3-D MicroEmulsion for Anaerobic Bioremediation:

1. Daniel Nunez, Regenesis
2. Glycerol esters of fatty acids and polylactates (3-D MicroEmulsion®(3DMe®), (3DMe®) Factory Emulsified, HRC® Advanced)
3. MSDS & Technical Data Sheet - Attached
4. Number of Field-scale Applications to Date: 1,000+ sites.
5. Case Studies – Attached
6. 3-D MicroEmulsion® is a pH neutral solution designed specifically to optimize anaerobic degradation of contaminants in subsurface environments. This structure incorporates esterified lactic acid (technology used in HRC) and esterified long chain fatty acids. The advantage of this structure is that it allows for the controlled-release of lactic acid (which is among the most efficient electron donors) and the controlled-release of fatty acids. Upon injection, the controlled release of lactic acid dominates serving to initiate and stimulate anaerobic dechlorination. Over time the controlled-release of fatty acids will dominate, acting to continue microbial stimulation. The expected single-injection longevity of this product is 3-5 years. This product has been on the market since 2005. 3DMe is factory emulsified is non-viscous liquid that incorporates a molecular structure composed of tetramers of lactic acid (polylactate) and fatty acids esterified to a carbon backbone molecule of glycerin. This product is approved on the general WDR permit listed as HRC-Advanced. This product is food grade material and there are no health and safety issues with this product.

3-D Microemulsion® Factory Emulsified
MATERIALS SAFETY DATA SHEET

Last Revised: November 15, 2011

Section 1 – Material Identification

Supplier:

REGENESIS

1011 Calle Sombra
San Clemente, CA 92673

Phone: **949.366.8000**

Fax: **949.366.8090**

E-mail: **info@regenesis.com**

Chemical Name(s):	Glycerides, tall-oil di-, mono [2-[2-[2-(2-hydroxy-1-oxopropoxy)-1-oxopropoxyl]-1-oxopropoxy]propanoates]
Chemical Family:	**Organic Chemical**
Trade Name:	**3-D Microemulsion® Factory Emulsified**
Synonyms:	**HRC Advanced®, HRC-PED (Hydrogen Release Compound – Partitioning Electron Donor)**
Product Use:	**Used to remediate contaminated groundwater (environmental applications)**

Section 2 – Chemical Identification

CAS#	**Chemical**
823190-10-9	**HRC-PED**
72-17-3	**Sodium Lactate**
7789-20-0	**Water**

Section 3 – Physical Data

Melting Point:	**Not Available (NA)**
Boiling Point:	**100 ºC**
Flash Point:	**> 93.3 ºC using the Closed Cup method**
Density:	**1.0 -1.2 g/cc**
Solubility:	**Soluble in water.**
Appearance:	**White emulsion.**
Odor:	**Not detectable**
Vapor Pressure:	**None**

Section 4 – Fire and Explosion Hazard Data

Extinguishing Media:	**Use water spray, carbon dioxide, dry chemical powder or appropriate foam to extinguish fires.**

Water May be used to keep exposed containers cool.

For large quantities involved in a fire, one should wear full protective clothing and a NIOSH approved self contained breathing apparatus with full face piece operated in the pressure demand or positive pressure mode as for a situation where lack of oxygen and excess heat are present.

Section 5 – Toxicological Information

Acute Effects:	May be harmful by inhalation, ingestion, or skin absorption. May cause irritation.
Sodium Lactate:	Toxicity to Animals: LD50: Not available. LC50: Not available. Chronic Effects on Humans: Not Available. Other Toxic Effects on Humans: Very hazardous in case of skin contact (irritant), ingestion and inhalation.
Soybean Oil:	Health Hazards (Acute and Chronic): Acute: none observed by inhalation. Chronic: none reported.
Inhalation Risks and Symptoms of Exposure:	Excessive inhalation of oil mist may affect the respiratory system. Oil mist is classified as a nuisance particulate by ACGIH.

Skin Absorption Health Risks and Symptoms of Exposure:	Sensitive individuals may experience dermatitis after long exposure of oil on skin.

Section 6 – Health Hazard Data

Handling:	Avoid continued contact with skin. Avoid contact with eyes.

In any case of any human exposure which elicits a reaction, a physician should be consulted immediately.

First Aid Procedures:

Inhalation:	Remove to fresh air. If not breathing give artificial respiration. In case of labored breathing give oxygen. Call a physician.
Ingestion:	No effects expected. Do not give anything to an unconscious person. Call a physician immediately. DO NOT induce vomiting.
Eye Contact:	Wash eyes with plenty of water for at least 15 minutes lifting both upper and lower lids. Call a physician.

Section 7 – Reactivity Data

Conditions to Avoid:	Strong oxidizing agents, bases and acids
Hazardous Polymerization:	Will not occur.
Stability:	Spontaneous combustion can occur.
Further Information:	Hydrolyses in water to form lactic acid and soybean oil.
Hazardous Decomposition Products:	None known.

Section 8 – Spill, Leak or Accident Procedures

After Spillage or Leakage:	Neutralization is not required. The material is very slippery. Spills should be covered with an inert absorbent and then be placed in a container. Wash area thoroughly with water. Repeat these steps if slip hazard remains.
Disposal:	Laws and regulations for disposal vary widely by locality. Observe all applicable regulations and laws. This material may be disposed of in solid waste. Material is readily degradable and hydrolyses in several hours.

No requirement for a reportable quantity (CERCLA) of a spill is known.

Section 9 – Special Protection or Handling

Should be stored in plastic lined steel, plastic, glass, aluminum, stainless steel, or reinforced fiberglass containers.

Protective Gloves:	Vinyl or Rubber
Eyes:	Splash Goggles or Full Face Shield. Area should have approved means of washing eyes.
Ventilation:	General exhaust.
Storage:	Store in cool, dry, ventilated area. Protect from incompatible materials.

Section 10 – Other Information

This material will degrade in the environment by hydrolysis to lactic acid and soybean oil. Materials containing reactive chemicals should be used only by personnel with appropriate chemical training.

This material is a non hazardous material in regards to USDOT shipping criteria.

The information contained in this document is the best available to the supplier as of the time of writing. Some possible hazards have been determined by analogy to similar classes of material. No separate tests have been performed on the toxicity of this material. The items in this document are subject to change and clarification as more information becomes available.

URS

Enhanced Anaerobic Biodegradation of Chlorinated Solvents at a Dry Cleaner

A large chlorinated solvent plume was discovered around the area of the Peter Pan Dry Cleaner facility in Santa Rosa, CA. The California DTSC tasked URS with finding a solution to treat the contaminated area which contained high amounts of PCE and TCE. A pilot study was undertaken to validate the use of enhanced anaerobic bioremediation to biodegrade the primarily dissolved-phase solvent plume. 3-D Microemulsion, an injectable, 3-stage electron donor release material was selected as the preferred bioamendment to accelerate the anaerobic biodegradation process.

Project Details:

- The remediation effort was covered by the California State Superfund under the direction of the DTSC and the URS, San Francisco office. This project was also within the jurisdiction of the North Coast Regional Water Quality Control Board (RWQCB).

- Elevated levels of chlorinated hydrocarbons including perchlorethene (PCE) and trichloroethene (TCE) were identified in the subsurface at the dry cleaning facility.

- Chlorinated contaminant levels as high as 4100 ug/L PCE and 120 ug/L TCE were measured and formed a plume migrating off-site and beneath a public school (Figure 1).

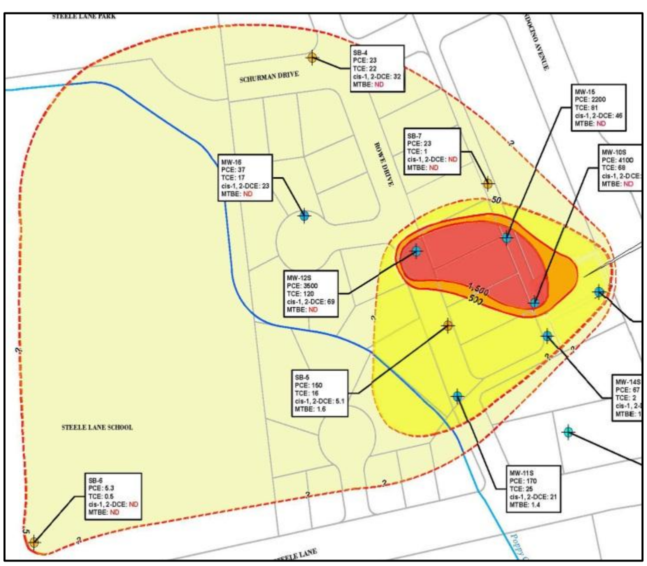

Figure 1. Contaminated Plume Pre-Injection

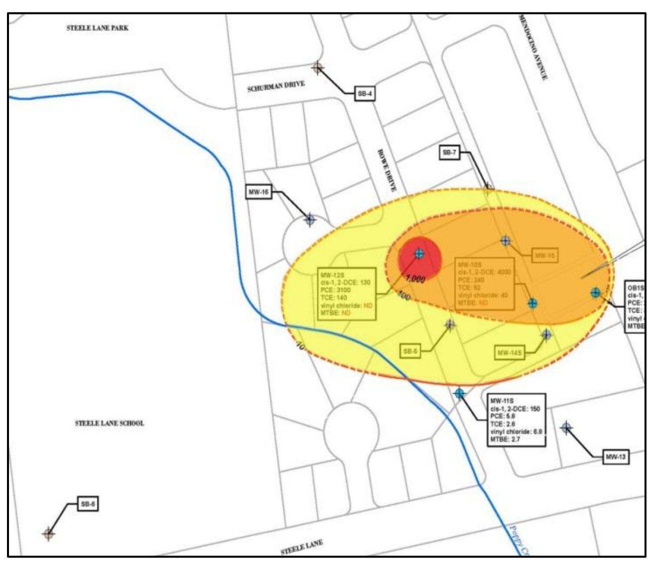

Figure 2. Contaminated Plume Area Post-Injection

- A total of 6300 lbs. of 3-D Microemulsion and 1080 lbs. of HRC® Primer were injected in a linear barrier configuration into approximately 25 direct-push points across the upper section of the plume.

- The remediation goal was to reduce chlorinated solvent levels (PCE and TCE) to below the CA MCL of < 5 ug/L in all monitoring wells.

Injection of 3-D Microemulsion®

- After 2 months post 3-D Microemulsion and HRC Primer injection, chlorinated solvent concentrations declined significantly in 7 of the 8 monitoring wells (Figure 2).

- One well, MW-15 showed an increase in contaminant concentrations. This uncharacteristic increase was identified to be the result of direct-injection activities and the mobilizing of an unidentified pocket of pure PCE which accumulated near a sewer line.

- Overall the pilot test results were favorable and the outlook for successful enhanced anaerobic bioremediation of the dissolved-phase chlorinated solvent plume is high. URS and the DTSC are currently reviewing the data and determining what the next steps are for treating the PCE source area that was recently identified.

For more information contact:

Project Manager: Giorgio Molinario
URS
Senior Environmental Chemist
(415) 896-5858
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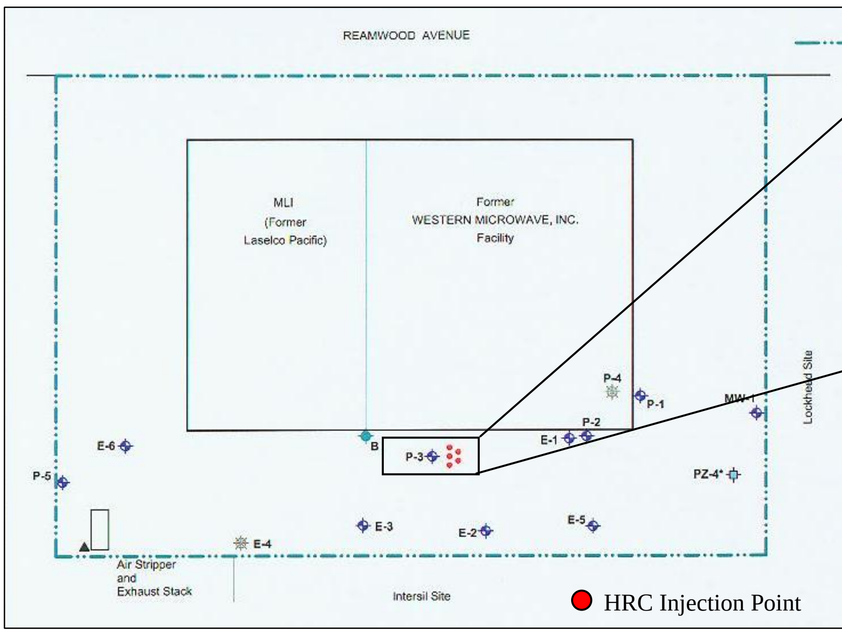
On-Site Direct-Push Rig

PES Environmental, Inc.
Engineering & Environmental Services

Successful Pilot Test Reduces Chlorinated VOCs

INTRODUCTION

Elevated concentrations of CVOCs were present in the subsurface as a result of plating operations which leaked PCE into the on-site groundwater. Additionally, an upgradient source of elevated TCE concentrations was contributing to the plume. Prior to remedial activities, PCE and TCE concentrations had reached 480 and 50 ug/L, respectively, while downgradient DCE and VC concentrations were close to 1500 and 790 ug/L, respectively. Since the rate of natural attenuation was not sufficiently able to remediate the on-site contamination, enhanced bioremediation using Hydrogen Release Compound (HRC®) was recommended. A pilot test was designed upgradient of impacted well P-3 to observe the effectiveness of HRC. A total of 250 pounds of HRC was applied at a rate of 5 lbs/ft. An injection spacing of 5 feet was used to inject 5 points upgradient of P-3 (Figure 1).



P-3 Concentrations Prior to Remedial Activities

Contaminant	Well P-3	Cleanup Goals
PCE	200 ug/L	5 ug/L
TCE	63 ug/L	5 ug/L
DCE	1000 ug/L	70 ug/L
VC	150 ug/L	2 ug/L

Figure 1. HRC Pilot Application Upgradient of P-3

HYDROGEN RELEASE COMPOUND (HRC®)

HRC® (Regenesis, San Clemente, CA; www.regenesis.com) is a controlled-release, polylactate ester mixture formulated to slowly release lactic acid upon hydration. When placed into a contaminated aquifer, the lactic acid from HRC enhances a multi-step process, known as reductive dechlorination. This mechanism biologically degrades chlorinated hydrocarbons under anaerobic conditions by substituting hydrogen for chlorine and reducing the contaminants to a harmless end product, ethene. Adding HRC within a reducing environment can accelerate this process which can occur naturally at a very slow unsustainable rate.

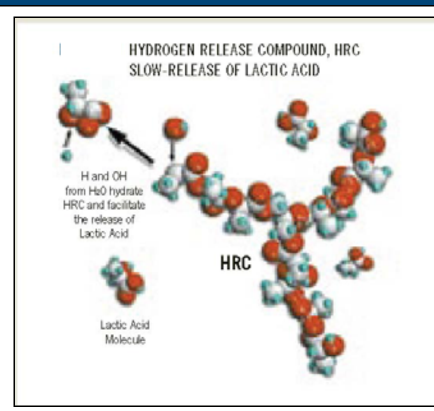


Figure 2. HRC Molecule

UPGRADIENT CONCENTRATIONS

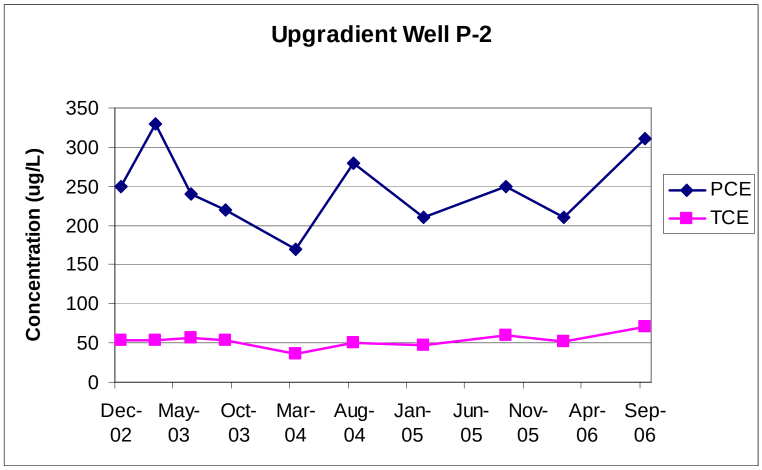


Figure 3. CVOC Concentrations in Untreated Well P-2

Monitoring on untreated well P-2 indicated consistent PCE concentrations above the cleanup criteria. Throughout the monitoring period levels remained elevated at 200 – 300 ug/L. No significant change was observed in TCE concentrations as well. It appeared that no reductive dechlorination process was occurring as levels of DCE or VC remained <10 ug/L and <2.5 ug/L, respectively.

HRC APPLICATION RESULTS

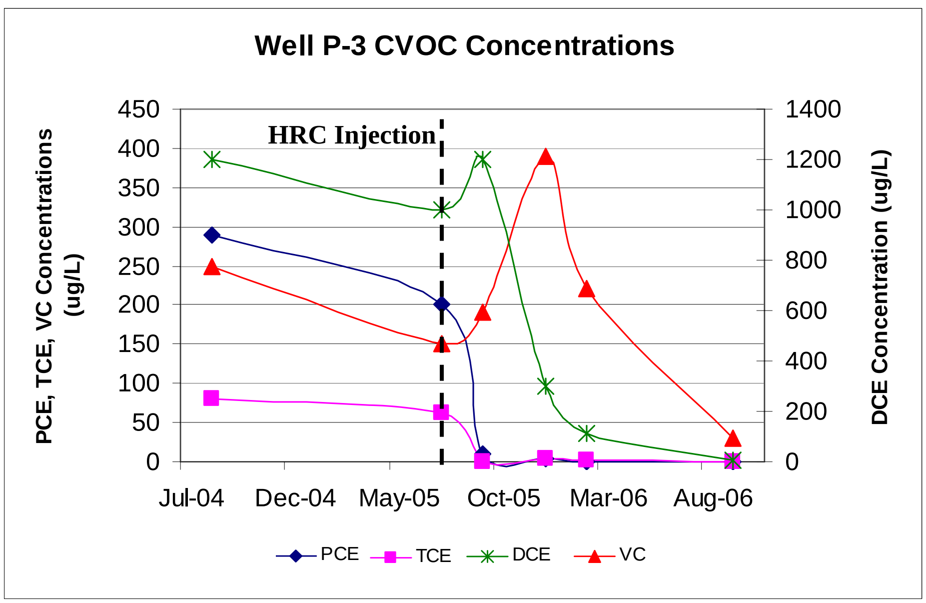


Figure 4. HRC Treated Well P-3

Initial parent concentrations of PCE and TCE were 200 and 63 ug/L, respectively, in P-3 and were reduced by more than 94% within 2 months. Continued reduction was observed as PCE and TCE concentrations reached non-detect levels. Past monitoring indicated significant DCE production, therefore, DCE degradation was monitored closely. As observed, HRC created a strong reducing environment for DCE reduction and resulted in a decrease of more than 90% within 6 months. With the significant reduction in DCE, a sequential increase in the production of VC was expected. Recent sampling results have shown a 92% reduction in VC from a high of 390 ug/L in December 2005. HRC was successful in not only creating a reducing environment for DCE but also in restraining the potential buildup of VC. A full-scale HRC application was applied and monitoring is ongoing.

PES Environmental, Inc. (PES) was formed in 1989 as an employee-owned company to provide cost-effective, innovative, and personalized solutions in the field of environmental consulting. PES has offices in Novato, California, Bellevue, Washington, and Eugene, Oregon. We have a highly qualified professional staff of environmental, civil, and chemical engineers; geologists; hydrogeologists; geochemists; industrial hygienists; and environmental scientists.

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HRC® CHOSEN OVER CHEM OX, IRON WALL AND LACTATE INJECTIONS FOR REMEDIATION AT INDUSTRIAL SITE

Introduction

FMC Corporation, working with GeoTrans engaged in the cleanup of shallow soils and groundwater contaminated with trichloroethylene (TCE) and other volatile organic compounds (VOC's). The former industrial site, now occupied by active, commercial and light industrial businesses, underwent a search for the least impacting and most in conspicuous treatment technology for both source and plume treatment.

Technology Selection

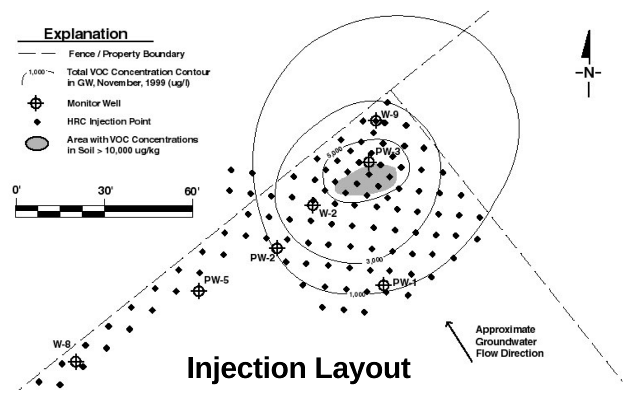
Several remediation options to address both the source and the migrating plume were explored including: Chemical Oxidation, Zero Valent Iron Walls, Lactate Injections and Hydrogen Release Compound (HRC) Injections. These applications ranged in cost from $922,000 for plume treatment only with an iron wall to $24,600,000 for chemical oxidation of both the source and plume area. Bioremediation options using lactate and HRC each at around $1,220,000 seemed to be the most cost-effective options for treating the entire site.

Of the two most cost-effective options HRC was chosen over lactate for several reasons:

- HRC is simply injected into the subsurface and left in-situ to release hydrogen and stimulate natural degradation pathways.
- Unlike lactate, HRC requires no obtrusive upgradient and downgradient pumping infrastructure to remove or inject water.
- HRC is a slow-release compound which provides a constant, balanced release of hydrogen to the aquifer.

Full Scale HRC Application

The full-scale application of HRC stimulated the reductive dechlorination process to bioremediate TCE to daughter products *cis*-1,2 DCE, vinyl chloride, and ethylene. The scope of the full-scale remediation included injecting approximately 12,000 pounds (or $66,000 worth) of HRC through 103 direct-push points to remediate an apparent TCE source area and to provide a barrier to downgradient TCE migration. The HRC application was conducted during 10 days of field work.



Injection Layout

Results

Microbial analyses were also performed in addition to the standard natural attenuation parameters. The results indicated that the application of HRC correlated to an increase in biomass and specific dehalogenating bacteria targeted in the analysis. These results provide further indication that the reductive dechlorination process will be successful in achieving the site remediation goals.

Follow Up Application

A second HRC application was completed during November 2001. It is anticipated that continued monitoring following this application will demonstrate complete reductive dechlorination of the vinyl chloride during the next one to three years.

More Information

For more information please contact Jeff Bensch at Geotrans (916) 853-1800, for information on HRC visit www.regenesis.com.

Table 1. Selected Data for Well W-8

Parameter	Units	May 2000	July 2000	Sep 2000	Nov 2000	Feb 2001	May 2001	Aug 2001
TCE	ug/L	224	570	51	34	13	23	21
cis-1,2 DCE	ug/L	6.4	960	120	96	87	77	89
Vinyl Chloride	ug/L	<2.0	<25.0	27	58	90	62	55
Ethylene	ug/L	<20.0	0.047	0.189	0.17	0.21	0.12	0.12
Sulfate	mg/L	270	243	214	214	208	239	255
Methane	ug/L	<10	16	188	740	2300	2400	2200
Hydrogen	nM	1.48	2.17	2.05	2.2	1.8	1.1	2.5
ORP	mV	-40	-121	3	97	-81	-169	-185

3-D Microemulsion (3DMe)™

TECHNICAL BULLETIN 2.0

Subsurface Transport Mechanisms

As described in 3-D Microemulsion Technical Bulletin 1.0 (Introduction), 3-D Microemulsion (3DMe)™, a form of HRC Advanced®, is a unique compound (patent applied for) which incorporates esterified lactic acid (the technology used in HRC), with esterified fatty acids. The unmatched advantage of this product is that it allows for the immediate and controlled-release of lactic acid which is among the most efficient electron donors. The controlled-release of proprietary fatty acids provides a cost-effective source of controlled-release hydrogen. This combination of organic acids, in turn, rapidly stimulates reductive dechlorination for extended periods of time up to 4+ years under optimum conditions (e.g. concentrated application in low permeability, low consumptive environments.).

3DMe is NOT Simple Emulsified Vegetable Oil

Vegetable oil is basically insoluble. Thus, to make it amenable to injection into the subsurface, some vendors have added commercial emulsifying agents to simple vegetable oils and produced emulsions claiming that the "stable" emulsion will transport the oil significant distances down-gradient from the injection point. Unfortunately, this is not the case.

When so-called "stabilized" oil-in-water emulsions are forced out of the injection point into subsurface aquifer materials the emulsifying agents are rapidly stripped from the oil droplet due to the zeta potential of subsurface materials (charges on the surfaces of soil particles) adhering to the hydrophilic "heads" of the emulsifying agents, and to organic matter within the aquifer matrix sorbing to the vegetable oil itself. Upon the stripping of the emulsifying agents the oil droplets rapidly coalesce in soil pores creating a separate phase (this is the basis for many de-emulsification filters used in the petroleum production industry). When this coalescence occurs in the aquifer, it retards further migration of any oil emulsion and, in fact, often blocks groundwater flow. Use of emulsified oil products can result in significant lowering of the aquifer hydraulic conductivity within aquifer settings (Edible Oil Barriers for Treatment of Perchlorate Contaminated Groundwater, Environmental Security Technology Certification Program, US Department of Defense, November 2005.)

3DMe has a balanced HLB

3DMe is composed of molecules that are surface active. That is to say the molecules behave as surfactants, with a hydrophilic or "water loving end", and a lipophilic or "oil loving end". As a result, the molecules tend to align themselves with the hydrophilic ends in the water matrix, while the lipophilic ends bind to organic compounds (such as the contaminant).

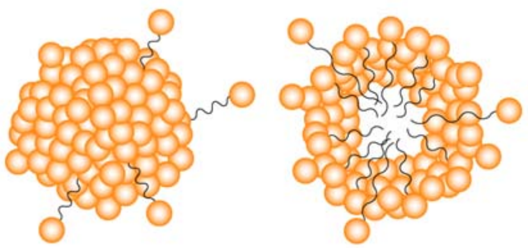
As a measure of the tendency for a molecule to move into water, chemists refer to the Hydrophile/Lipophile Balance index (HLB). The greater the HLB, the higher the tendency for dissolution in water, thus, low HLB molecules are generally pushed out of the water matrix and sorb onto surfaces and to organic compounds within the aquifer material.

3DMe was designed to have a low, yet positive HLB. This gives 3DMe the advantage of being able to sorb organic contaminants (partition), yet have a significant solubility in water allowing for aqueous transport (unlike emulsified oils). A comparison of estimated HLBs for substrates is listed below.

Substance	HLB
Sugars	30
Lecithin	20
3DMe	6
Vegetable Oil	-6

3DMe Forms Micelles

When 3DMe is in water in concentrations in excess of about 300ppm, dissolved molecules of 3DMe begin to spontaneously group themselves into forms called “micelles”. In colloidal chemistry this concentration is referred to as the “critical micelle concentration” or CMC. The grouping of the micellar structure is very orderly, with the charged or hydrophilic ends (heads) of the fatty acids facing out to the water matrix and the hydrophobic ends (tails) facing in together. The micellar structures formed from 3DMe are generally spherical, but under certain circumstances can become lamellar. A depiction of a 3DMe micellar structure is shown below:



After B. Lindman

Depiction of 3-D ME Micellar Structure

The size of the 3DMe micelles formed is very small, on the order of .02 to .05 microns in diameter. These will spontaneously form in aquifer waters when the CMC is exceeded. Thus, by loading the aquifer with volumes of injection water containing 3DMe in excess of approximately 300 ppm, micelles will spontaneously form carrying the 3DMe product further down-gradient.

Mixing and Application

Concentrated Delivery

When applied to the subsurface in concentrated form, 3DMe will behave much like HRC. Once installed the material remains stationary and slowly releases soluble lactic acid and fatty acids which diffuse and advect away from the point of application. In this fashion the engineer is assured of a long-term, constant supply of electron donor emanating from the point of application for a period of up to 4+ years (under optimal conditions). This is particularly attractive when used to treat a flux of contamination from an up-gradient source or when a very long term supply of electron donor is required.

High Volume Delivery

3DMe can also be used to treat large areas in a short period of time by using a high-shear pump to mix the 3DMe with water prior to injection. This mixing generates a large volume of a 3DMe colloidal suspension. The actual suspension of 3DMe generated by this mixing ranges in size from micelles on the order of .02 microns to .05 microns in diameter to "swollen" micelles, also termed "microemulsions", which are on the order of .05 to 5 microns in diameter.

Once injected into the subsurface in high volumes followed by water the colloidal suspension mixes and dilutes in existing pore waters. The micelles/microemulsions on the injection front will then begin to sorb onto the surfaces of soils as a result of zeta potential attraction and organic matter within the soils themselves. As the sorption continues, the 3DMe will "coat" pore surfaces developing a layer of molecules (and in some cases a bilayer). This sorption continues as the micelles/microemulsion moves outward.

Unlike emulsified oil, however, the sorbed 3DMe has a significant capacity to move beyond the point of initial sorption. As the high concentration of 3DMe present in the initial injection volume decreases, bound material desorbs. As long as this concentration exceeds the CMC, micelles will spontaneously form, carrying 3DMe further out in to the contaminated aquifer through advection and diffusion.

Additional Research Underway

Regenesis is currently undertaking a series of laboratory studies and in-field research efforts to further define the extent to which 3DMe suspensions transport under various aquifer conditions. These studies will generate information which will aid in understanding the limitations to the transport of colloidal suspensions under realistic injection/aquifer dispersion conditions.

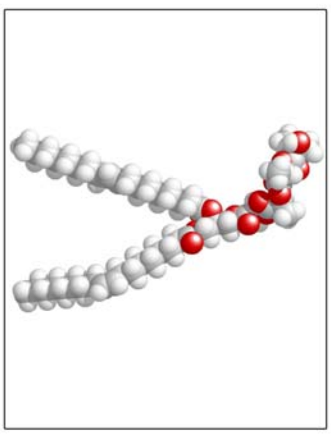
3-D Microemulsion (3DMe)™

TECHNICAL BULLETIN 1.0

Introduction

3-D Microemulsion (3DMe)™, a form of HRC Advanced®, is the new paradigm in time-release electron donors for groundwater and soil remediation. 3DMe is based upon a new molecular structure (patent applied for) designed specifically to optimize anaerobic degradation of contaminants in subsurface environments. This structure incorporates esterified lactic acid (technology used in HRC) and esterified long chain fatty acids. The advantage of this structure is that it allows for the controlled-release of lactic acid (which is among the most efficient electron donors) and the controlled-release of fatty acids (a very cost effective source of slow release hydrogen). Upon injection, the controlled-release of lactic acid dominates serving to initiate and stimulate anaerobic dechlorination. Over time the controlled-release of fatty acids will dominate, acting to continue microbial stimulation. The expected single-injection longevity of this product is 1-2 years and in excess of 4 years under optimal conditions, e.g. concentrated application in low permeability, low consumptive environments.

3DMe is a slightly viscous liquid that incorporates a molecular structure composed of tetramers of lactic acid (polylactate) and fatty acids esterified to a carbon backbone molecule of glycerin.



The image to the left illustrates a ball-and-stick version of the glycerol ester in 3DMe. Oxygen atoms are shown in red, carbon atoms in grey, and hydrogen atoms in white. The long chains represent the fatty acid components of the molecule.

REGENESIS

Advanced Technologies for Groundwater Resources

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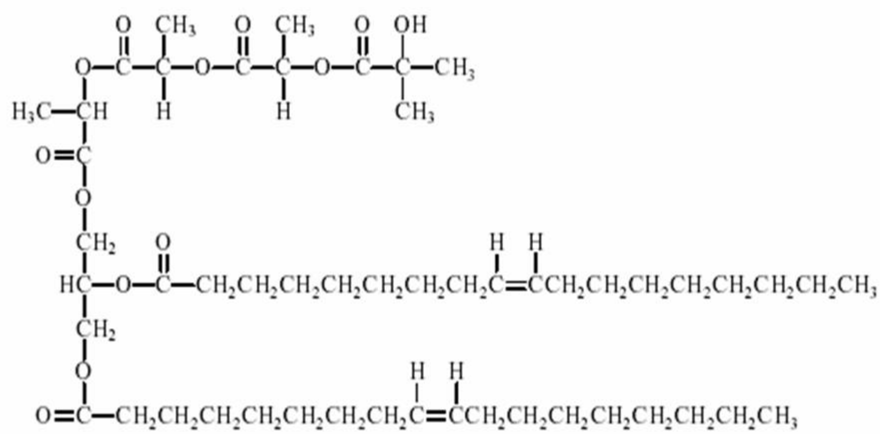
When 3DMe is placed in water, free lactic acid immediately begins to ferment which initiates reductive dechlorination and subsequent contaminant treatment. Over time the ester bonds begin to cleave, producing dissolved-phase lactic acid and fatty acids. 3DMe also contains free fatty acids for additional electron donating capacity. Thus, 3DMe provides the benefits of lactic acid, a rapidly fermented substrate and excellent hydrogen source, as well as fatty acids, which are slower to ferment and provide hydrogen to a contaminated site over extended time periods. This combination of lactic acid and fatty acids provides a functional longevity of 1-2 years for most sites (>4 years under optimal conditions). 3DMe creates an anaerobic system in a redox range where bacteria known to be responsible for reductive dechlorination flourish. Maintaining these conditions provides maximum utilization of the electron donor for reductive dechlorination, rather than simply providing excess carbon per unit time which can result in excess methane production, as simple soluble substrates often do.

3DMe Attributes:

- Incorporates proven Hydrogen Release Compound (HRC®) base materials
- Provides a persistent and significant source of hydrogen
- Typical single-injection longevity of 1-2 years and over 4 years under optimal conditions
- Achieve wide subsurface distribution when applied as microemulsion
- Easily applied with readily available direct injection equipment

Molecular Diagram

The following chemical structure shows the glycerol ester (patent applied for). The top "prong" is the tetramer of polylactate (look for 4 double bonded O atoms). The middle and bottom "prongs" are fatty acids.



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