

Catalyzed Enhanced Reductive Bioremediation

Wednesday, May 3, 2023

12:00 PM - 1:00 PM EDT

tersusenv.com • surbec.com



Agenda

Bioremediation
Biological Reductive Dechlorination

01

Catalyzed Enhanced
Reductive Bioremediation

03

Transesterification of Vegetable Oils



02

Emulsified Vegetable Oils
History and Advancements

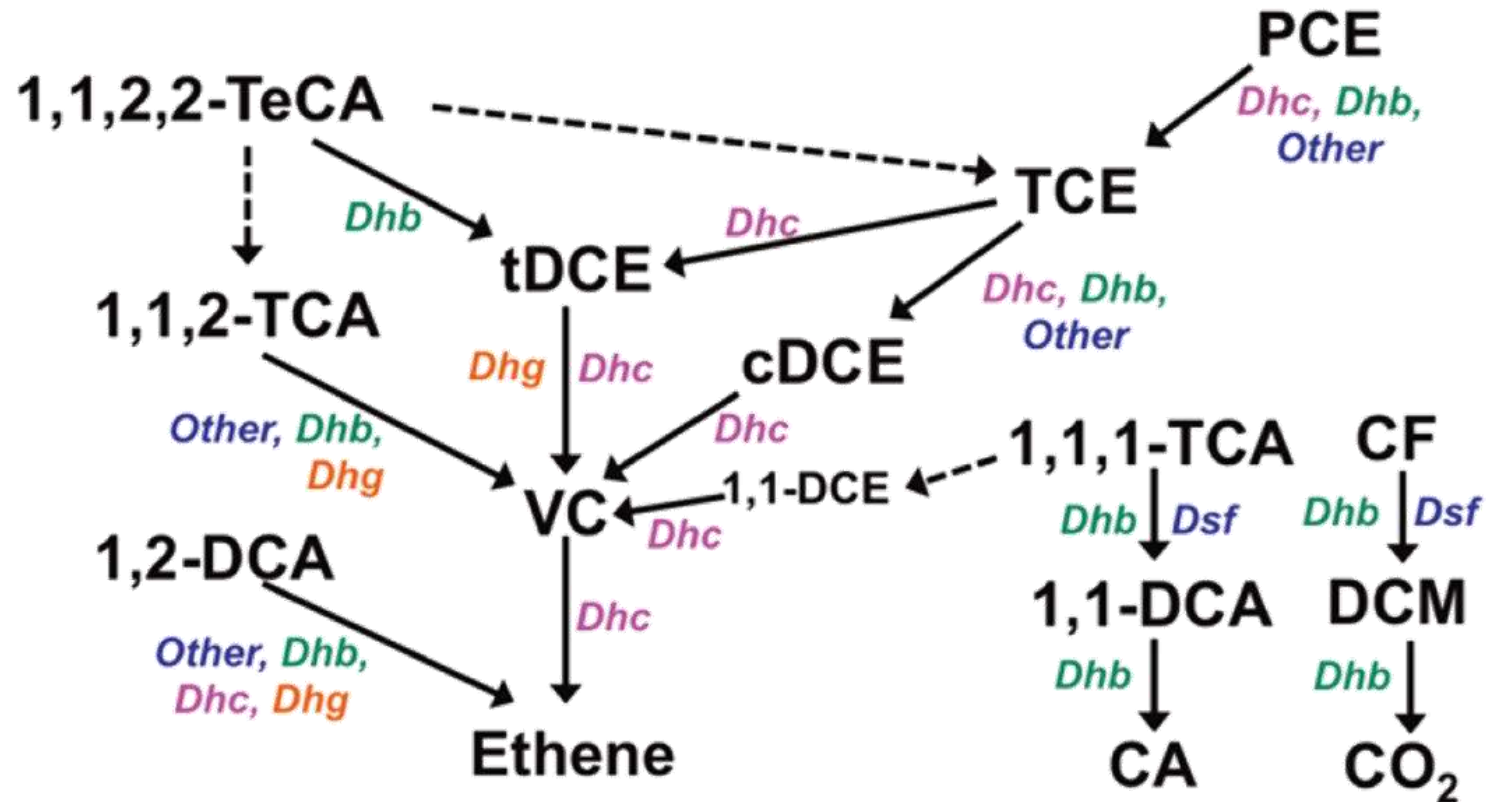
04

Enhancement Options
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

Dorin Kaya,^{1,2,3,4*} Britte V. Kjellerup,⁵ Karuna Chourry,^{1,2,3} Robert L. Hettich,⁶ Dora M. Taggart,⁷ and Frank E. Löffler^{1,2,3,4,6*}

¹Center for Environmental Biotechnology, ²Department of Microbiology, ³Department of Civil and Environmental Engineering, and ⁴Department of Bioreactors Engineering & Soil Science, University of Tennessee, 656 Dabney Hall, 1416 Circle Drive, Knoxville, Tennessee 37996, United States

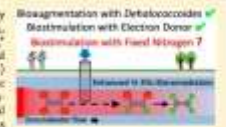
⁵BioScience Division and ⁶Chemical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, United States

⁷Department of Civil and Environmental Engineering, University of Maryland College Park, College Park, Maryland 20742, United States

⁸Microbial Insights, Inc., Knoxville, Tennessee 37932, United States

* 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) compared to incubations without NH₄⁺ ((4.1 ± 0.8) × 10⁷ copies). 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 avoid 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.^{1–3} Enhanced anaerobic bioremediation at sites impinged with chlorinated ethenes relies on biostimulation with fermentable substrates to increase hydrogen flux.^{4–6} Hydrogen is the key electron donor for organohalide-oxidizing *Dehalococcoides* (Dhc) strains capable of dechlorination to environmentally benign ethene.⁷ *In situ* growth of Dhc in response to biostimulation with fermentable substrates has been documented,^{8,9} 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.^{10,11} 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.¹² Unavailable nitrogen (N₂) must be reduced to ammonium (NH₄⁺) to serve as a biogenic nitrogen source; N₂ fixation is an energetically expensive process (16 ATP consumed per N₂ molecule reduced to NH₄⁺) and only occurs when NH₄⁺ is limiting.¹³ The nitrogenase enzyme complex Nif, encoded by nifH, nifD, and nifK (nif operon), catalyzes the reduction of N₂ to NH₄⁺.¹⁴ 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 dechlorination 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>



Tote Photograph Courtesy of Legacy Remediation, Inc.

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

(54) ENHANCED REDUCED
BIODEGRADATION METHOD USING
IN-SITU ALCOHOLYSIS

TERPUS PATENT DOCUMENTS

(71) Applicant: Tersus Environmental LLC, Wake
Forest, NC (US)

US 107449 * 4/99
WO 20107849 * 6/2011

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

(73) Assignor: Tersus Environmental LLC

OTHER PUBLICATIONS

(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 300 days.

Office of Science "DOE Lecture" Catalysis 2012 (Year
2012)*
Sauer et al., Chemistry for Environmental Engineering, McGraw-
Hill Inc., 1994 (Chapter 4, pp. 119-121, Chapter 5, pp. 112-114, and
Chapter 6, pp. 107-109)
Hernandez et al., Isolation of a Secreted Free Redoxively
Dissimilating Tetrahydrofuran to Ethanol, Science (1997) 279: 1508-
1511
Mee et al., Biobased Production of Bioethanol, Industrial, 79
(1999) pp. 1-15
Wu et al., Electron Acceptor Limitations Between Hydrophobic-
Soluble and/or Insoluble, Composites, and Inhibitors,
Department of Chemical Engineering and Applied Chemistry, Uni-
versity of Toronto, Toronto, ON, Canada, 2016
Tand et al., Overview on the Current Trends in Synthetic Pro-
duction, Large Volume, Storage, 52 (2001) pp. 2741-2744
MCP, Technical Report: White Paper, A Review of Redoxing
Conditions for Treatment of Soil Microbiology of (petroleum), 12,
2003, pp. 1-63
Coville et al., Effect of Aqueous Conditions on the Apparent Solu-
bility of Hydrophobicity, Proceedings of the 2003 Conference on
Environmental Biotechnology, pp. 32-46
Johal et al., American Journal of Energy Science (AJES), 3 (2014),
pp. 49-53
Dittmann et al., 2017 AIEE Conference Proceedings (AIEE), 92(01),
1207, https://doi.org/10.1109/1.5200824, pp. 1-6
Non-ferrous Recovery Technology Certification Program, Train-
ing for Enhanced In-Situ Bioremediation Using Insoluble Cells
Oil, Arlington, VA, May 2006, pp. 1-69
European Search Report EP 21 151549 A1, Jul. 13, 2021

(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.

- B99C 1/00 (2006.01)
- B99F 31/00 (2006.01)
- B99F 21/00 (2006.01)
- B99F 35/00 (2006.01)
- B99F 35/04 (2006.01)
- B99C 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.

- B99C 1/00 (2006.01), B99F 31/00
(2006.01), B99F 35/00 (2006.01), B99C
1/00 (2006.01), B99C 1/02 (2013.01), B99C
1/08 (2013.01), C02F 1/22 (2013.01), C02F
1/28 (2013.01), C02F 1/34 (2013.01),
B99C 21/00 (2013.01), C02F 21/01 (2013.01)

(58) Field of Classification Search

See application file for complete search history.

(50) References Cited

U.S. PATENT DOCUMENTS

7,657,964 A *	4/97	Delbec	1,111,130
7,800,000 B2 *	1/09	Osley	1,012,221
7,800,000 B2 *	1/09	Osley	1,011,117
7,800,000 B2 *	1/09	Osley	1,011,234
7,800,000 B2 *	1/09	Osley	1,011,254
7,800,000 B2 *	1/09	Osley	1,011,274
7,800,000 B2 *	1/09	Osley	1,011,294
7,800,000 B2 *	1/09	Osley	1,011,314
7,800,000 B2 *	1/09	Osley	1,011,334
7,800,000 B2 *	1/09	Osley	1,011,354
7,800,000 B2 *	1/09	Osley	1,011,374
7,800,000 B2 *	1/09	Osley	1,011,394

29 Claims, 9 Drawing Sheets

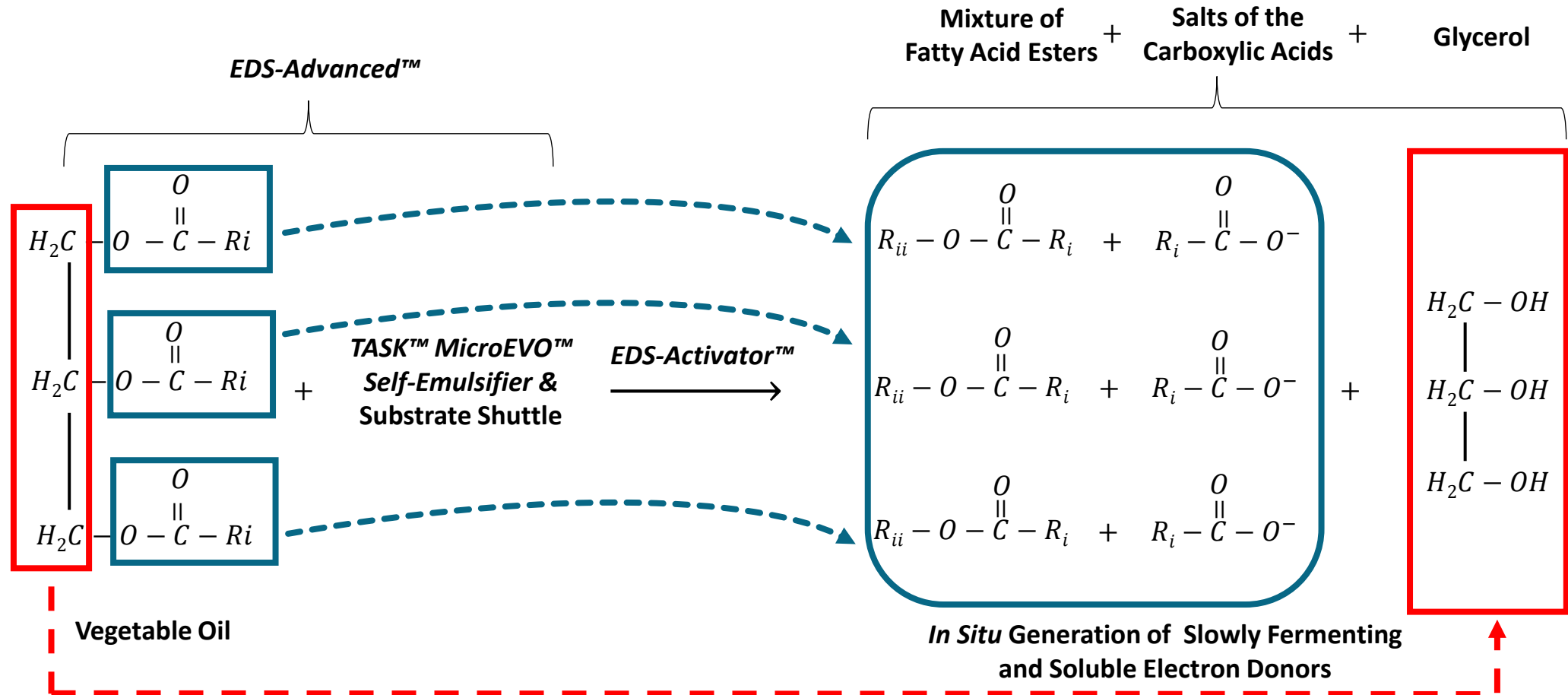
Deploying Electron Donor via In Situ Alcoholysis



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)

pH Plays a Key Role in VFA Production

Systems under alkaline conditions

- Enhances the activity of fatty acid-producing bacteria
- Inhibits methanogens
- Increases production of VFAs



Image by brgfx on Freepik,
www.freepik.com/free-vector/ph-scale-diagram_4453068.htm

Typical Application Rates

EDS-ER™ (Soybean Oil and TASK™ MicroEVO™ Self-Emulsifier)	6 to 8 g/L
EDS-Activator™	16 to 20% of EDS-ER™ Dose
EDS Substrate Shuttle (Co-Solvent)	0.4 g/L
EDS-ME™ (Alcohol Blend)	0 to 2.4 g/L*

*Based on average sum of chlorinated solvents across treatment zone

Example Projects



TCE Site

- 2,830 $\mu\text{g/L}$ TCE (highest conc.)
- Primawave[®] Pressure Pulse
- Injected 75,000L of EDS-Advanced[™] into the source zone over two weeks



Photos courtesy of Justin Kerr, Kerr Environmental, Greater Adelaide Area, SA, AUSTRALIA



Injection Setup

Four injection lines with individual flow and pressure control and monitoring



**Primawave® Pressure Pulse
for injection into clays**



Photos and video courtesy of Justin Kerr, Kerr Environmental, Greater Adelaide Area, SA, AUSTRALIA

TCE Results

(Approximately 95% mass reduction)

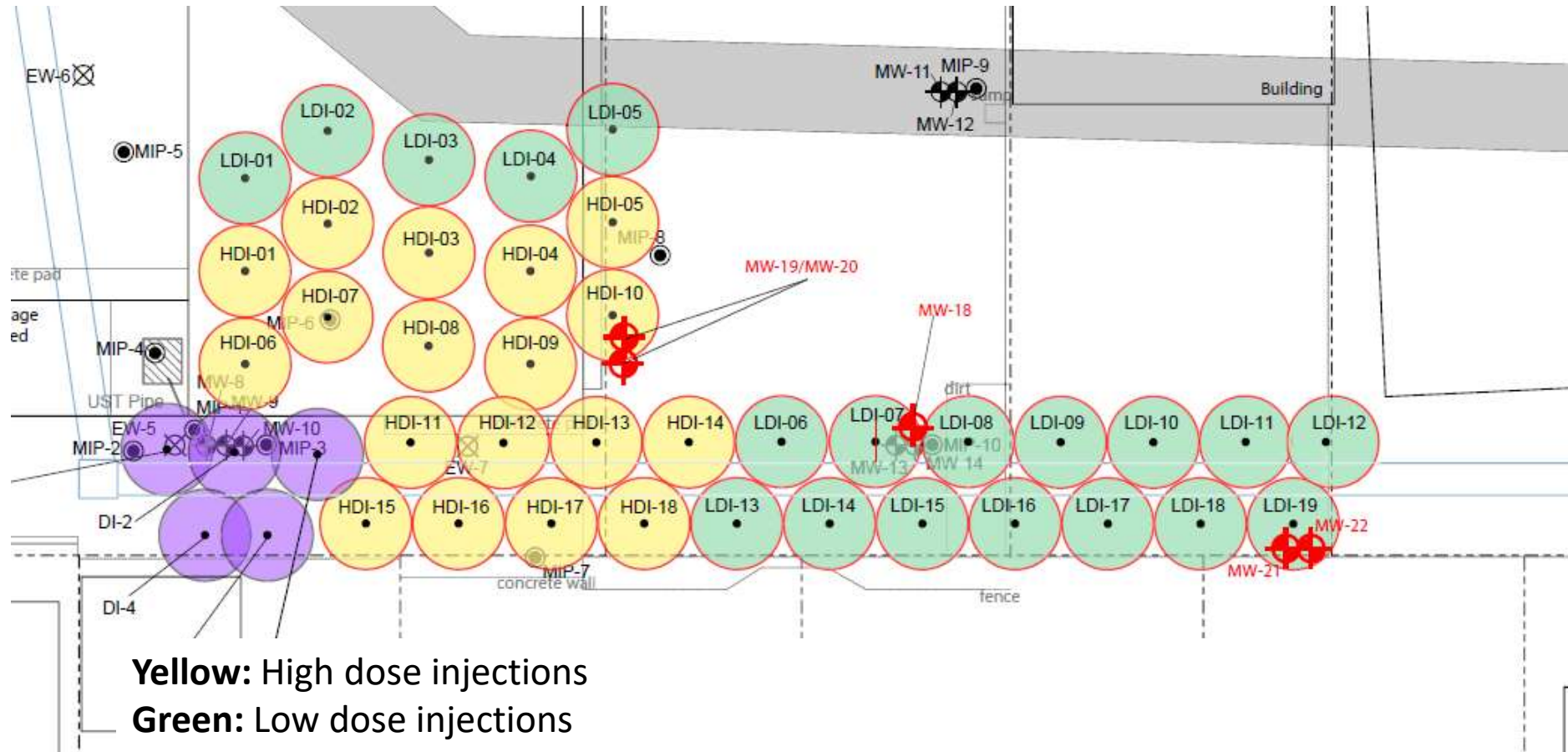


MWS_BSE_17_11
Baseline 2,830 µg/L
Week 13 33.1 µg/L

MWS_BSE_17_24
Baseline 1,380 µg/L
Week 13 55.8 µg/L

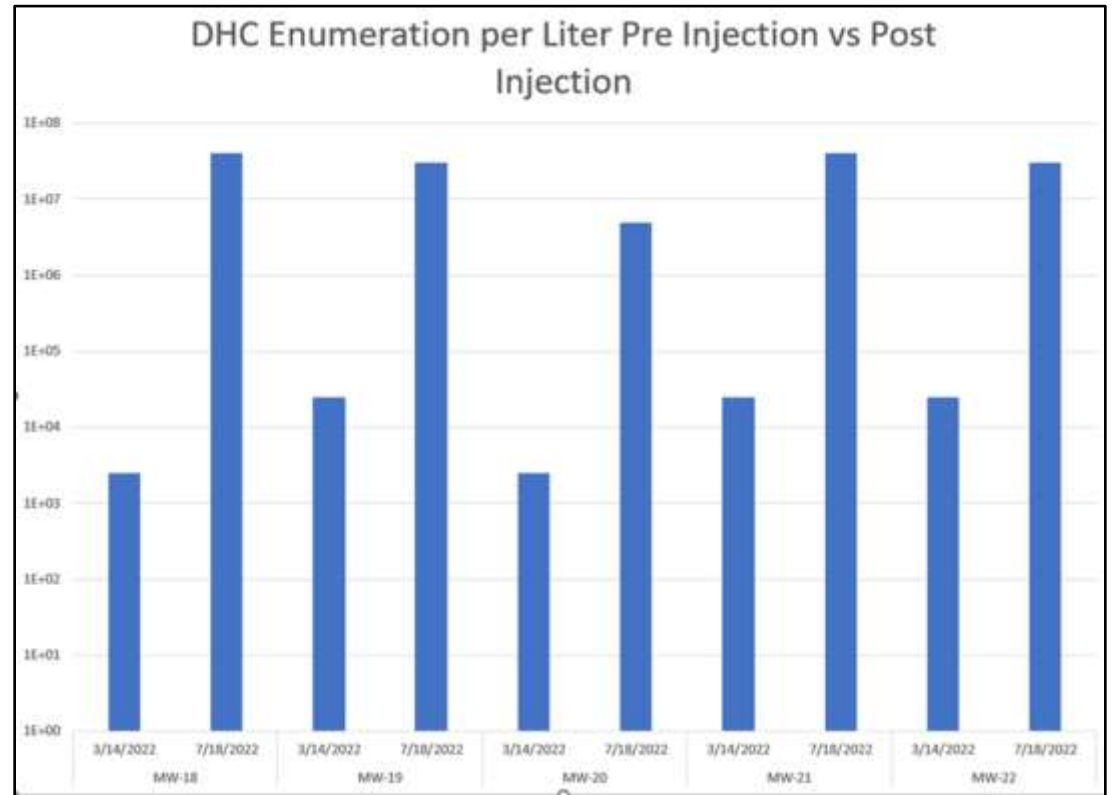
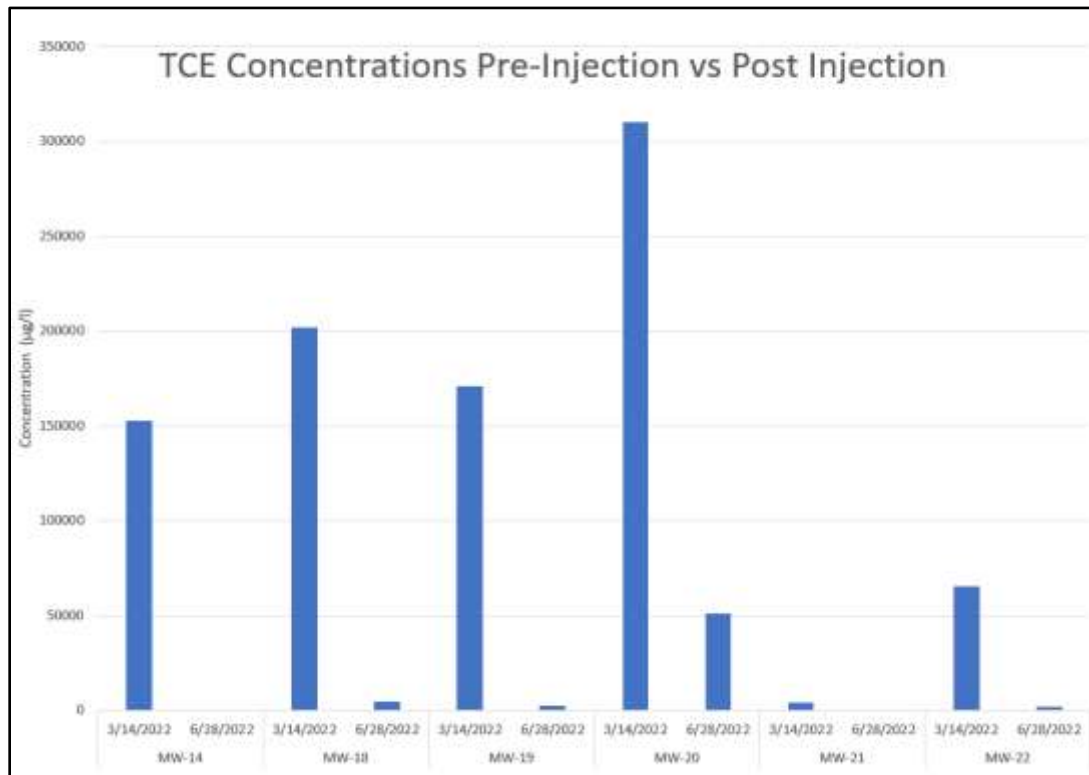
Data courtesy of Justin Kerr, Kerr Environmental, Greater Adelaide Area, SA, AUSTRALIA

TCE Site



Reference: Remediation Seminars Webinar, *How to Integrate Bench Scale Tests, Molecular Diagnostic Tools (MDT), and Compound-Specific Isotope Analysis (CSIA) to your Field Pilot Test*, Dec. 7, 2022

Injection Results



Reference: Remediation Seminars Webinar, *How to Integrate Bench Scale Tests, Molecular Diagnostic Tools (MDT), and Compound-Specific Isotope Analysis (CSIA) to you Field Pilot Test*, Dec. 7, 2022

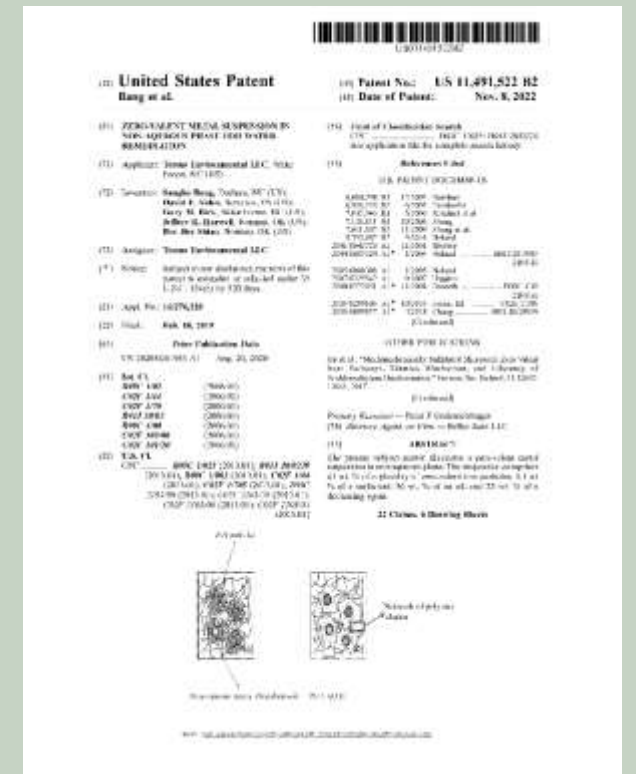
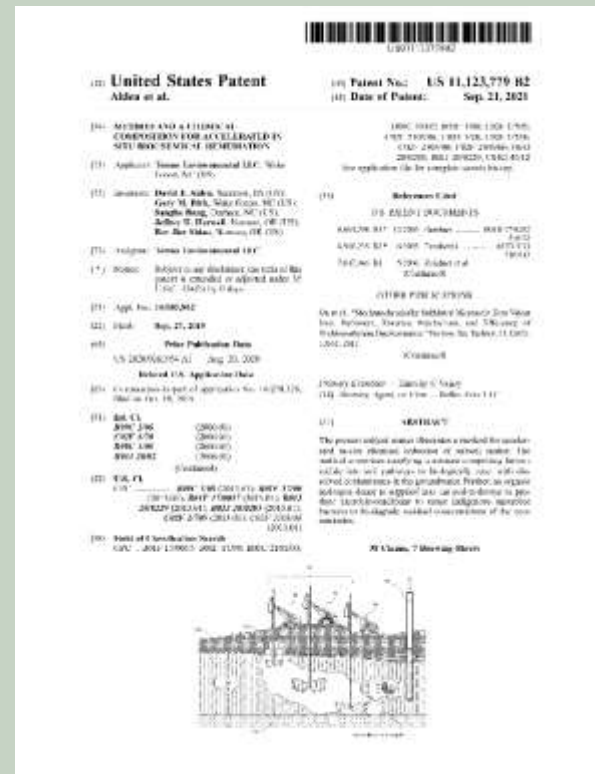
Advantages

Catalyzed Enhanced Reductive 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

Abiotic Enhancement Options

Zero-Valent Metal Particle Suspension Patents



ZVI in Water vs iron-Gel™



Iron Sulfide Reagent



Provides

- Benefits of sulfidated ZVI
- Higher contaminant removal efficacy
- Lower cost

Specifications

- Physical form: colloidal suspension
- Specific gravity: 1.15 - 1.22
- ORP: -700 to -1300 mV

Thermal 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

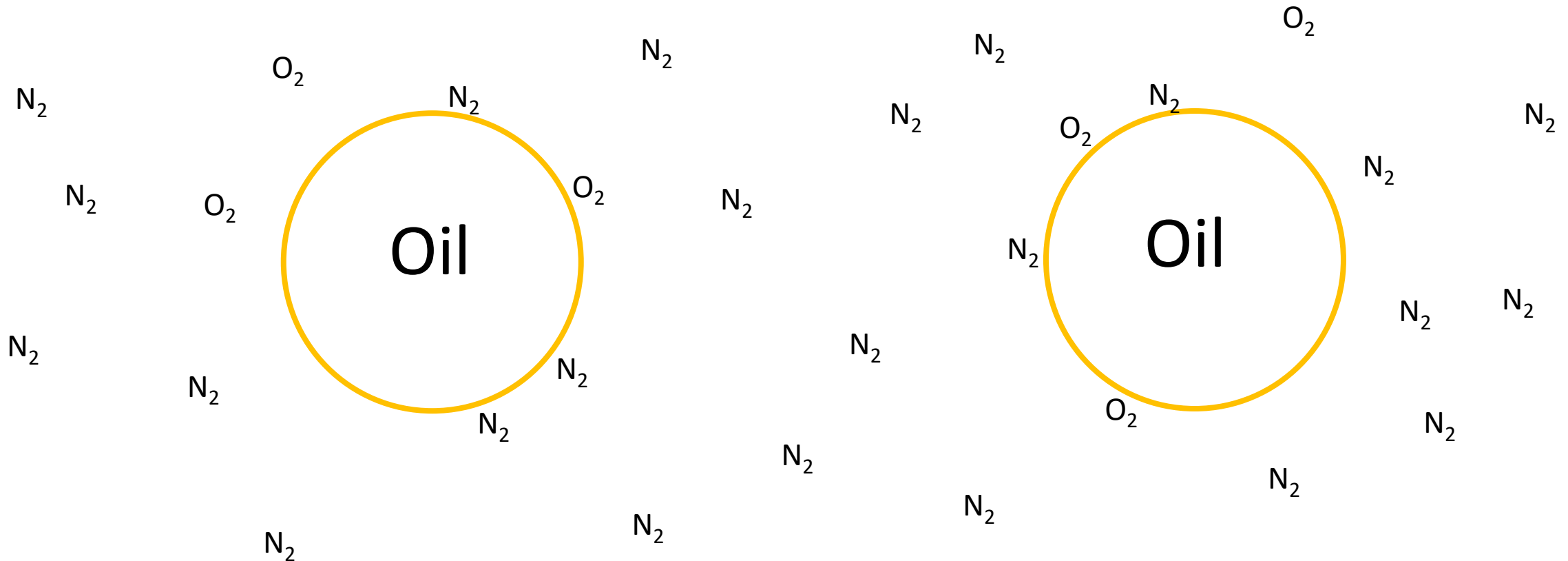
- 25-30°C hydrogenotrophic Dhc strains (Löffler et al., 2013)
- <40–45°C biotic or abiotic destruction
- > 50°C very little biotic or abiotic destruction (Stroo et al., 2013; Costanza et al., 2009)

Hot water vs cold water

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

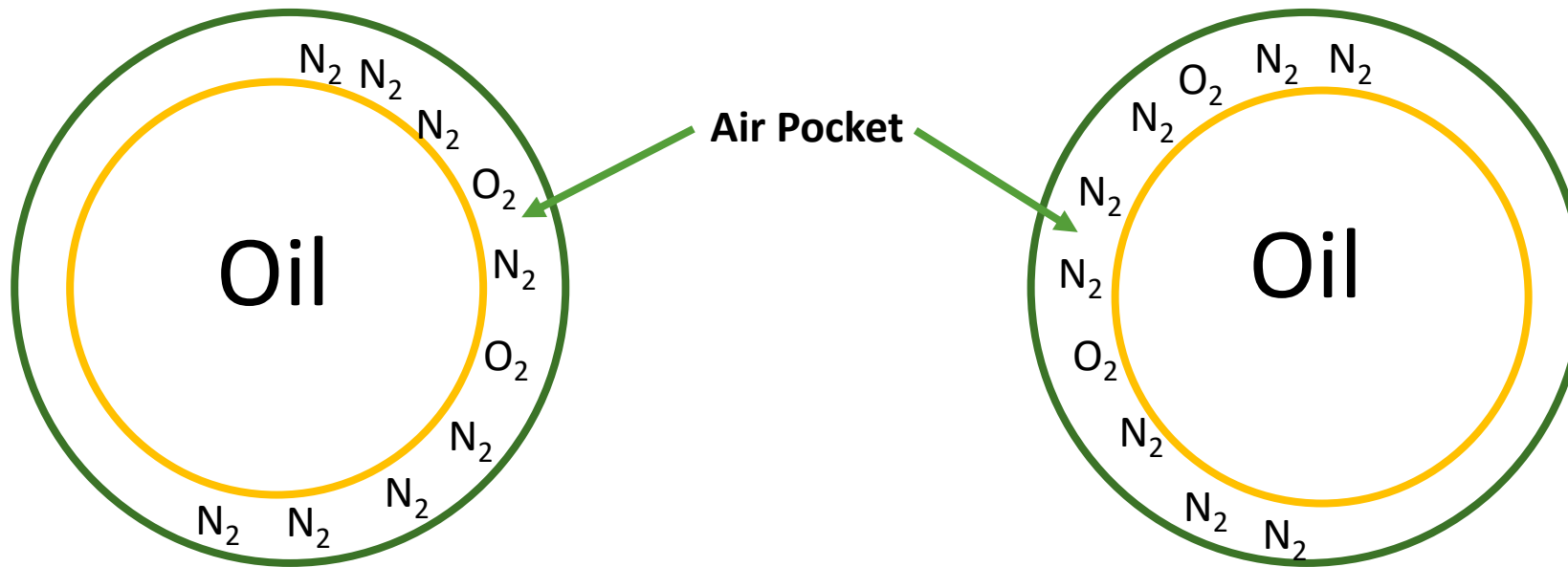
Example of dissolved gas in water with oil

Water



The dissolved gas adsorbs to the surface of the oil

Water



Surface tension pushes oil droplets together to form one big droplet

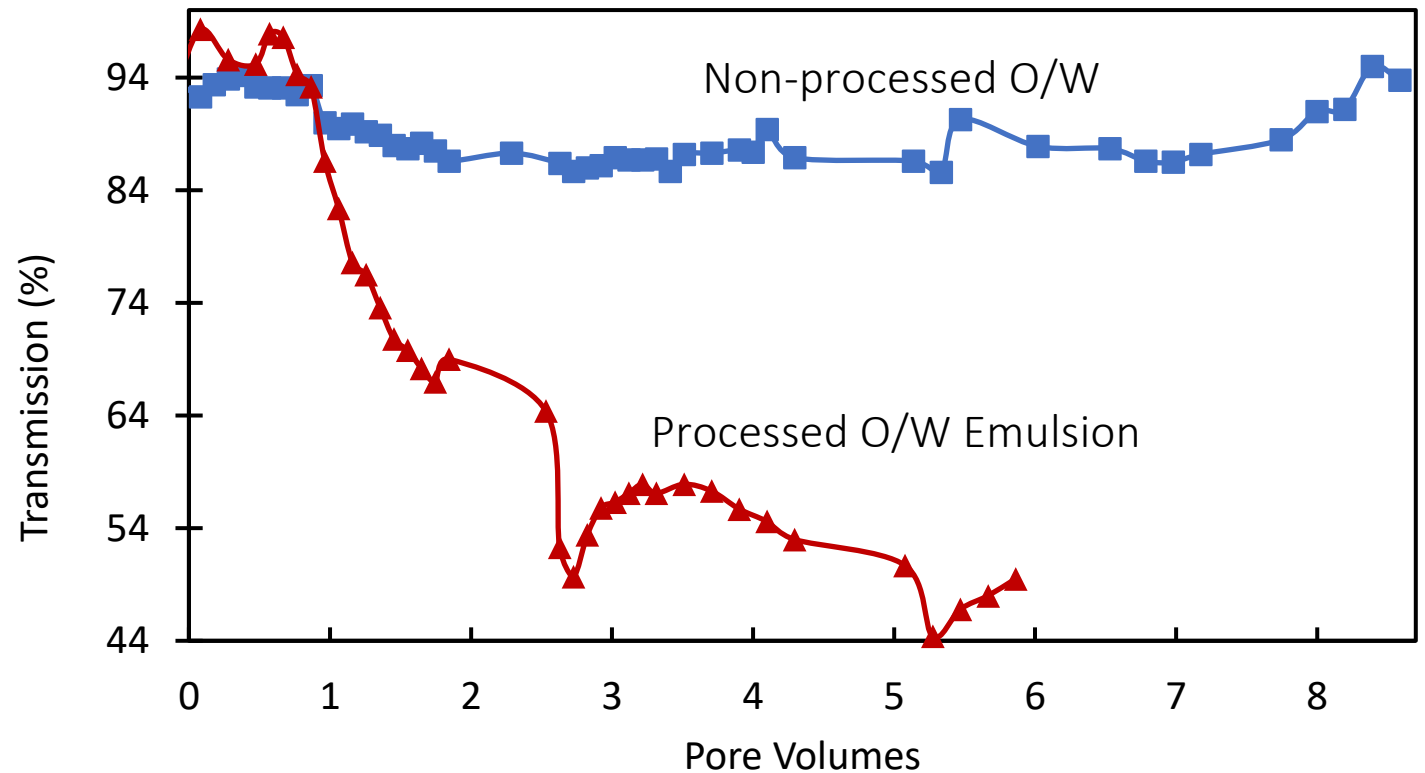
Water



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

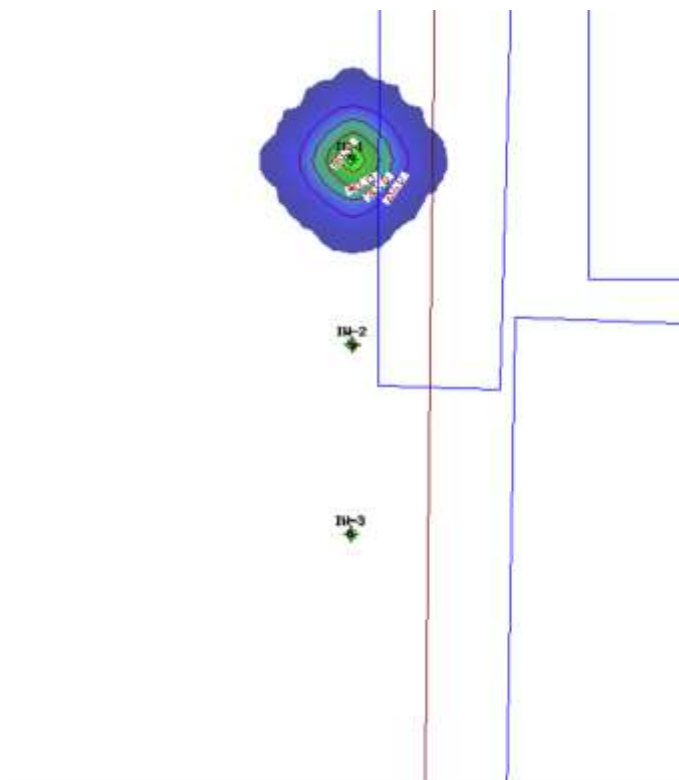
- Site lithology: sand
- Porosity: 0.33
- Aquifer hydraulic conductivity K of 1×10^{-2} cm/s
- Hydraulic gradient: 0.002 feet/feet

Injection

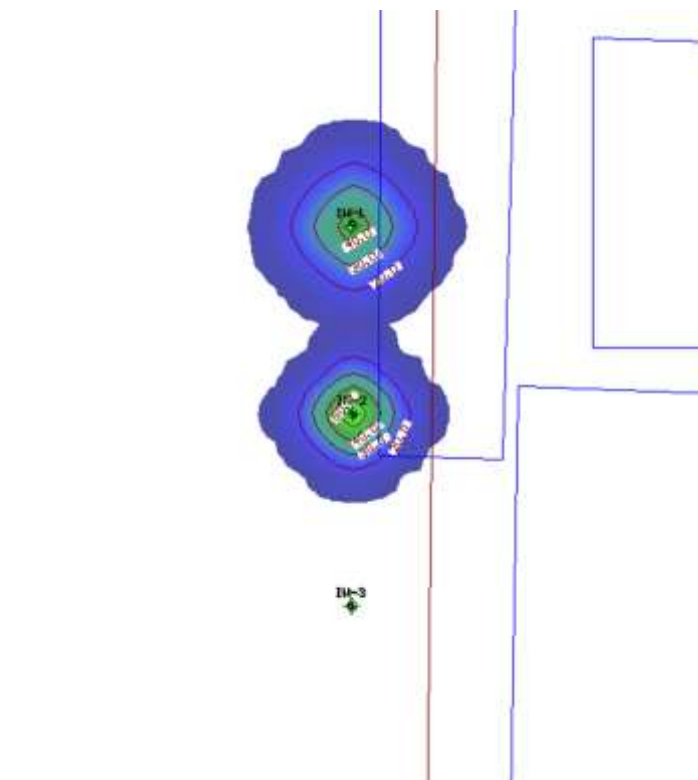
- 12-hour injection event
- 75 m³ (19,813 gallons) of water heated to 90°C
- 150 m³/d (27.5 gpm) flow rate

Model Results

Time = 0.5 days

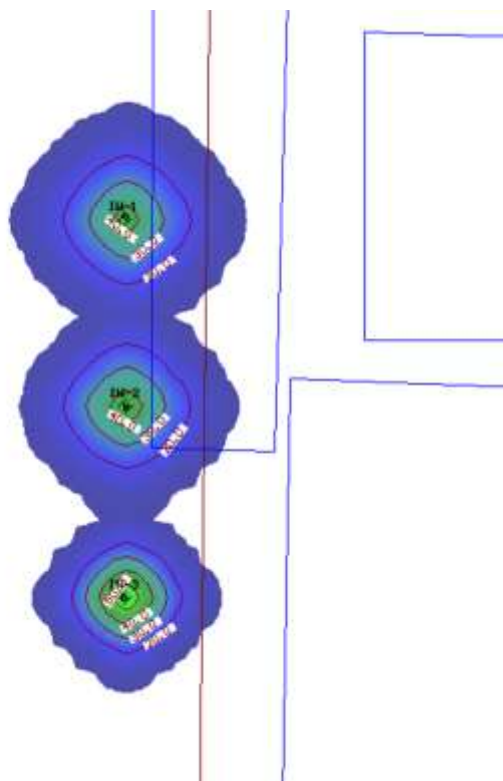


Time = 1 day

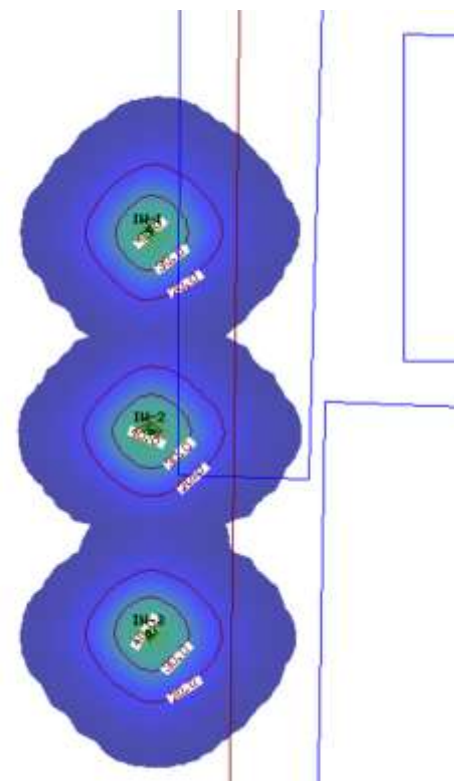


Model Results (continued)

Time = 1.5 days

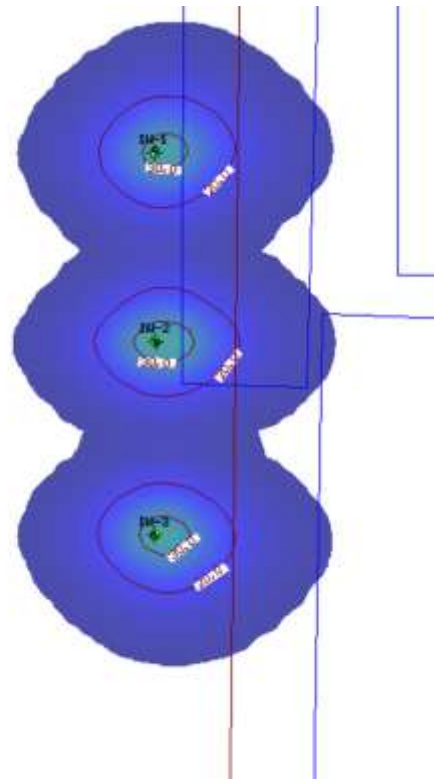


Time = 5 days

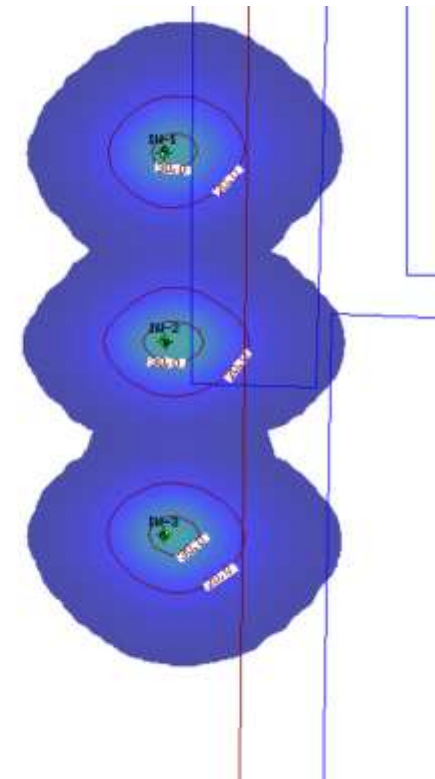


Model Results (continued)

Time = 10 days

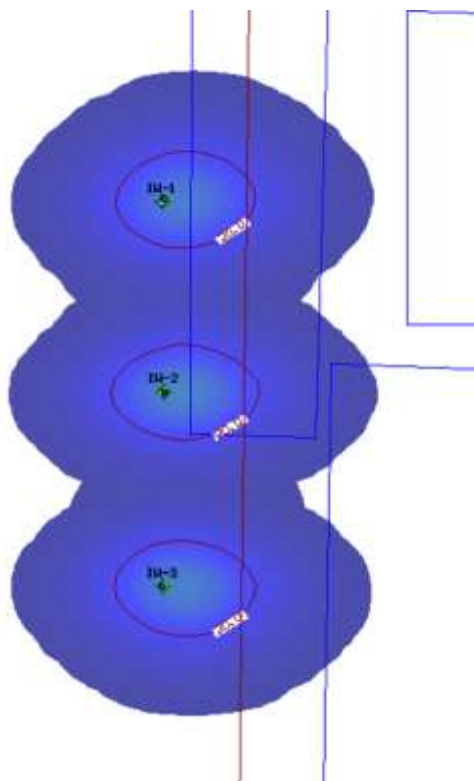


Time = 30 days

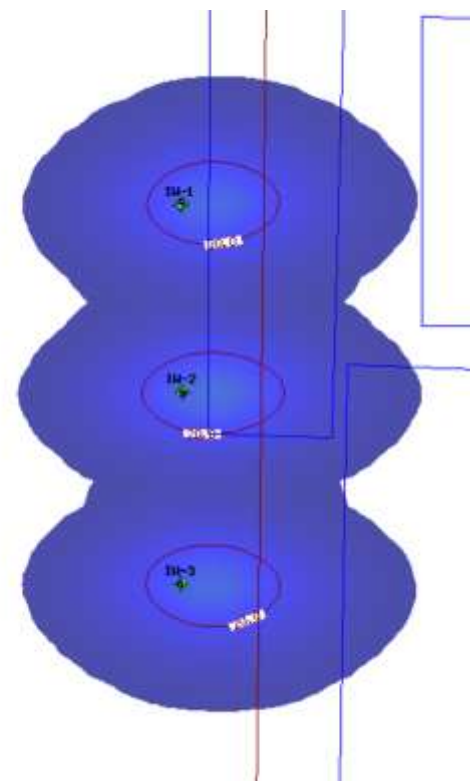


Model Results (continued)

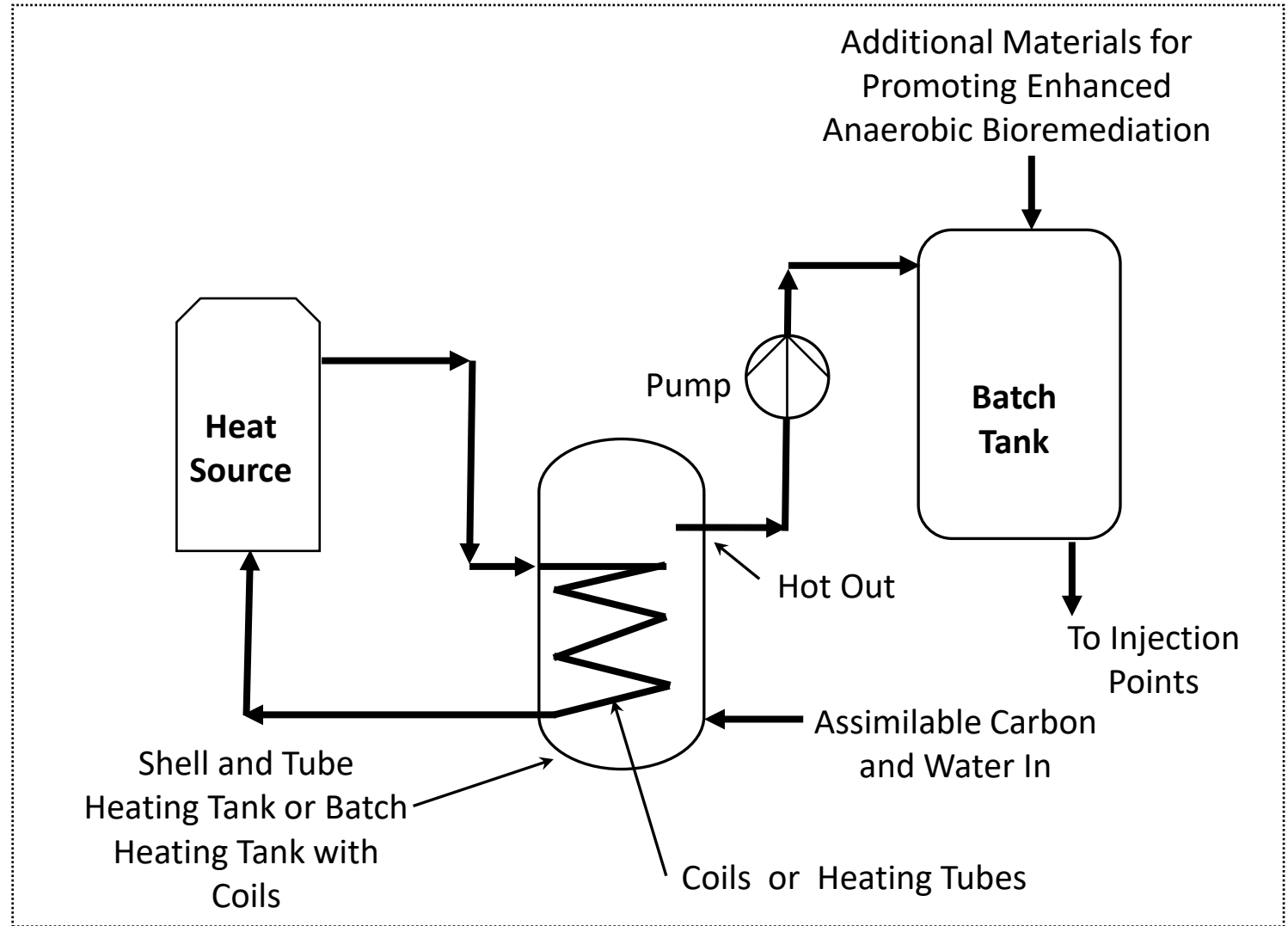
Time = 60 days



Time = 90 days



Shell and Tube Heating Tank or Batch Heating Tank with Coils





Galaxy S22 Ultra
6 de marzo de 2023 5:39 p. m.



Photograph Courtesy of Corporativo SIRON S de RL de CV

Tankless Heaters



Water Heating Capacity Data	
Temperature Rise (°F)	Flow Rate (GPM)
35	12.1
45	9.4
55	7.7
65	6.5
75	5.6
90	4.7
100	4.2
120	3.5
140	3.0

} Potential Reaction
Temp of 30 to 40°C

Heat Enhanced Reductive Bioremediation

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

Thank you



Gary M. Birk, P.E. (NC, VA, & FL)

Tersus Environmental

T. + 1 919 453 5577 x2001 | M. + 1 919 638 7892

gary.birk@tersusenv.com | tersusenv.com



Working With Us

Request a Site Evaluation and Cost Estimate

tersusenv.com/support

