, the upgradient well should be designated as the control well and the more downgradient well should be used to monitor impacts of the emulsified oil injection.

2. Extract groundwater from the emulsified oil test well, collect in a single storage tank, and sample for contaminant and geochemical characteristics. It is desirable to extract and re-inject at least 10 gallons of groundwater per foot of well screen. Assuming an effective porosity of 20%, this will provide a 1.5-ft radius of influence around the well screen. For a 10-foot well screen, a common approach is to fill two new 55-gallon drums with extracted groundwater.
3. Add the edible oil emulsion and a conservative tracer to the groundwater in each drum and mix. In previous studies, 250 to 500 mg/L of NaBr has been adequate to provide a clear signal. The amount of emulsion to inject will depend on the formation properties and can be calculated using procedures presented in Section 4. For a typical silty or clayey sand, 0.35 lbs of emulsified oil per gallon of injection water should be sufficient. The emulsion/tracer mix is then injected into the test well. During the injection process, injection pressure, flow rate, and general operating conditions should be monitored.
4. Groundwater from the test and control well should then be sampled periodically for the target contaminants and biogeochemical parameters. A typical monitoring schedule would be to sample at 1, 3, 6 and 12 months after emulsion injection. Changes in concentrations of the contaminant, organic carbon, and alternate electron acceptors over time are used to estimate rates of substrate utilization and contaminant degradation. Measurement of conservative tracers can be used to normalize the data to account for dilution.

Figure 5.1 shows results of a single well push-pull test conducted at Altus AFB OU-1 in 2001-02. In November '01, the test well (TS-IW-6) and a parallel control well (WL-250) were monitored to establish background conditions prior to substrate addition. In December '01, well TS-IW-6 was then treated with two drums of a dilute soybean oil-in-water emulsion (Emulsified Oil Substrate[®] or EOS[®]) followed by two drums of groundwater. The soybean oil emulsion was designed to stick to the formation, providing a long-term source of slow-release organic substrate to support reductive dechlorination. WL-250 is a nearby well that was not treated and was monitored as a control. Treatment of well TS-IW-6 with emulsified soybean oil enhanced anaerobic biodegradation processes in the immediate vicinity of TS-IW-6, stimulating complete dechlorination of TCE to ethene and ethane. In comparison, there was no significant change in contaminant concentrations in the untreated control well.

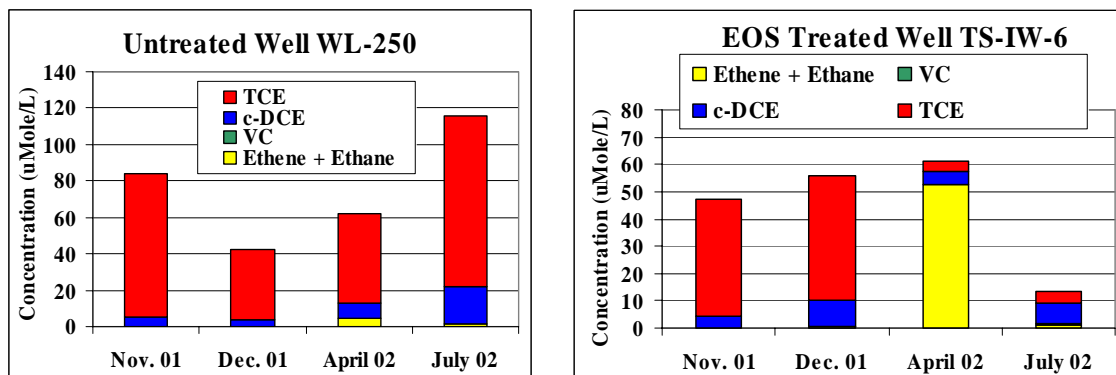


Figure 5.1 Variation in chlorinated ethenes in untreated control well WL-250 and emulsion treated well TS-IW-6 at Altus AFB OU-1. TCE and *cis*-DCE were dechlorinated to ethene and ethane in emulsion treated well compared to no significant change in untreated well.

The relatively simple push-pull test provided clear evidence that: (1) emulsified oil addition to the aquifer at the OU-1 site could stimulate reductive dechlorination of TCE, and (2) addition of a bioaugmentation culture was not needed to stimulate complete dechlorination to ethene and ethane. Because the emulsified oils attached to the aquifer solids, they provided a long-lasting substrate for reductive dechlorination and were not washed downgradient with the flowing groundwater. In contrast, more soluble and readily biodegradable substrates, such as lactate or yeast extract added with the oil, could be transported away from the well with the flowing groundwater. Partitioning of chlorinated solvents to the oil did not substantially interfere with interpretation of the test results since a large portion of the original TCE was recovered as ethene and ethane during the April '02 sampling.

While the single well push-pull test can generate very useful results, there are several important limitations to this method.

1. In areas with high groundwater velocities, the small radius of influence around the injection well may not provide sufficient contact time between the flowing groundwater and the sorbed edible oil for extensive contaminant biodegradation. This will particularly be a problem at sites with high levels of competing electron acceptors (e.g., sulfate > 500 mg/L).
2. For contaminants that do not generate measurable degradation products, it will be difficult to distinguish between contaminant mass partitioning into the edible oil and biodegradation. However, when contaminants generate degradation products that can be monitored (e.g., *cis*-DCE, VC, and ethene), this is less of an issue.
3. In many states, an Underground Injection Control (UIC) permit or equivalent will be required prior to any injection. This can substantially increase the time and cost of the push-pull test.
4. Preparation of small quantities of emulsion with appropriate chemical characteristics and droplet size distribution can be time consuming. Given the small quantities of material required, it may be useful to purchase a pre-mixed emulsion for a single push-pull test.

In certain cases, it may be useful to conduct a two-stage push pull test. In the first stage, the test well is treated with an emulsified oil substrate as described above and highly anaerobic conditions are allowed to develop (on the order of several weeks to a few months). Groundwater is then extracted, characterized,

spiked with contaminants, if necessary, amended with tracers, and re-injected. The groundwater is then periodically extracted over a period of a few days to a week and analyzed as usual. The advantages of a two-stage push-pull test are that the system is allowed to acclimatize and become highly anaerobic before the contaminant rate phase is measured. Contaminant degradation rates under this scenario should be optimal and sufficient to observe the effectiveness of substrate addition over a much shorter period of time.

5.3.2 MULTI-WELL INJECTION TESTS

In some cases, it will be appropriate to conduct larger scale pilot tests using a series of injection wells or direct-push well points configured in a grid or linear barrier configuration. Multi-well injection field tests are utilized to develop a larger reaction zone than a single well test. Monitoring of the injection zone and the effects on downgradient water quality can be observed by sampling a monitoring well network over time. Benefits of a multi-well field test include the following:

- Closer representation of full-scale system performance and costs.
- Larger monitoring network providing evaluation of the downgradient extent of the reaction zone and impacts on downgradient water quality.
- Monitoring results will be representative of a greater aquifer zone (i.e., not limited to a single well point that may or may not be representative of aquifer conditions).

5.4 MONITORING DURING THE INJECTION PROCESS

Monitoring during injection of the emulsified oil is required to optimize the injection system and to determine critical design parameters for full-scale application. Operating parameters that should be documented include the following:

- Substrate preparation including a description of the mixing system; measured concentrations of oil, water and amendments in the substrate mixture; and emulsion stability.
- Injection pressures throughout system operation and the corresponding flow rate.
- Significant injection thresholds. For example, minimum pressure required to obtain a desired flow rate, or a drop in pressure and increase in flow rate indicating fracturing of the formation.
- Amount of substrate and water push injected per injection point.
- Substrate breakthrough at monitoring points as an indicator of the radius of influence or the presence of preferential flow paths (i.e., breakthrough beyond the theoretical ROI).
- Safety issues including failure of well seals, leaks, or failure in the injection system.

Field observations may be used to optimize the injection process during the field test and to provide data for future full-scale operations.

5.5 GROUNDWATER TRACERS

Groundwater tracers are often used when conducting pilot tests. If properly implemented, tracers in the water introduced immediately before, during, or after emulsion injection can provide valuable information on the following:

- The direction of movement and seepage velocity of groundwater that has been in contact with the emulsion.
- The effects of dilution or degradation of the organic substrate with groundwater migration.
- The impacts of emulsified oil on dispersivity, hydraulic conductivity, and porosity.

Knowing the direction of movement and seepage velocity of the groundwater that has been in contact with emulsified oil is important because it will provide information on the potential treatment area and behavior of the organic carbon introduced into the groundwater.

In order to trace groundwater migrating from the immediate vicinity of the injection wells (i.e., zone of influence), water used for injection can be amended with a conservative tracer such as sodium bromide or sodium iodide. Bromide has a low adsorptive potential, migrates at approximately the rate of advective groundwater flow, and can be tracked in groundwater after injection to estimate advective groundwater flow in the treatment area.

The migration of organic carbon in groundwater (from dissolution of the edible oil) also can be measured as total organic carbon (TOC, unfiltered samples) or dissolved organic carbon (DOC, filtered samples) at monitoring well locations. TOC is typically used with edible oil applications because the oil may migrate as colloids or small droplets suspended in water. Thus, TOC can be tracked and used to determine the zone of influence of the emulsion. Emulsified oil is a non-conservative tracer as organic carbon is retarded (relative to migration of a conservative tracer such as bromide) due to its higher adsorptive potential and is also subject to biodegradation.

5.6 MONITORING NETWORKS

Monitoring networks are necessary to document the performance of the enhanced bioremediation system. Section 6 describes typical monitoring programs for pilot and full-scale field projects. Monitoring network design includes consideration of the location and depths of the monitoring wells and soil gas monitoring points and the frequency of monitoring events.

5.6.1 NUMBER AND LOCATION OF MONITORING POINTS

Process monitoring wells should be located both upgradient of the reaction zone and at locations within and downgradient of the reaction zone parallel to the direction of groundwater flow. These wells are used to monitor changes in groundwater chemistry over time along the groundwater flowpath through the treatment area. Evaluation of changes in contaminant concentrations allows estimation of biodegradation rate constants. Cross-gradient well locations are useful to define the lateral extent of treatment and provide greater accuracy in mapping hydraulic gradients.

Consideration should be given to groundwater seepage velocity and the desired frequency of process monitoring when determining monitoring locations and spacing. In general, the screened interval of the

monitoring wells should be similar to the injection interval. It is beneficial to have at least one monitoring location within the injection area screened at multiple depths to determine vertical hydraulic gradients and the potential for vertical migration of dissolved substrate. Downgradient monitoring locations screened at deeper or shallower depths may be necessary to monitor the downgradient contaminant flow path in the presence of vertical gradients.

Injection wells and monitoring wells should target intervals of elevated contaminant concentrations. However, injected fluid will primarily flow through preferential flow paths. A site-specific monitoring well network should be configured to allow the measurement of radius of influence, injected oil, and treatment zone. The following example calculations in **Table 5.1** show how one can calculate the dimensions of the injectant distribution zone based on different assumed contaminated interval thicknesses.

Table 5.1. Calculating the Dimensions of Injectant Distribution Zone

Injectant Volume/Well (gal)	Porosity	Injection Well Screened Interval (ft)	Volume of Aquifer Affected (gal)	Maximum Potential Radius of Influence (ft)
10,577	0.25	5.0	42,308	18.98
10,577	0.25	10.0	42,308	13.42
10,577	0.25	15.0	42,308	10.96

The calculation above is useful in selecting monitoring well locations radially outward from injection wells based upon the estimated thickness of the target treatment interval and the injection volume. At a minimum, two monitoring wells should be placed within the estimated radius of substrate distribution. This information is also useful in evaluating if injected substrate moved into the aquifer in a more uniform and symmetric pattern or not. The addition of a conservative tracer like sodium bromide is also recommended to evaluate substrate distribution patterns.

When vapor accumulation is a potential concern, soil gas monitoring points should be installed within the reactive zone to evaluate the presence of methane. Soil gas monitoring points should also be installed at background locations to provide a baseline for comparison. Measurements should be collected before injection and during each process monitoring event to evaluate changes over time.

5.6.2 MONITORING FREQUENCY

Process monitoring sampling frequency will depend on many factors including, but not limited to: well spacing, groundwater seepage velocity, aquifer heterogeneity, and the efficacy of biodegradation. It is important to ensure that enough time has passed to see changes in groundwater geochemistry and changes in the ratios of parent compound(s) to daughter products.

The use of slow-release emulsified oil systems requires no operational component, and quarterly to semi-annual performance monitoring is typically sufficient. Typical lag times to stimulate measurable increases in the rate of degradation of chlorinated ethenes (e.g., PCE and TCE to DCE, and DCE to VC to ethene) may be on the order of 6 to 12 months or more. In these cases, frequent sampling on the order of weeks to a few months may yield unsatisfactory early results and an unjustified lack of confidence in the effectiveness of the system.

SECTION 6 MONITORING AND DATA EVALUATION

Several methods are available to assess the effectiveness of using emulsified oils for enhanced anaerobic bioremediation. This section provides a brief overview of typical monitoring and data evaluation procedures for emulsified oil projects. Protocols used to evaluate MNA (e.g., USEPA, 1998; AFCEE, 2000; AFCEE, 2003) provide references for many of these methods and techniques.

6.1 MONITORING

Monitoring of emulsified oil projects typically consists of soil and/or groundwater sampling to evaluate the substrate distribution after injection, groundwater sampling to monitor the emulsified oil performance and geochemical changes, soil gas sampling to monitor the accumulation of vapors in the soil, and aquifer testing to evaluate permeability effects.

6.1.1 SUBSTRATE DISTRIBUTION EVALUATION

Given the ever-present heterogeneity of the subsurface, control and measurement of the distribution of injected fluids is always challenging. Uniform distribution of the organic substrate throughout the target treatment zone is a critical factor. Thus, a monitoring strategy must be developed to measure substrate distribution. The evaluation of substrate distribution should incorporate multiple approaches. These approaches are based on the concept that the injected fluid has different chemical characteristics than the site groundwater. The radius of influence of the injection includes both the physical distribution of the emulsified oil and the migration of dissolved substrate constituents, including highly soluble metabolic acids produced by degradation of the edible oil. Methods used to evaluate the substrate distribution include groundwater and soil sampling. As mentioned in Section 5.5, groundwater tracers are often used in pilot tests to determine the distribution of oil in the subsurface and the radius or zone of influence. These data can be useful in developing the full-scale design. **Table 6.1** outlines a variety of techniques that have been used to evaluate substrate distribution and longevity.

Table 6.1. Methods to Measure Distribution of Organic Substrates

Measured Parameter	Detection Approach	Data Interpretation
Oil emulsion	Periodic visual inspection of groundwater in monitoring wells	The appearance of visible emulsion in a well indicates breakthrough in that region. The time versus distance relationship indicates whether uniform or channelized flow occurred.
Dissolved total organic carbon, edible oil fatty acids	Laboratory chemical analysis of groundwater and/or soil samples	Same as visual and tracer methods above
Geochemical indicators (e.g., dissolved oxygen, redox, nitrate, ferrous iron, sulfate, methane, etc.)	Field meters and laboratory analyses can be used to determine if monitoring location is within or downgradient of substrate distribution zone.	These techniques are indirect and require that enough time has passed to allow for biodegradation to occur. Differentiating zones that are directly within the substrate distribution zone versus areas immediately downgradient may not be possible.

Soil samples may be collected to determine the radius of influence. Soils may be analyzed for TOC by EPA Method 415.1 as an indicator of the amount of substrate present in the aquifer matrix. Alternately, because the long-chain organic compounds present in edible oils are detected by analyses for total petroleum hydrocarbons (TPH), simple field immunoassay methods may be used as a semi-quantitative indicator of the amount of oil substrate present. This method was used for a site at the former Newark AFB, Ohio, where direct-push techniques were used to collect soil samples. Field analyses were confirmed by shipping a limited number of duplicate samples for fixed-base laboratory analyses. Soil samples collected by direct-push techniques were sufficient to evaluate the effective distribution of the edible oil emulsion in the subsurface.

In addition, soil (or groundwater) samples can be analyzed for the presence of edible oil fatty acids. Fatty acid biomarkers (e.g., phospholipids) have been primarily used for evaluating microbial responses but can also be an effective molecular biological tool (MBT) for monitoring the distribution of edible oil substrates within a site. The presence of edible oil fatty acids can be confirmed through assessment of the total concentrations of fatty acids along with the presence of high proportions of signature fatty acids common to edible oils (including oleic and linoleic acids) from either groundwater or soil samples. Results are typically reported in micrograms of edible oil per gram or ml of sample. Further calculations based upon analyzed standards of the specific edible oil injected can be used to estimate the concentration of substrate found within a particular sample.

6.1.2 PROCESS MONITORING PROTOCOLS

Biodegradation of organic compounds stimulated by substrate addition brings about measurable changes in the chemistry of groundwater in the treated area. By measuring these changes, it is possible to document and quantitatively evaluate the effect of adding emulsified oil to the subsurface to enhance anaerobic biodegradation at a site. Guidance on developing protocols for emulsified oil projects is included in the following sections of this chapter and can also be found in various publications on MNA and enhanced bioremediation including AFCEE (2004), USEPA (1998), National Academy of Sciences (2000), ITRC (1999), and Morse *et al.* (1998).

Ongoing process monitoring of key contaminant and biogeochemical characteristics of the site is critical to evaluating the effectiveness of the system to meet remedial objectives. Process monitoring should typically follow the protocol used for baseline geochemical characterization that was selected for the site. Primary groundwater parameters that should be sampled regularly for process monitoring include contaminants and daughter products, biogeochemical indicators of redox conditions, and the strength and distribution of organic substrate.

These parameters provide basic information on the efficacy of substrate delivery to the treatment zone and the prevailing redox conditions. Contaminants of concern and their degradation products must be measured to determine treatment effectiveness. Certain monitoring parameters may be dropped if they provide little or no useful information. For example, denitrification will not be a significant redox reaction for a site with naturally low levels of nitrate (e.g., less than 1 mg/L). Therefore, continued monitoring of this parameter yields little information on the predominant redox reactions that are occurring. Caution is advised for regulated parameters that may be expected to change with a lowering of the redox potential. For example, it may take several months for the system to evolve to reducing conditions that may result in elevated levels of metals. In this case, groundwater monitoring for select metals should not be discontinued if initial metal concentrations are low under initial aerobic conditions.

The following sections describe the contaminant and geochemical analytes that should be monitored to assess the performance of emulsified oils in stimulating contaminant biodegradation. Monitoring should

be conducted prior to emulsified oil injection to provide baseline conditions and during multiple process monitoring events to evaluate the degree to which biodegradation has been stimulated.

6.1.2.1 Contaminants and Biodegradation Products

The target analytes and metabolic daughter products are used to determine the type, concentration, and distribution of contaminants and degradation products in the aquifer. In addition, the ratio of the parent and daughter compounds should change as biodegradation is stimulated. At a minimum, VOC analysis by USEPA Method SW8260B should be used for CAHs. Final dechlorination products of the dechlorination sequence (ethene and ethane) are also recommended. In cases where abiotic reactions may be significant, analyses of optional degradation products (such as acetylene) may be warranted.

6.1.2.2 Biogeochemistry

Biogeochemical parameters are measured to determine whether conditions are suitable for enhanced anaerobic biodegradation to occur. Profound changes in redox processes may occur as a result of substrate addition, and the predominant electron acceptor being utilized by microbial activity often varies in zones across the site. Addition of emulsified oil is intended to deplete competing electron acceptors and to maintain anaerobic conditions that are optimal for high rates of anaerobic biodegradation to occur. Excessive levels of competing electron acceptors (e.g., DO and sulfate) may limit the effectiveness of substrate addition. Therefore, groundwater geochemical conditions across the site should be measured in order to identify any undesirable geochemical conditions. At a minimum, parameters that should be measured include DO, redox, nitrate, Fe(II), sulfate, methane, alkalinity, and pH.

6.1.2.3 Indicators of Organic Carbon

Indicators of organic substrate available for biodegradation processes includes total organic carbon (TOC, for unfiltered samples), dissolved organic carbon (DOC, for filtered samples), metabolic or volatile fatty acids (VFAs), and edible oil fatty acids (EOFA). TOC is more commonly measured than DOC for emulsified oil systems, because the oil substrate may be present in suspended or colloidal form. Total inorganic carbon (TIC) can be measured as an indicator of organic carbon that has been degraded to inorganic byproducts.

TOC and VFAs should be monitored over time to evaluate longevity of the edible oil. Levels of TOC and VFAs should be expected to decline over time as microbial growth and activity increases and the substrate is consumed. EOFA analysis can also be used to evaluate the distribution of substrate in the subsurface. Fatty acid biomarkers (e.g., phospholipids) have been primarily used for evaluating microbial responses but can also be an effective molecular biological tool (MBT) for monitoring the distribution of edible oil substrates within a site. The presence of edible oil fatty acids can be confirmed through assessment of the total concentrations of fatty acids along with the presence of high proportions of signature fatty acids common to edible oils (including oleic and linoleic acids) from either groundwater or soil samples. Results are typically reported in micrograms of edible oil per gram or ml of sample. Further calculations based upon analyzed standards of the specific edible oil injected can be used to estimate the concentration of substrate found within a particular sample.

6.1.2.4 Molecular Techniques for Microbiological Characterization

In many cases, favorable contaminant and geochemical data may suffice for site selection purposes. However, sites exhibiting marginal or difficult biogeochemical conditions may benefit from the use of a variety of microbial screening methods. Molecular biology tools (MBTs) can be used to characterize the structure, function, and activity of *in situ* microbial communities based on macromolecules present within

each cell (e.g., nucleic acids, protein, and lipids). Advances in molecular biology have had a profound effect on laboratory studies of bioremediation processes and are used extensively in the research community.

At present, there has been limited use of MBTs in the design and implementation of field bioremediation system. Technical barriers to field implementation include insufficient knowledge of key biomarkers, limited ability to develop rate information, limited understanding of physiology, and limited availability of databases. Other barriers include subsurface sampling difficulties, limited decision-making impact, insufficient confidence in results, and limited commercial interest (SERDP and ESTCP, 2005).

While current use of MBTs is limited, this technology is evolving very rapidly and there is tremendous potential for these tools to improve the design, implementation, field performance, and monitoring of remediation technologies. The Strategic Environmental Research and Development Program (SERDP) and Environmental Technology Certification Program sponsored an Expert Panel Workshop on Research and Development Needs for the Environmental Remediation Application of Molecular Biological Tools (SERDP-ESTCP, 2005) which identified high priority areas for further research. Those planning and implementing *in situ* bioremediation systems are strongly encouraged to consult the SERDP (www.serdp.org) and ESTCP (www.estcp.org) websites to remain abreast of current research in this emerging area.

6.1.2.5 Soil Gas

Soil gas is collected for two general reasons. First, soil gas data may be collected and analyzed to better characterize soil contamination and to identify potential source areas. Depending on the status of the site, this work may have been completed during previous remedial investigation work. Second, soil gas is used to monitor the accumulation in the vadose zone of gasses that pose a health or safety risk or the presence of noxious gasses.

Monitoring for methane should be conducted when emulsified oils are applied near the water table surface and in close proximity to occupied buildings. There is a potential for the formation of methane as a result of oil injection. Biodegradation will occur rapidly in the presence of oxygen, and soil gas oxygen concentrations should be measured to determine if methane is likely to be biodegraded *in situ*. Soil gas carbon dioxide concentrations should also be measured because carbon dioxide is a precursor of methane and is indicative of anaerobic conditions and possibly methanogenic conditions.

6.1.2.6 Downgradient Groundwater Quality and Noxious Gases

Secondary impacts of anaerobic bioremediation on downgradient water quality and production of noxious gases may also need to be evaluated as part of the process monitoring protocol.

While anaerobic biodegradation may be effective in degrading the target contaminants, secondary degradation of groundwater quality may occur. Incomplete dechlorination of CAHs may lead to accumulation of more toxic daughter products (e.g., VC). Degradation reactions and excessive changes in groundwater pH and redox conditions may lead to solubilization and mobilization of metals (e.g., arsenic, iron, and manganese). The presence of naturally-occurring heavy metals in the aquifer matrix should be evaluated both prior to and during implementation of an anaerobic bioremediation application, as many metals are more soluble and mobile in an extremely reducing environment. Fermentation effects also may create conditions conducive to formation of aldehydes, ketones, and mercaptans that have taste and odor impacts.

Stimulating biodegradation also may enhance generation of gaseous byproducts (e.g., methane and hydrogen sulfide) that may degrade groundwater quality or accumulate in the vadose zone. In particular, caution must be exercised when operating near structures where these gases could accumulate.

6.1.3 EFFECT OF EMULSIFIED OIL INJECTION ON FORMATION PERMEABILITY

Formation permeability may change due to biomass growth, trapped gas bubbles, and/or residual oil. Aquifer tests, such as slug tests or well drawdown (specific capacity) tests, should be conducted in select injection and monitoring wells within the treatment area both before and after injection and periodically during process monitoring (Wilson *et al*, 1997). Estimates of hydraulic conductivity from these aquifer tests can be used to determine whether substrate addition has had an impact on aquifer permeability. It should be noted that bio-clogging of well screens between the interface of the aquifer matrix and the open borehole may cause an apparent decrease in hydraulic conductivity. Therefore, caution should be used in interpreting these results. A non-reactive tracer test can be conducted to evaluate the potential for flow bypassing around the treatment zone.

6.2 DATA EVALUATION

Several methods are available for evaluating data from emulsified oil projects to assess the effectiveness of the process for enhancing anaerobic bioremediation. Among these assessments are changes in contaminant concentration and mass over time, changes in groundwater geochemistry, and an increase in contaminant biodegradation rates.

6.2.1 CHANGES IN CONTAMINANT CONCENTRATION AND MASS

The primary objective of enhanced anaerobic bioremediation is a decrease in contaminant concentration and mass by anaerobic degradation processes. Measurement of contaminant concentrations in groundwater both before and after edible oil addition that demonstrate a reduction in contaminant mass can be used to show that enhanced bioremediation is an effective remedy. In addition, a change in the molar ratios of parent compounds to biodegradation products can be useful in evaluating the extent to which anaerobic bioremediation is occurring.

It is important when evaluating the attenuation of a contaminant plume that the data demonstrate a clear and meaningful trend in contaminant concentration and/or mass over time at appropriate monitoring locations. Both visual and mathematical methods can be used to evaluate mass reduction and plume attenuation.

There are several ways to present data showing changes in contaminant concentrations and plume configuration over time after emulsified oil injection. Common visual techniques include the use of concentration isopleth maps and concentration versus time and distance plots. Isopleth maps of contaminant concentrations can be prepared over space and/or time. Pre-injection and post-injection maps can be compared to evaluate changes in contaminant concentrations and determine if enhanced biodegradation is effectively degrading the target contaminants. Another useful method to present data showing changes in contaminant concentrations and plume configuration is to plot contaminant concentrations versus time for individual monitoring wells or to plot contaminant concentrations versus distance downgradient for several wells along a groundwater flow path over several sampling events. Traditional data presentations show the changes in concentration of each target compound or indicator parameter. After treatment is initiated, plots of individual contaminants including parent compounds and daughter products also can be useful in evaluating the effectiveness of the enhanced anaerobic

bioremediation. Trends in the data can be analyzed by plotting concentration data versus time. **Figure 6.1** shows conceptually how concentrations of individual compounds change as sequential anaerobic reductive dechlorination proceeds. It is important to keep in mind that this is a conceptual model only.

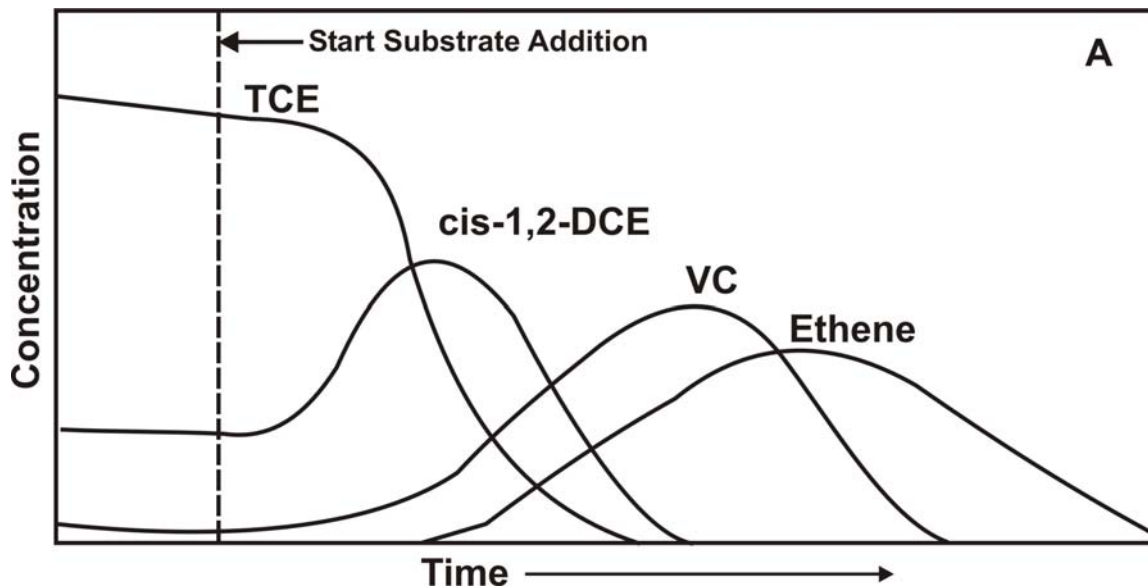


Figure 6.1. Conceptual Model of Changes in Chlorinated Ethenes over Time due to Sequential Reductive Dechlorination.

Evaluating the change in the molar concentrations and fractions (or ratios) of parent compounds to degradation products also can be very useful in determining the efficacy of biodegradation brought about by edible oil injection. During biodegradation, the molar ratios of the compounds involved in the reaction chain will change. Looking at molar concentrations is more accurate and informative than evaluating changes in concentration alone for the parent/biodegradation products because of the different molecular weights of the compounds.

Plotting the molar fraction or ratio over time is often used when there is a constant or continuing source of contaminant mass entering a treatment zone. In this case, the total molar concentration may remain elevated or even increase because of continuing mass inputs, but an increase in the molar ratio of dechlorination products will demonstrate that sequential anaerobic reductive dechlorination is occurring (AFCEE, 2004). By converting concentration to molar concentration and plotting these values versus distance from the treatment zone allows the practitioner to evaluate the effectiveness of the treatment and its influence along the groundwater flowpath. Plotting changes to the molar fraction or total molar concentration at one location in the treatment zone is a way of determining the effectiveness of the treatment at that location.

Figure 6.2 presents data illustrating the changes in molar concentration of chlorinated compounds over time from a source area emulsified oil pilot test conducted at the Tarheel Army Missile Plant in Burlington, NC. The data presented are averages from four injection/monitor wells. The principle contaminant prior to injection of substrate was TCE with some *cis*-DCE present. Soon after injection, TCE was reduced substantially with production of *cis*-DCE. By 287 days post-injection, much of the *cis*-DCE was converted to VC. Consistent with the conceptual model above in **Figure 6.1**, as the less

chlorinated compounds are formed, they are degraded and lost to the system, resulting in an overall decrease in the total molar concentration in the system.

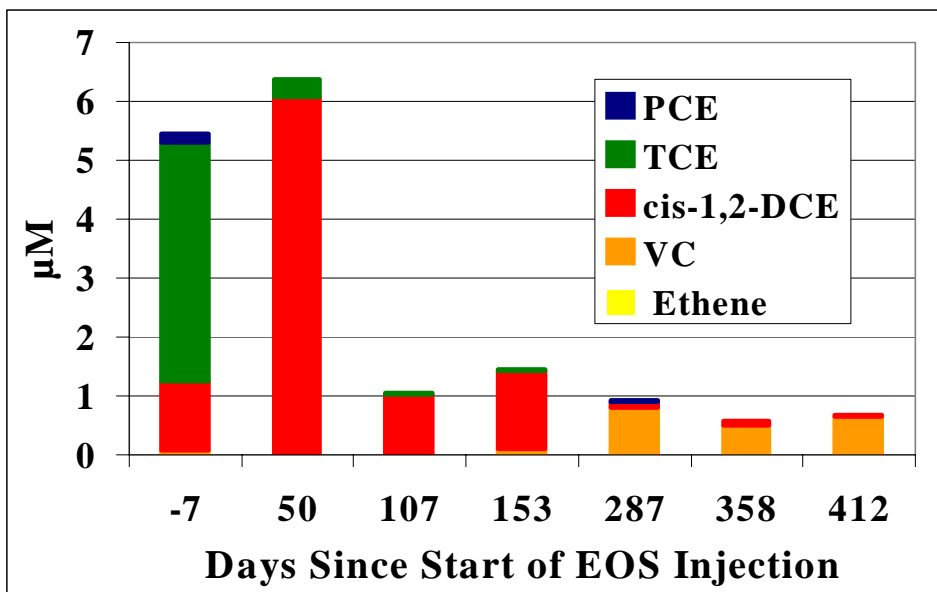


Figure 6.2. Changes to Molar Concentrations of Chlorinated Compounds in Groundwater after Injection of Emulsified Oil Substrate (from Beckwith *et al.*, 2005)

6.2.2 BIODEGRADATION RATE CONSTANT CALCULATIONS

Biodegradation rate constants should be estimated prior to substrate addition (if possible) and during performance monitoring to determine whether emulsion addition has resulted in an increase in the biodegradation rate. Biodegradation rate constant estimates can be calculated by many methods. The reader is referred to such documents as USEPA (1998) and Newell *et al.* (2003) for a detailed discussion of biodegradation rate constant estimation.

While monitoring contaminant biodegradation rates can be very useful, accurate estimation of biodegradation rate constants can be difficult due to partitioning of chlorinated solvents between the sediment, injected oil, and aqueous phases. Since monitor wells preferentially sample the higher permeability zones, groundwater sampling results will also be influenced by the slow diffusion of contaminants out of lower permeability zones.

In contaminant source areas, there are no generally accepted methods for estimating overall average contaminant biodegradation rates. The point decay approach described by Newell *et al.* (2003) can be used to estimate rates of contaminant decline in individual monitor wells. However, these rates may not be representative of the entire treatment zone. NAPL oil and oil-in-water emulsions are preferentially transported through the higher permeability (K) zones. As a consequence, biodegradation rates may be greater in the higher K zones than low K zones. Contaminant concentrations often decline very rapidly in monitor wells (which preferentially sample the high K zones), even though some contaminants remain in the lower permeability layers. In a strongly heterogeneous site in the North Carolina Piedmont, Beckwith *et al.* (2005) showed that TCE was reduced from approximately 1,000 µg/L to below detection within 50 days of emulsion injection. However, *cis*-DCE, VC, and ethene continued to be produced for over twelve months indicating additional TCE was slowly diffusing out of lower permeability zones and being degraded. Slow diffusion of contaminants out of low K zones is not a problem as long as some oil

remains to support contaminant biodegradation. However, if the oil is depleted before both the high and low K zones are remediated, additional oil injections may be necessary to maintain biodegradation rates.

In barrier systems, mass transfer between high and low K zones is less of an issue, and degradation rates can be calculated once geochemical and microbiological conditions stabilize. To be considered 'stable', important indicators of biogeochemical conditions (pH, Eh, DO, SO₄, CH₄) and contaminant biodegradation (contaminant molar ratios, CI#) should be reasonably constant over 3 or more sampling events. Once conditions stabilize, degradation rates can be estimated by adjusting the rate constants in BIOCHLOR (Aziz *et al.*, 2002) until model simulations approximately match average concentrations (after conditions stabilize) in monitor wells at various locations upgradient and downgradient of the barrier. Typically, degradation rates are assumed equal to background conditions, except in areas directly impacted by edible oils (indicated by DOC > 20 mg/L). Accurate estimates of hydraulic gradient, permeability, and effective porosity will also be required for calibration of BIOCHLOR. Once accurate estimates of degradation rates are available, BIOCHLOR can be used to determine the required barrier width. Typically, a range of groundwater velocities is used in this analysis to account for seasonal variations in groundwater flow.

Users of this protocol should be aware that it may take several years after oil injection for biogeochemical and microbiological conditions to stabilize and to collect sufficient data for accurate estimation of degradation rates. If this extended data collection period is not practical, preliminary degradation rate estimates can be developed using monitoring data collected before conditions stabilize. However, these preliminary rate estimates may be lower than actual long-term degradation rates. Monitoring results from multiple sites treated with edible oils indicate that degradation rates can slowly increase over several years as the microbial community gradually adapts to the increased level of organic substrate and competing electron acceptors are depleted.

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APPENDIX A
SUBSTRATE CALCULATIONS

Excel spreadsheets are provided for calculating substrate requirements based on site-specific conditions. There are two spreadsheets: one for a permeable reactive barrier design and one for an areal treatment design. Each spreadsheet contains seven sections (A through G). A brief description of each section is provided below. Additional information is provided in Section 4.5.1 of the Protocol.

Section A: Barrier or Treatment Area Dimensions

The user enters the dimensions of the barrier or treatment area.

Section B: Site Hydrogeologic Data

Site-specific hydrogeologic data is entered and is used to calculate the groundwater flow rate through the barrier or treatment area.

Section C: Design Lifespan for One Application

The user enters a design lifespan which is used along with the data in Sections A and B to calculate the total volume of groundwater to be treated. When selecting a design life, users should be aware that the spreadsheets assume the barrier or source area treatment will operate at 100% efficiency until the day when the organic substrate runs out. On that day, the treatment efficiency is assumed to drop to zero. However, in practice, treatment efficiency will begin to decline as substrate is depleted from the more permeable/contaminated zones. Consequently, users should include an appropriate factor of safety when selecting the design life. In addition, users should take into account project cost, contaminant source(s) and concentrations, and remedial objectives when selecting a design life.

Section D: Electron Acceptors

In this section, the user enters concentrations of contaminants and competing electron acceptors and the spreadsheets calculate the hydrogen demand, which is used to determine the amount of substrate.

Section E: Additional Hydrogen Demand and Carbon Losses

The user estimates other demands that effect the amount of substrate required. Typical values are provided for guidance.

Section F: Substrate Requirement Based on Hydrogen Demand and Carbon Losses

The user enters information about the substrate (pure edible oil or emulsified edible oil), and the spreadsheets calculate the amount of substrate required based on the hydrogen demand and carbon losses.

Section G: Substrate Requirement Based on Adsorptive Capacity of Soil

The user estimates the adsorptive capacity of the soil. The spreadsheets then calculate the amount of substrate required to distribute edible oil or emulsified edible oil throughout the targeted treatment area based on the treatment zone dimensions and the oil retention by the aquifer material.