



32nd Annual International Conference
on Soil, Water, Energy, and Air

Tuesday, March 21 , 2023

2:30 PM – 3:00 PM

**Optimization and
Advances in
Amendments for
Chlorinated Solvent Sites**



Agenda

Bioremediation

Biological Reductive
Dechlorination

01

In Situ Alcoholysis

Transesterification of Vegetable
Oils

03



02

Emulsified Vegetable Oils

History and Advancements

04

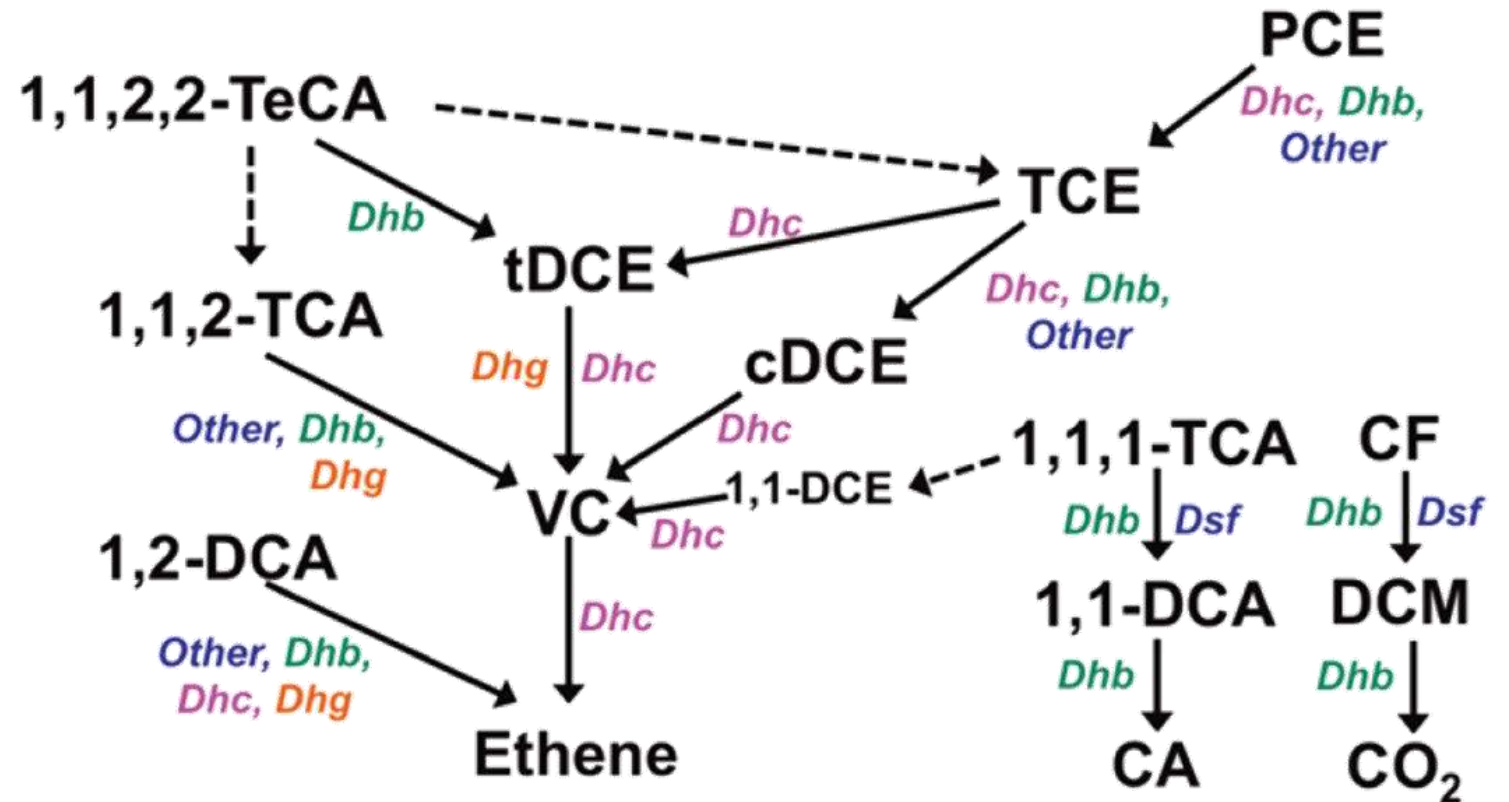
Heat Enhanced Reductive Bioremediation

Benefits of this approach

What is needed for enhanced reductive dechlorination?

Vegetable oils ferment to acetic acid and hydrogen

Overview chlorinated solvent dechlorination pathways and organisms responsible



Dhc = *Dehalococcoides*

Dhg = *Dehalogenimonas*

Dhb = *Dehalobacter*

Other = e.g., *Desulfitobacterium (Dsf)*, *Sulfurospirillum*, *Geobacter*

----- Dashed lines are abiotic reactions

Wei K., Grostern A., Chan W.W.M., Richardson R.E., Edwards E.A. (2016) Electron Acceptor Interactions Between Organohalide-Respiring Bacteria: Cross-Feeding, Competition, and Inhibition. In: Adrian L., Löffler F. (eds) Organohalide-Respiring Bacteria. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-662-49875-0_13

EDS-ER™

Electron Donor Solution – Extended Release

Water soluble vegetable oil



Impact of Fixed Nitrogen Availability on *Dehalococcoides mccartyi* Reductive Dechlorination Activity

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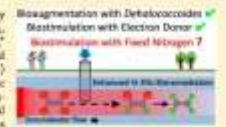
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* Supporting Information

ABSTRACT: Biostimulation to promote reductive dechlorination is widely practiced, but the value of adding an exogenous nitrogen (N) source (e.g., NH₄⁺) during treatment is unclear. This study investigates the effect of NH₄⁺ availability on organohalide-oxidizing *Dehalococcoides* (Dhc) growth and reductive dechlorination in enrichment cultures derived from groundwater (PW4) and river sediment (TC) spiked with chlorinated ethenes. In PW4 cultures, the addition of NH₄⁺ increased cis-1,2-dichloroethane (cDCE)-to-ethene dechlorination rates about 5-fold (20.6 ± 1.6 versus 3.8 ± 0.5 μM CT⁻¹ d⁻¹), and the total number of Dhc 16S rRNA gene copies were about 43-fold higher in incubations with NH₄⁺ ((1.8 ± 0.8) × 10⁹ and 10⁸ copies mL⁻¹) compared to incubations without NH₄⁺ ((4.1 ± 0.8) × 10⁷ copies mL⁻¹). In TC cultures, NH₄⁺ also stimulated cDCE-to-ethene dechlorination and Dhc growth. Quantitative polymerase chain reaction (qPCR) revealed that Coriell-type Dhc capable of N₂ fixation dominated PW4 cultures without NH₄⁺, but their relative abundance decreased in cultures with NH₄⁺ amendment (i.e., 98 versus 54% of total Dhc). Parallel-type Dhc incapable of N₂ fixation were responsible for cDCE dechlorination in TC cultures, and diazotrophic community members met their fixed N requirement in the medium without NH₄⁺. Responses to NH₄⁺ were apparent at the community level, and N₂-fixing bacterial populations increased in incubations without NH₄⁺. Quantitative measurement of Dhc nitrogenase genes, transcripts, and proteomic data linked Coriell-type Dhc nifH and nifK expression with fixed N fixation. NH₄⁺ addition also demonstrated positive effects on Dhc *in situ* dechlorination activity in the vicinity of well PW4. These findings demonstrate that biostimulation with NH₄⁺ can enhance Dhc reductive dechlorination rates; however, a “do nothing” approach that relies on indigenous diazotrophs can achieve similar dechlorination end points and avoids the potential for useful dechlorination due to inhibitory levels of NH₄⁺ or transformation products (i.e., nitrous oxide).



INTRODUCTION
Groundwater aquifers are often oligotrophic and cannot sustain high-rate reductive dechlorination desirable at sites contaminated with chlorinated solvents.^{1–7} Enhanced anaerobic bioremediation at sites impinged with chlorinated ethenes relies on biostimulation with fermentable substrates to increase hydrogen flux.^{8–12} Hydrogen is the key electron donor for organohalide-oxidizing *Dehalococcoides* (Dhc) strains capable of dechlorination to environmentally benign ethene.¹³ *In situ* growth of Dhc in response to biostimulation with fermentable substrates has been documented,^{14–17} however, a decline in dechlorination rates and incomplete reductive dechlorination at sites that receive sufficient electron donor is a common challenge to meet remedial goals.^{18,19} While hydrogen and chlorinated ethenes meet Dhc's energy require-

ment and acetate generated in fermentation reactions serves as a carbon source, fixed nitrogen (N) availability may limit Dhc growth and reductive dechlorination activity.¹⁴ Unavailable nitrogen (N₂) must be reduced to ammonium (NH₄⁺) to serve anabolic purposes; however, N₂ fixation is an energetically expensive process (16 ATP consumed per N₂ molecule reduced to NH₄⁺) and only occurs when NH₄⁺ is limiting.²⁰ The nitrogenase enzyme complex Nif, encoded by nifH, nifD, and nifK (nif operon), catalyzes the reduction of N₂ to NH₄⁺.²¹ The nifH gene has been used as a biomarker for

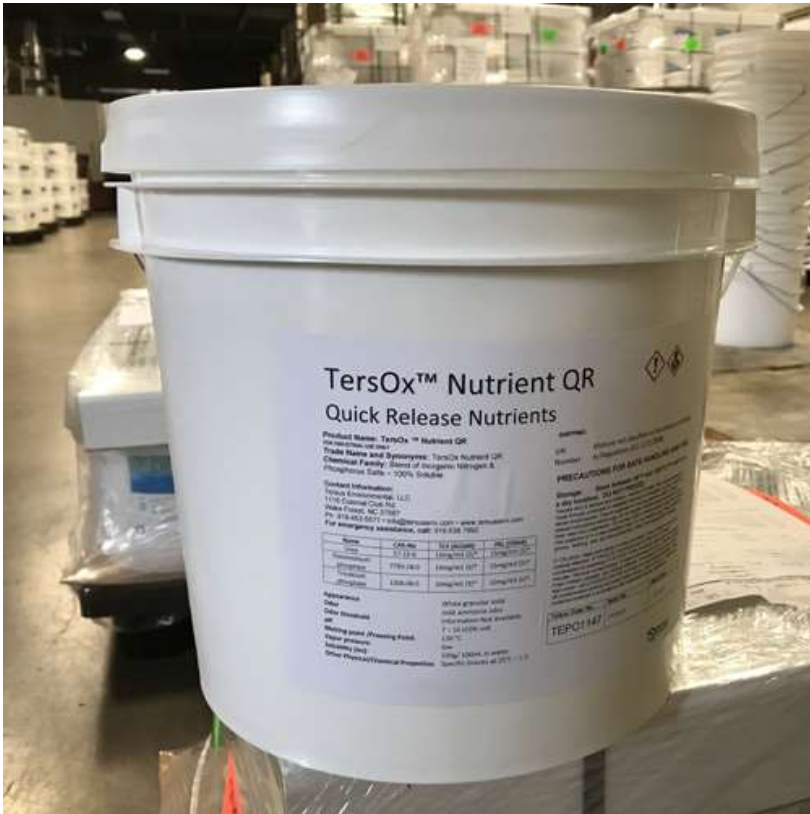
Nutrients

- Biostimulation benefits from adding an exogenous nitrogen (N) source (e.g., NH₄⁺)
- Addition of NH₄⁺ increased cis-1,2-dichloroethene (cDCE)-to-ethene dichlorination rates about 5-fold
- Typical target dosing:
 - 20:1 BOD to NH₃ – N ratio
 - 100:1 BOD to PO₄ – P ratio

Environ. Sci. Technol. 2019, 53, 24, 14548–14558



TersOx™ Nutrients-QR



- Fast-acting soluble nutrient blend for bioremediation
- Blend of nitrogen, phosphorous and microbial growth enhancers that provide a source of urea, phosphate and potassium

Vitamin B₁₂

- *Dehalococcoides mccartyi* strains require vitamin B₁₂ (Yan et al, 2013)
- Reported concentration for optimal dechlorination and growth: 25 to 50 µg/L (Stroo et al., 2013)

Stroo et al., 2013, Bioaugmentation for Groundwater Remediation, edited by Stroo, H.F., Leeson, A., Ward, C.H. HydroGeoLogic, Inc., Ashland, OR, USA

Yan et al, 2013, Yan J, Im J, Yang Y, Löffler FE. 2013 Guided cobalamin biosynthesis supports *Dehalococcoides mccartyi* reductive dechlorination activity. Phil Trans R Soc B 368: 20120320. <http://dx.doi.org/10.1098/rstb.2012.0320>



Distribution of the Correct Type of Fatty Acids is Essential

Acetate

- Slow consumption
- Will migrate downgradient
- Stimulates PCE -> TCE -> cDCE
- Will not stimulate cDCE -> VC -> ethene

Hydrogen (H₂)

Produced from linolenic acid, propionate, butyrate, etc.

- Rapid consumption
- Does not migrate beyond injection zone
- Required for cDCE -> VC -> ethene



US 11,577,231 B2

(12) United States Patent
Birk et al.

(10) Patent No.: US 11,577,231 B2
(45) Date of Patent: Feb. 14, 2023

(51) ENHANCED REDUCTIVE
BIOREMEDIATION METHOD USING
IN-SITU ALCOHOLYSIS

OTHER PUBLICATIONS:

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Forest, NC (US)

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WO 20107849 * 6/2011

(72) Inventor: Gary M. Birk, Wake Forest, NC (US);
David H. Allen, Spartanburg, SC (US)

(73) Assignor: Tersus Environmental LLC

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(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 30 days

(21) Appl. No.: 16/797,617

(22) Filed: Feb. 21, 2020

(45) Prior Publication Data

US 2021/020166 A1 Aug. 26, 2021

(51) Int. Cl.

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- B01F 15/00 (2006.01)
- B01F 21/00 (2006.01)
- B01F 25/00 (2006.01)
- B01F 25/04 (2006.01)
- B01C 1/00 (2006.01)
- C02F 1/22 (2021.01)
- C02F 1/28 (2021.01)
- C02F 1/34 (2021.01)
- C02F 1/36 (2006.01)

(52) U.S. Cl.

- CPC: B01C 1/00 (2013.01), B01F 15/00
(2013.01), B01F 25/00 (2013.01), B01C
1/00 (2013.01), B01C 1/02 (2013.01), B01C
1/08 (2013.01), C02F 1/22 (2013.01), C02F
1/28 (2013.01), C02F 1/34 (2013.01),
B01C 1/00 (2013.01), C02F 1/22 (2013.01),
C02F 1/28 (2013.01), C02F 1/34 (2013.01)

(58) Field of Classification Search

See application file for complete search history.

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7,800,000 B2 *	1/09	Doddy	1,010,224
7,800,000 B2 *	1/09	Doddy	1,010,224
7,800,000 B2 *	1/09	Doddy	1,010,224
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29 Claims, 9 Drawing Sheets

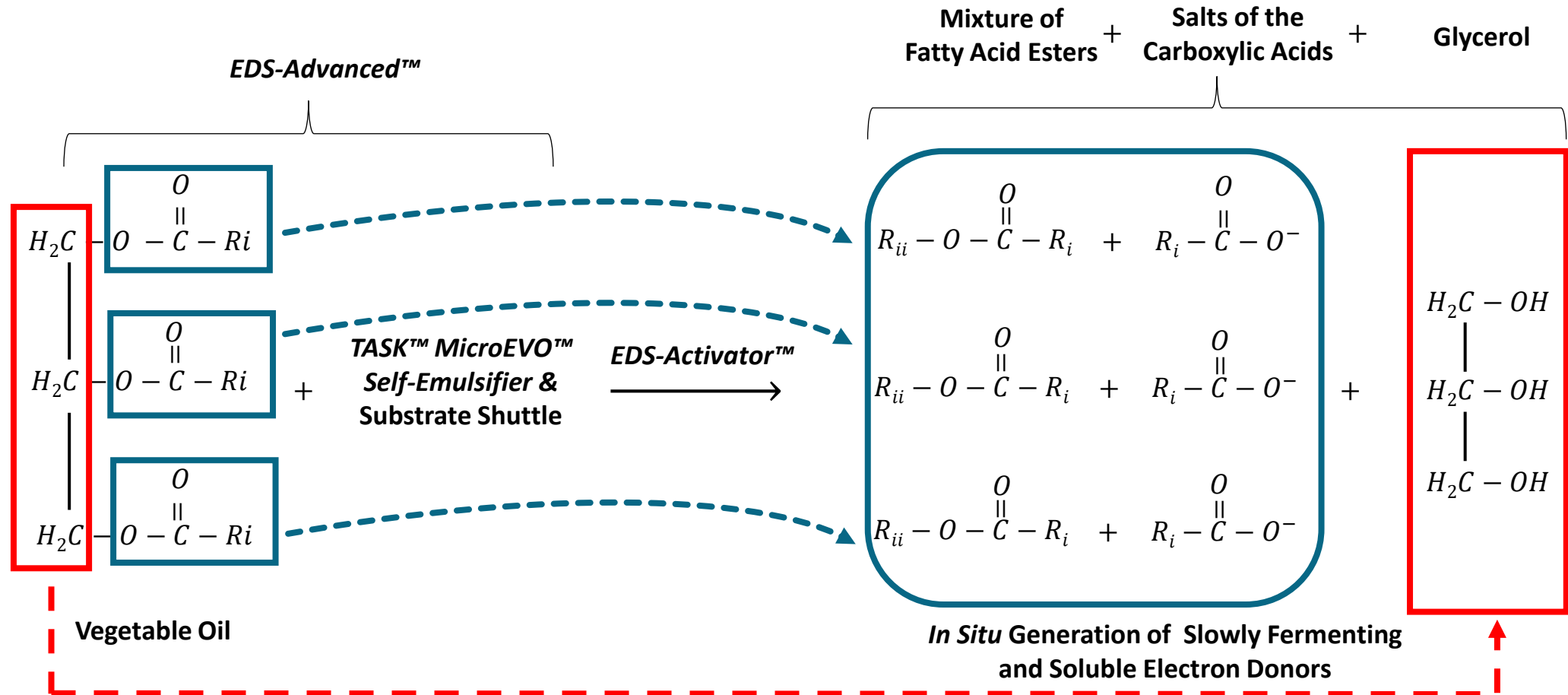
Deploying Electron Donor via In Situ Alcoholysis



Definitions

- **Alcoholysis:** A reaction in which an alcohol is a reactant and becomes part of the reaction product.
- **Transesterification:** The chemical conversion process of triglycerides with alcohol into fatty acid esters with the help of a catalyst.

In Situ Transesterification of Vegetable Oils



Activator Options

Hydroxide base-catalyzed transesterification of triglycerides

- Homogeneous Alkaline Catalyst
 - Alkyl oxides (RO⁻)

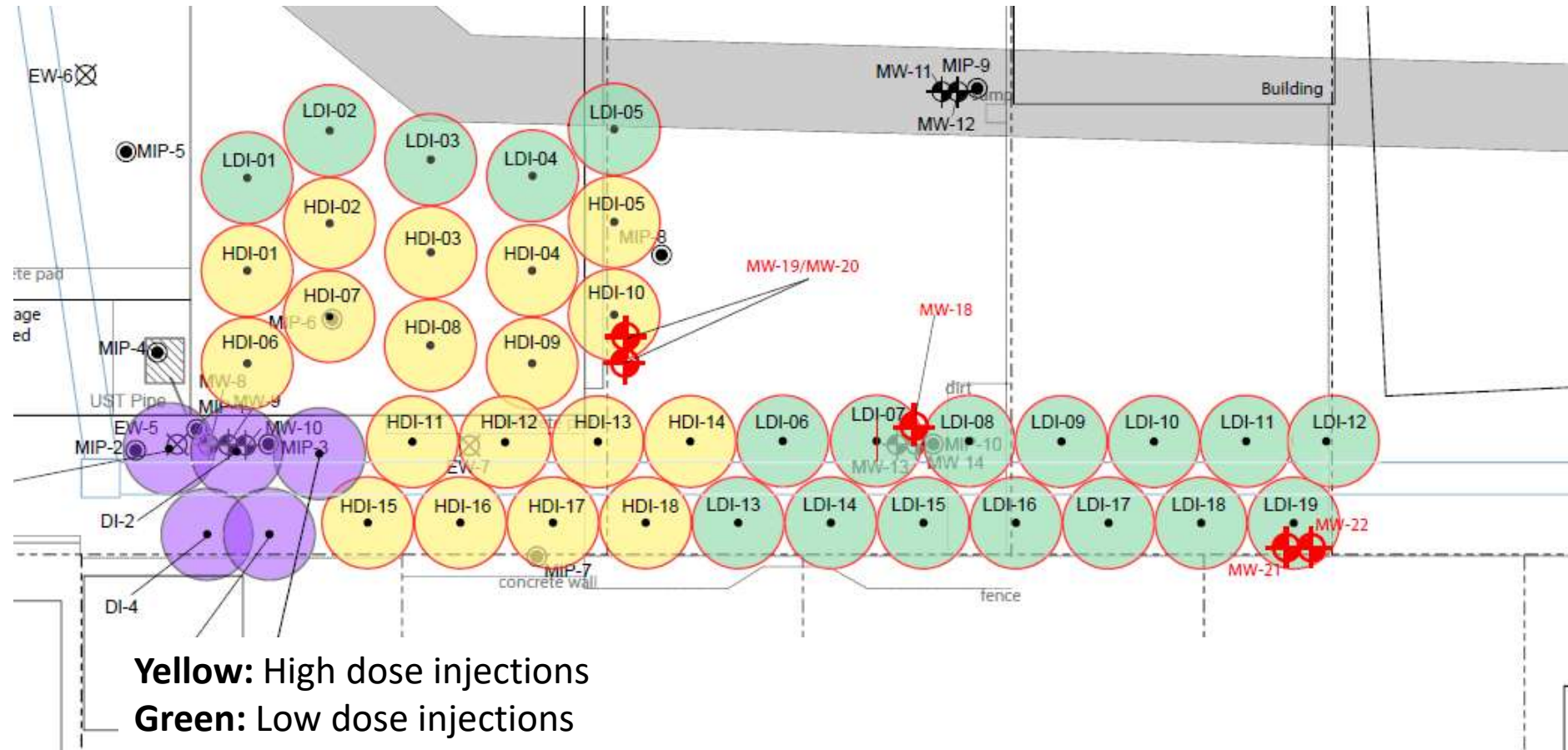
Lipase-catalyzed hydrolysis of triglyceride

- Biocatalyst
 - Enzyme (triglyceride lipases)

Typical Application Rates

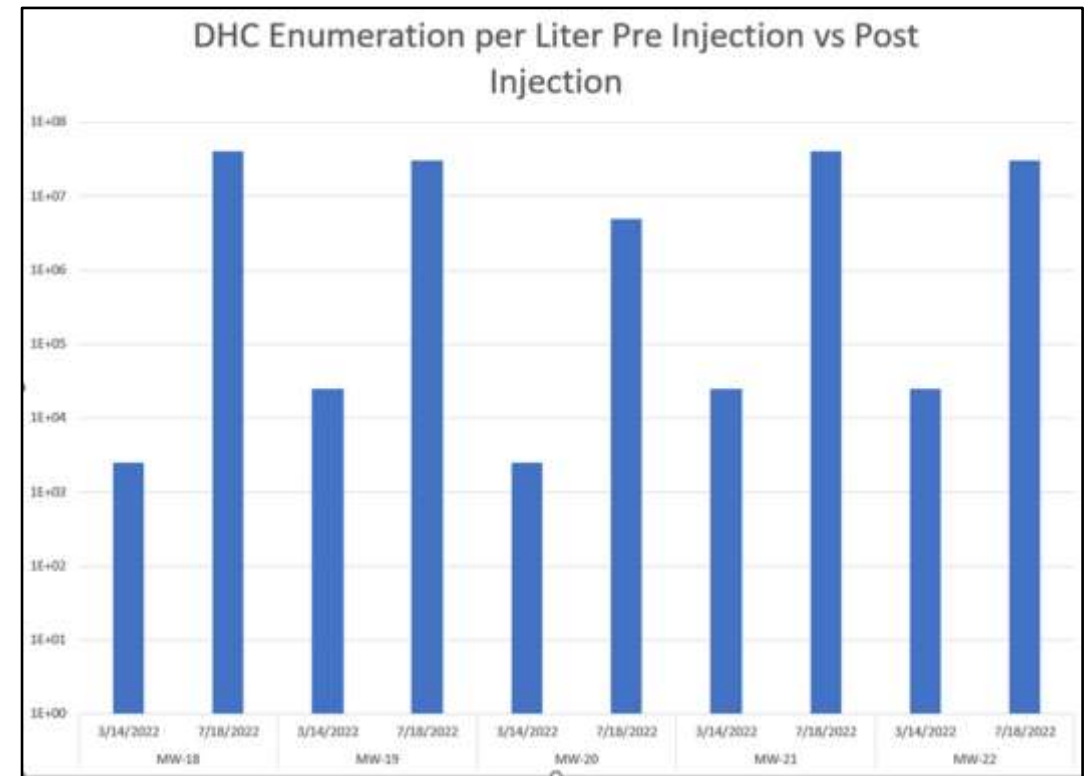
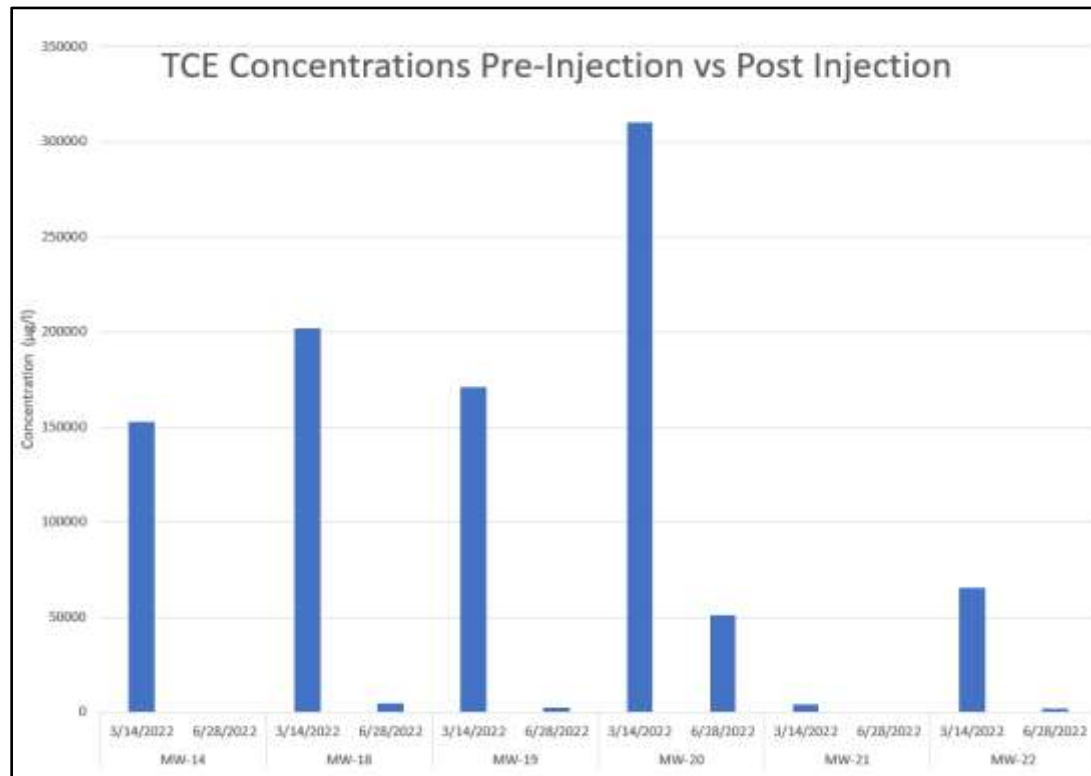
EDS-ER™ (Soybean Oil and TASK™ MicroEVO™ Self-Emulsifier	2 to 8 g/L
EDS-Activator™	16 to 20% of EDS-ER™ Dose
EDS Substrate Shuttle (Co-Solvent)	0.4 g/L
Microscale Zero-Valent Iron (mZVI)	4 to 6 g/L

TCE Site



Yellow: High dose injections
Green: Low dose injections
Purple: Low dose injections excluding KB-1®

Injection Results



EDS-Advanced™

Unrestricted Electron Donor Subsurface Distribution for Anaerobic Bioremediation

- Improved subsurface distribution of a vegetable oil-based electron donor
- Improved ROI, fatty acid distribution and TOC when compared to EVO
- Eliminates dependence on EVO droplet size
- Aids in reducing cVOC inhibitory concentrations by sequestering DNAPL
- High alcohol content and high solubility reduces injection well biofouling risk

Enhancement Options

Apply Heat

- Enhances transesterification reaction
- Reduces time from days to months to minutes to hours
- 6 minutes
 - ✓ 90°C – 94% yield
 - ✓ 32°C – 64% yield

Optimum Growth Temperature

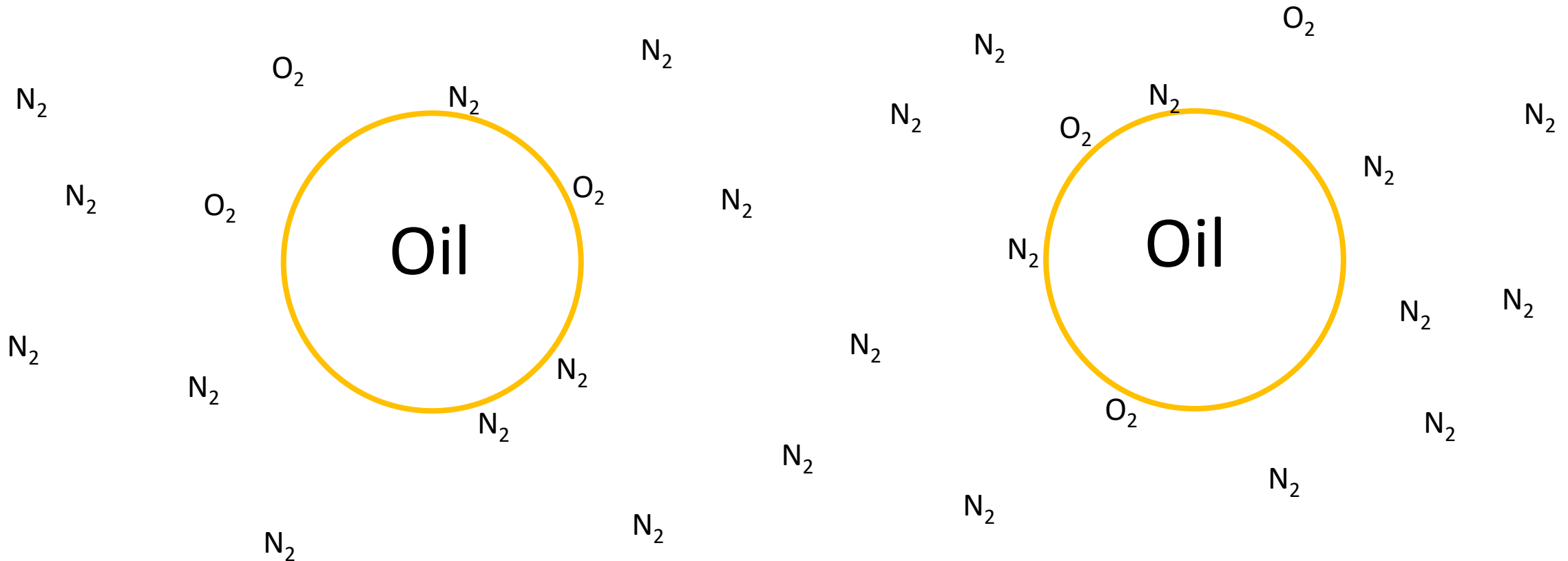
- 25-30°C hydrogenotrophic Dhc strains (Löffler et al., 2013)
- <40–45°C biotic or abiotic destruction
- > 50°C very little biotic or abiotic destruction (Stroo et al., 2013; Costanza et al., 2009)

Hot water vs cold water

- Hot water dissolves fewer gases (e.g., oxygen or carbon dioxide)
- Hot water dissolves more solids (e.g., sugars)

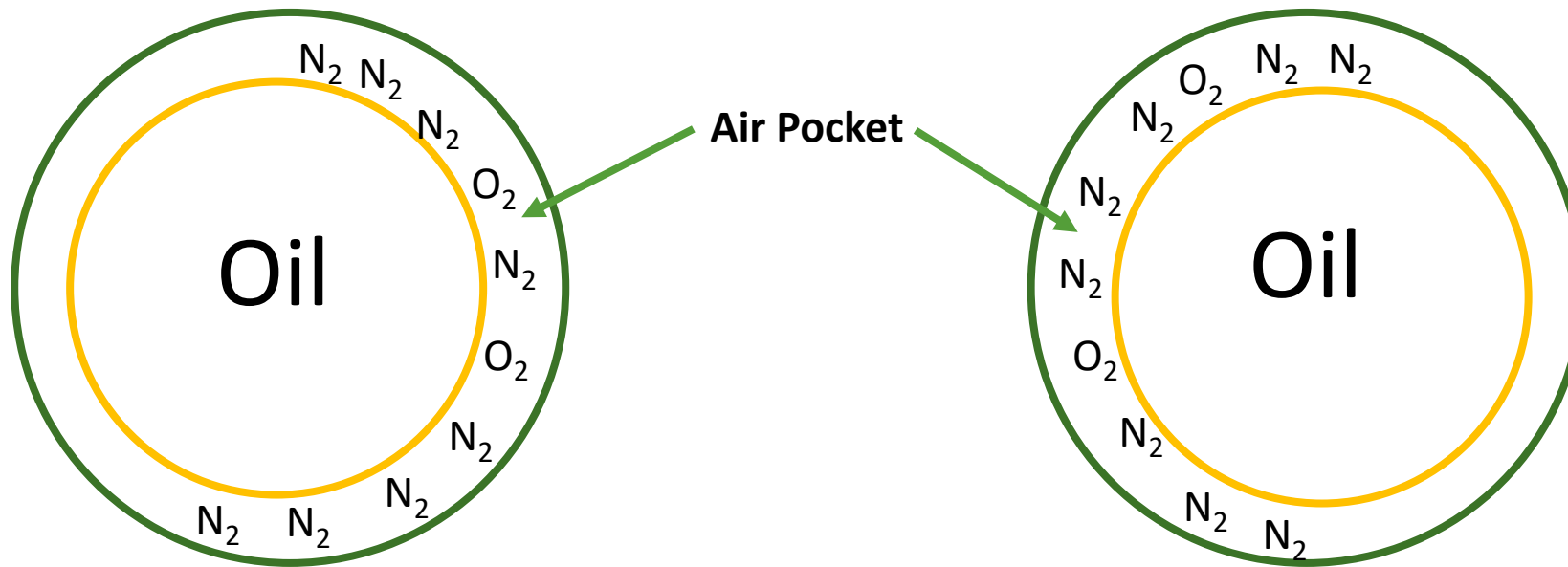
Example of dissolved gas in water with oil

Water



The dissolved gas adsorbs to the surface of the oil

Water



Surface tension pushes oil droplets together to form one big droplet

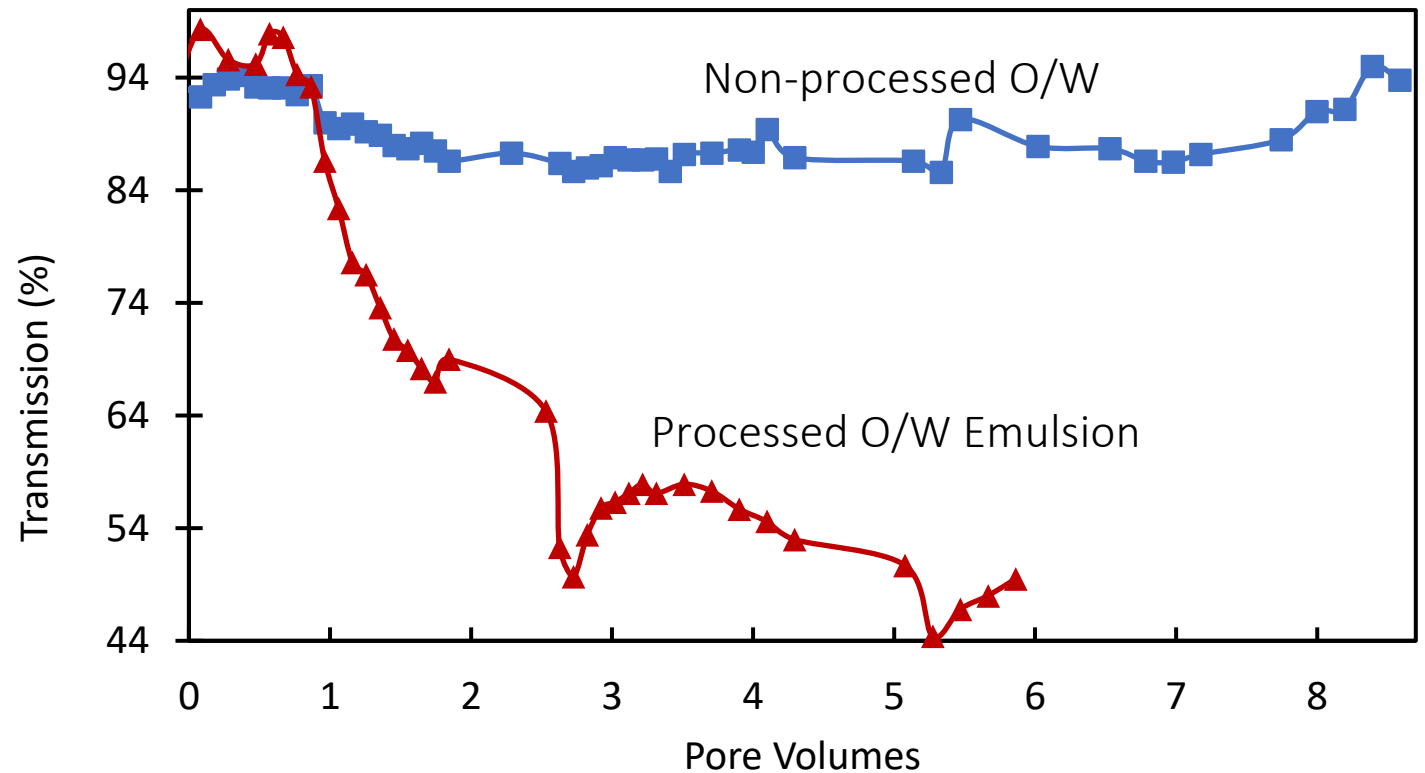
Water



Processed Emulsions Remain Stable in Flow Through Porous Media



Photo of processed O/W emulsion after 1 hour



Heating Options

Conventional

- Residual heat from an *in situ* thermal remediation project
- Electrical resistance heating
- Thermal conduction heating

Heat amendments / water and inject

- Hot water boiler
- Shell and tube heating tank or a batch heating tank with coils
- Solar collector, thermal storage tank with a submerged heat exchanger and an auxiliary heat exchanger

Hot Water Injection

Hydrogeological parameters

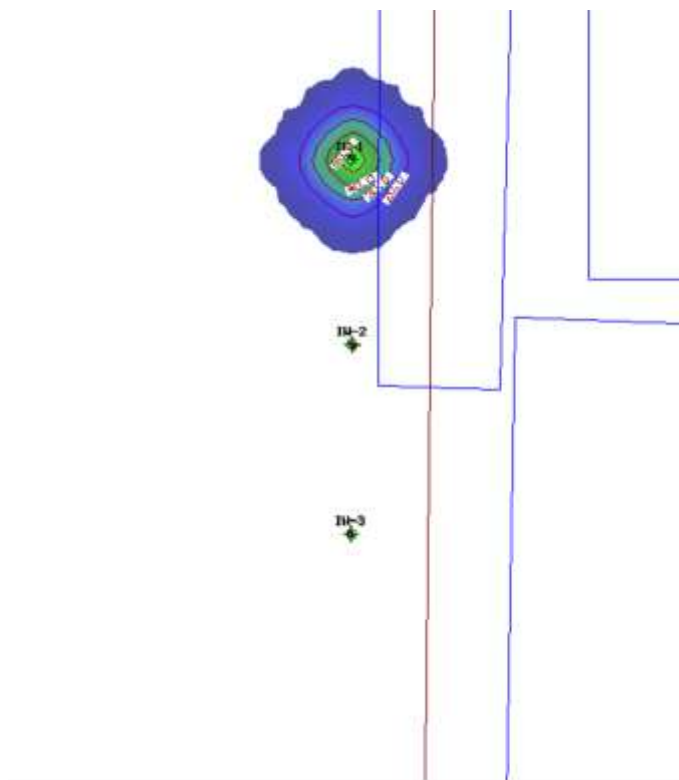
- Site lithology: sand
- Porosity: 0.33
- Aquifer hydraulic conductivity K of 1×10^{-2} cm/s
- Hydraulic gradient: 0.002 feet/feet

Injection

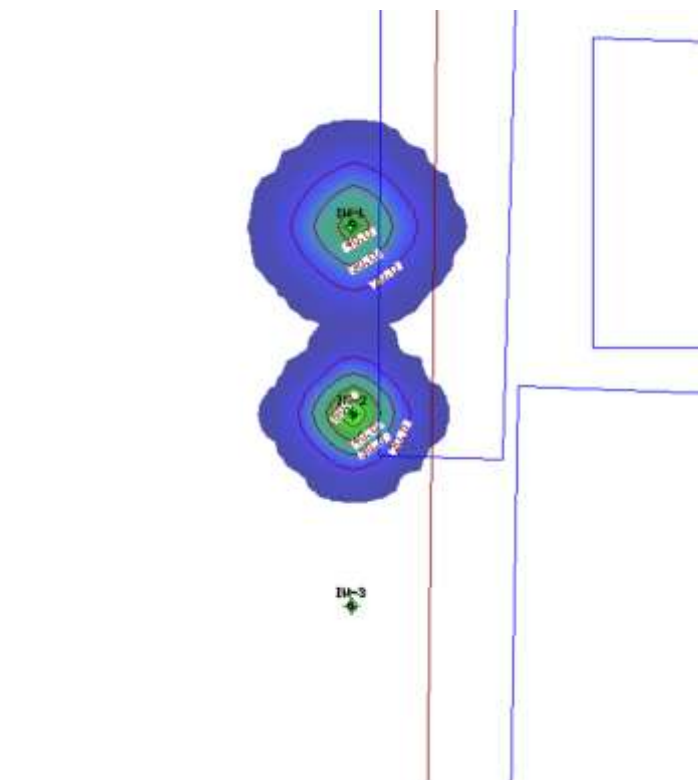
- 12-hour injection event
- 75 m³ (19,813 gallons) of water heated to 90°C
- 150 m³/d (27.5 gpm) flow rate

Model Results

Time = 0.5 days

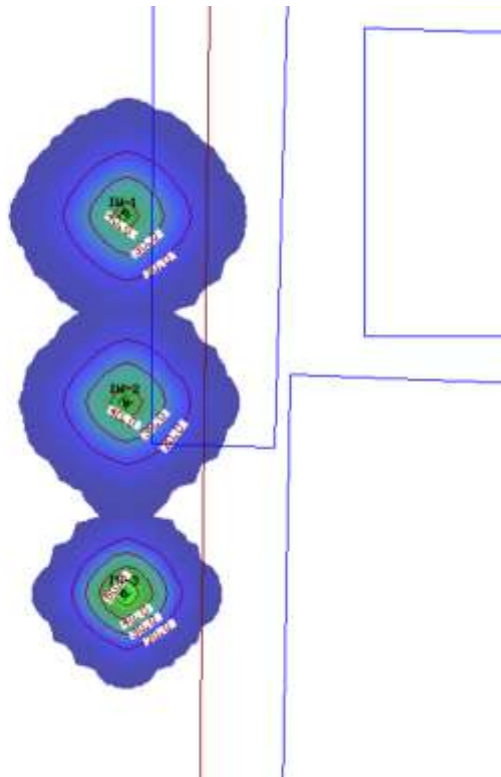


Time = 1 day

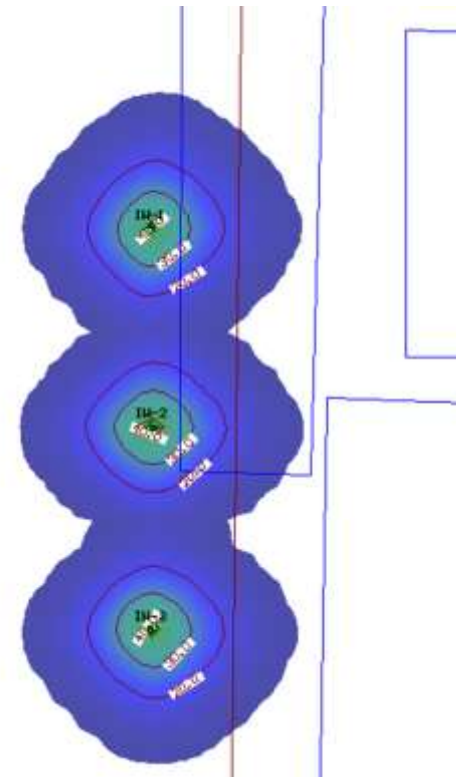


Model Results (continued)

Time = 1.5 days

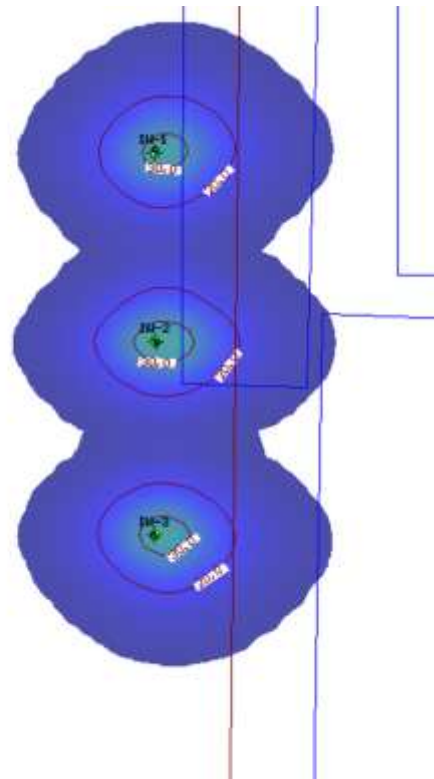


Time = 5 days

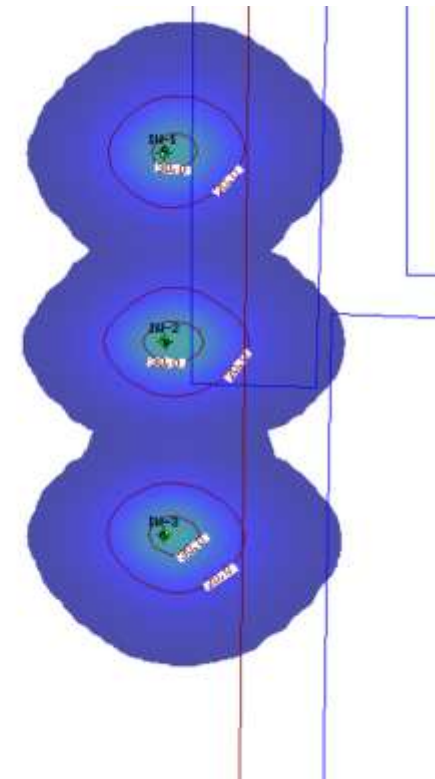


Model Results (continued)

Time = 10 days

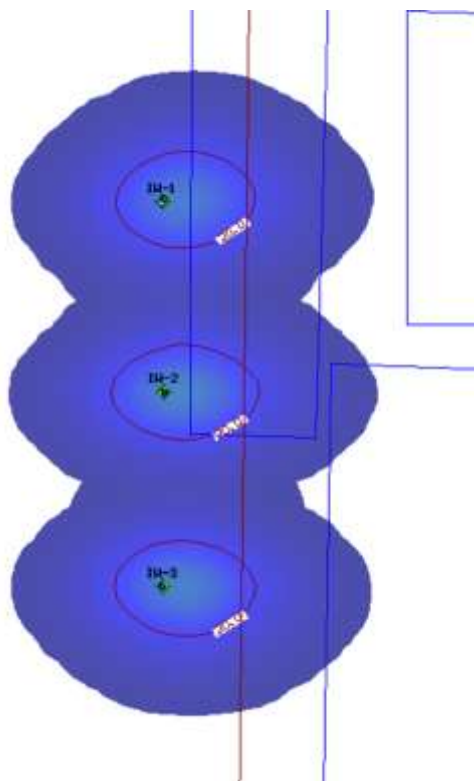


Time = 30 days

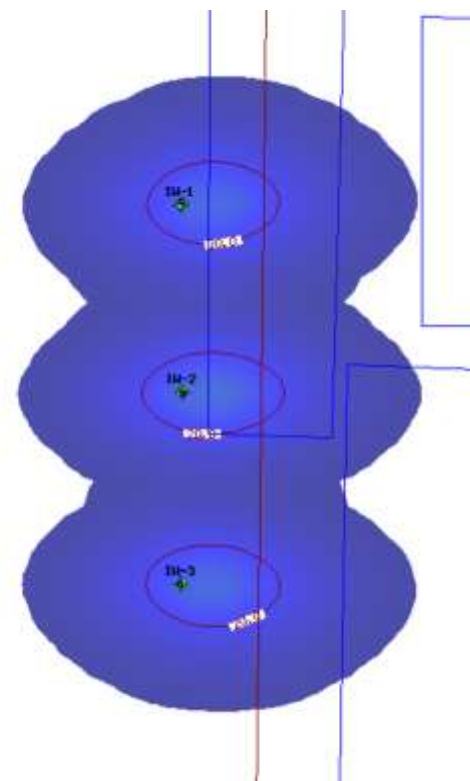


Model Results (continued)

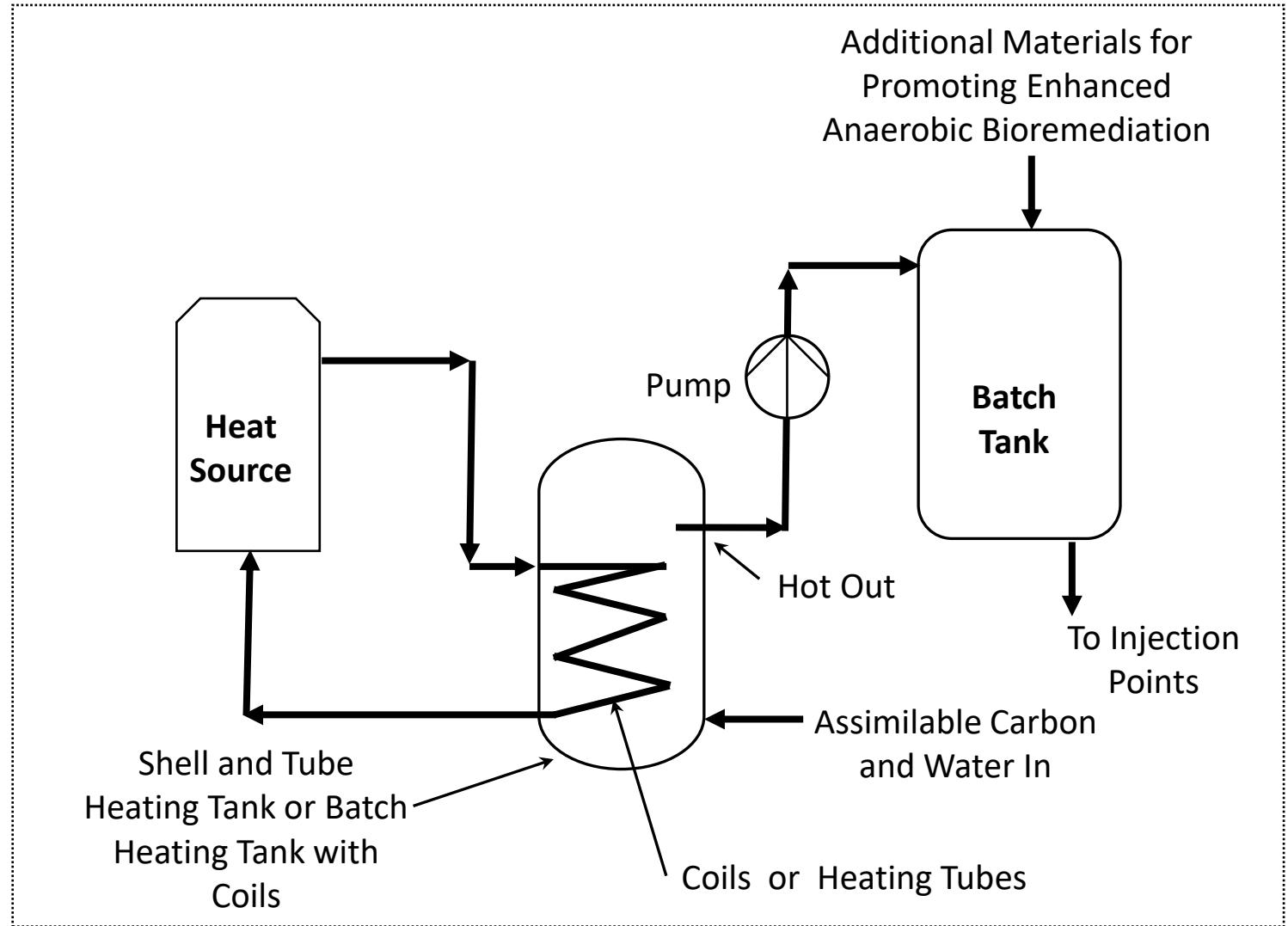
Time = 60 days



Time = 90 days



Shell and Tube Heating Tank or Batch Heating Tank with Coils



Heat Enhanced Reductive Bioremediation

- Microbes that do all the work like a warm environment
- Warm water has lower dissolved gases
- Heating increases the alcoholysis reaction rates

Thank you

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