Design and Evaluation of *in situ* Bioremediation Applications

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What is Bioremediation?

Emulsified Oil Substrate

Fermentation

Electron Donor

+ Electron Acceptor

Respiration

PCE + TCE = Waste CO₂, Water

Energy

Multiply

Aquifer pH Adjustment

CoBupH₄Mg

BAC-9

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What is Bioremediation?

Electron Donor + Electron Acceptor = Energy Multiply

ΔG°r

Fe(III) SO₄²⁻ O₂ NO₃

Calcium Peroxide (O₂) Sulfate Salt (Food Grade)

Waste CO₂, Water
Choosing the Right Substrate

Some considerations to take into account:

- Source Treatment or Bio-Barrier or Both
- Lithology and Heterogeneity Dictates Distribution and Injection Rate
- Groundwater Velocity
- Oxidation/Reduction Potential
- Competing e\(^-\) Acceptors
- Commingled Contaminants: Treatment Train
- Microbiology (MBT): Primary or Reinjection Event
- Substrates Degrade at Various Rates
- Injection Method (Site Activity): Traditional Wells, DPT, Existing Infrastructure or Placement Prior to Backfill
Hydrogen vs. Acidity

Not all electron donors are created equal

Low $H^+$ to $H_2$ ratio
Least acid for reducing equivalents

High $H_2$ to Kg ratio
Most reducing equivalents per mass substrate

Not all electron donors are created equal.
Biodegradability

- Short term or soluble substrates (e.g., sodium lactate) provide a quick initial burst of electron donor

  ![Quick degradation](#)  
  - Substrate
  - Fermentation occurs 3-6 months
  - Reducing conditions (low ORP)
  - H⁺  
  - Acetate
  - H⁺

- Slow release substrates (e.g.: soybean oil) provide steady electron donor, useful to treat source area, matrix diffusion, and construct barriers

  ![Slow fermentation](#)  
  - Substrate
  - Fermentation occurs 2-4 years
  - H⁺  
  - Acetate
  - H⁺

Happy bugs!
Emulsified Oil Process

- Install temporary (DPT) or permanent injection points
  - Grids or barriers
- Prepare and inject emulsions 5:1 to 20:1 (water:EOS)
- Inject water to distribute emulsion throughout treatment zone 25-50% of the pore vol.
- Mix in other additives, buffers, vitamin B12, soluble iron
- Oil slowly ferments to \(H_2\) and acetate, 3-6 month post-injection monitoring frequency
- Better contact \(\rightarrow\) better treatment
Design Considerations

- **Treatment zone dimensions**
  - Width perpendicular to flow (x)
  - Length along GW flow direction (y)
    - Contact time (velocity of GW)
    - Effective vertical height (z)

- **Amount of oil**
  - Oil required for biodegradation, including competing e- acceptors
  - Oil droplet retention by sediment

- **Number and spacing of injection wells**
Design Considerations

Oil requirement

Mass of oil = x * y * z * ne * \( \rho_B \) * \( O_R \)

- \( x \) = Treatment zone length parallel to GW flow (ft)
- \( y \) = Design width perpendicular to GW flow (ft)
- \( z \) = height (ft)
- \( ne \) = Effective porosity (unit less)
- \( \rho_B \) = Sediment bulk density (lb./ft\(^3\))
- \( O_R \) = Oil retention (wt./wt.)

Oil Retention: the amount of oil that sorbs to an aquifer grain
What is Oil Retention

Oil retention is a function of

- **Droplet size**
- **Zeta potential of sediments and droplets**
  - Most clays have a net negative charge
  - Negatively charged droplets will have lower retention
- **Surfactant type**
  - Non-ionic typically have lower sorption
  - Ionics have higher sorption (lecithin sorption is very high)
# Substrate Properties

### Properties of “water-less” oil products (EOS 100)

- High vegetable oil content (~80 to 95% by wt.)
- Emulsifiers and other additives
- Once mixed with water have a large droplet diameter (~5-10 microns)

![Mean Droplet 5-10 microns](image)

### Properties of traditional EVO products (EOS Pro)

- Low to medium vegetable oil content (45%-60% by wt.)
- Include nutrients & vitamin B12
- Droplets as delivered ~1 micron

![Mean Droplet ~ 1 micron](image)
Particle Size Distribution

![Graph showing particle size distribution for EOS 100 and EOS Pro](image)

**EOS 100 Suspension**
Column Studies: Oil Retention

- Column studies were conducted to compare:
  - Traditional vegetable oil emulsions (EOS Pro) to water mixable oils (EOS 100)
  - Measured the effective oil retention on two different types of soil:
    - Silty sand (field sand) $K = 30 \text{ ft/day}$
    - Clean sand (washed masonry sand) $K = 100 \text{ ft/day}$
Oil Retention Results

![Diagram showing oil retention results for EOS 100 and EOS Pro in clean sand and silty sand samples.](chart_image)

- **Clean Sand**:
  - EOS 100: 0.020 g/g dry soil
  - EOS Pro: 0.000 g/g dry soil

- **Silty Sand**:
  - EOS 100: 0.040 g/g dry soil
  - EOS Pro: 0.010 g/g dry soil
Life Cycle Cost Analysis

ESTCP Emulsion Tool Kit:
200ft Barrier
GW Velocity 1.6 ft./day
Geology: medium sand with silt

http://www.microbe.com/webinars/
When Do You Need to Reinject?

- Soluble substrate systems (lactate, molasses, etc.)
  - Maintain 50 – 100 mg/L TOC

- Emulsified Oil Systems
  - Oil droplets rapidly attach to solid phase
  - > 99% of TOC is ‘sorbed’ to aquifer material
  - Aqueous TOC < 20 mg/L
  - Continue to get good treatment

Lab Column Experiment (HRT= 3 weeks, Long and Borden, 2006)
TOC & VFA

- Total Organic Carbon (TOC)
  - Initial spike
  - Stabilized at relatively constant value
  - Rapid decrease with distance downgradient
  - TOC dropped during last sampling indicating substrate is starting to be exhausted

ESTCP demonstration project
Daughter Products & Performance

AFCEE, 2004, Demonstration of Bioaugmentation at Kelly AFB, TX.
Note: Electron donor injected Feb 2000 and bioaugmentation culture was injected in May 2000.

Lendvay et al., 2003, Bioreactive Barriers: A Comparison of Bioaugmentation and Biostimulation for Chlorinated Solvent Remediation
Note: Shaded areas represent same time frame of treatment
Molecular Biological Tools (MBTs)

1) Quantitative Polymerase Chain Reaction (qPCR): Sample is analyzed for specific microorganisms or target functional genes.
   - Can’t tell if cells are alive or dead or if genes actively being expressed.
   - Can use to enumerate baseline and monitor changes over time or in response to treatment.

2) Phospholipid Fatty Acid (PLFA): Used to assess viable (but non-specific) biomass concentrations, profile microbial community composition (e.g., iron or sulfate reducers, fermenters); provides index of metabolic activity.

3) Stable Isotope Probing (SIP): Used to conclusively demonstrate biodegradation.
   - Quantifies incorporation of C\textsuperscript{13} labeled contaminant into microbial biomass and dissolved organic carbon.

Pictures courtesy of Microbial Insights, Inc. www.microbe.com
Calculating Culture

How to calculate: (Can use our design tool)

- Determine the area for treatment (ft²)
- Determine the saturated thickness (ft)
- Using the effective porosity calculate the pore volume (ft³)
- Convert the pore volume into liters (L)
- BAC-9™ culture has a cell concentration of $10^{11}$ cell/L
- Typical target cell concentration in an aquifer is $10^7$ cell/L

\[
\text{Pore volume} \times \text{target cell concentration} / \text{BAC-9 cell concentration} = \text{amount of culture required}
\]

Example: Pore volume of 200,000L

\[
200,000L \times 1.0 \times 10^7 \text{ cell/L} / 1.0 \times 10^{11} \text{ cell/L} = 20L \text{ of BAC-9™}
\]
Calculating Culture

BAC-9™ is sold in 19 L soda kegs. If you require more than 19 L the culture is concentrated. The concentration factor can range from 0-10x.

To convert the concentrated culture into actual volume to inject:
Divide the unconcentrated culture value per injection well by the concentration factor.

Example:
If you determine you need 30 L of culture and you have 11 injection wells, then plan to inject 2.72 L per well.

EOS sends a keg of BAC-9™ with 19 L of culture concentrated 1.6x

Take 2.72/1.6 = 1.7 L of concentrated culture to inject per point.
Injection Procedure

Important Field Parameters to Consider:

- DO levels: <0.5 mg/L
- ORP: < -200 mv
- pH: 5.5 – 8.5 with 6.6 – 7.4 yielding the best degradation rates
- SO\textsubscript{4} Concentrations: <50 mg/L (per SABER project) – There are a number of examples where sulfate levels have been elevated (~100 mg/L to 1,000+ mg/L) and VC and ethene were detected (Battelle, 2004 and Dockum et al., 2013)
- CVOC concentrations: Complete dechlorination has been observed when TCE concentrations were between 400 and 800 mg/L (Harkness et al., 2006) and in DNAPL remediation (Schaefer et al., 2010b)
Managing Aquifer pH

C₂Cl₄ (PCE) + H₂ → C₂HCl₃ (TCE) + HCl
C₂HCl₃ (TCE) + H₂ → C₂H₂Cl₂ (DCE) + HCl
C₂H₂Cl₂ (DCE) + H₂ → C₂H₃Cl (VC) + HCl
C₂H₃Cl (VC) + H₂ → C₂H₄ (ethene) + HCl

C₂Cl₄ (PCE) + 4H₂ → C₂H₄ (ethene) + 4HCl

Optimum pH for PCE reduction appears to be 6.0 – 7.0

<table>
<thead>
<tr>
<th>Base</th>
<th>Formula</th>
<th>MW</th>
<th>OH- per Mole</th>
<th>OH- per Kg</th>
<th>pH</th>
<th>Solubility (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brucite (milk of magnesia)</td>
<td>Mg (OH)$_2$</td>
<td>58.3</td>
<td>2</td>
<td>34.3</td>
<td>10.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Caustic Soda (Lye)</td>
<td>NaOH</td>
<td>39.9</td>
<td>1</td>
<td>25.0</td>
<td>14</td>
<td>1,100</td>
</tr>
<tr>
<td>Baking Soda</td>
<td>NaHCO$_3$</td>
<td>84</td>
<td>$\alpha$</td>
<td>11.9</td>
<td>8.3</td>
<td>78</td>
</tr>
<tr>
<td>Calcite</td>
<td>CaCO$_3$</td>
<td>100</td>
<td>1</td>
<td>10.0</td>
<td>8.3</td>
<td>0.02</td>
</tr>
</tbody>
</table>
Magnesium Hydroxide

\[ \text{Mg(OH)}_2(s) \leftrightarrow \text{Mg}^{2+}(aq) + 2\text{OH}^-(aq) \]

\[ \text{Ksp} = 10^{-10.74} \]

- Magnesium Hydroxide is a “sparingly” soluble base and occurs in nature as the mineral brucite.
- Its solubility is controlled by the pH of the system.

Faure; 1998, Principles and Applications of Geochemistry, Figure 9.2, pg123
CoBupH-Mg

Colloidal suspension of magnesium hydroxide

Specifications:

- Alkaline Buffer (% by wt.) 45%
- Dispersant (% by wt.) 1%
- Stabilizer (% by wt.) 0.5%
- Mean Particle Size (um) ~1
- Bulk Density (g/mL) ~1.4
- pH (SU) 9-10
CoBupH-Mg Titration

- Ambient pH is too low for microorganisms to function/live
- ~13 g/Kg needed to reach pH 7
- Equilibrium pH of ~8 reached with double the CoBupH Mg needed

Data provided by CB&I (2012)
Field Demonstration

If you are considering aquifer pH adjustment collect/understand:

- Soil Acidity
- Groundwater Acidity
- CVOC Concentrations
- Substrate selection

CoBupH\textsubscript{Mg}

Colloidal Suspension of Magnesium Hydroxide designed for maximum transport in the aquifer