INTRODUCTION TO ENHANCED ANAEROBIC BIOREMEDIATION (EAB)

Enhanced anaerobic reductive dechlorination with non-emulsified and emulsified vegetable oils has been implemented at thousands of commercial and military field sites globally. There are a wide variety of compounds that can be anaerobically biodegraded using vegetable oils including chlorinated ethenes, chlorinated ethanes, halomethanes, perchlorate, nitrate, certain metals, and explosives. In practice, the organic substrate amendments are initially fermented to molecular hydrogen and low-molecular weight fatty acids such as acetate, lactate, propionate, and butyrate. The short-chain, low molecular weight fatty acids then provide carbon and energy to the microorganisms which in turn facilitate reductive dechlorination. During reductive dechlorination, the parent chlorinated ethene, for example perchloroethylene (PCE), is sequentially dechlorinated whereby a chlorine atom is removed and replaced with a hydrogen atom to form less-chlorinated daughter products trichloroethene (TCE), 1,2-cis-dichloroethene (cDCE) and vinyl chloride (VC). When this process goes to completion ultimately ethene is formed as the non-toxic end product (Figure 1).

During reductive dechlorination of chlorinated volatile organic compounds (CVOCs) the CVOCs serve as the electron acceptor and the hydrogen from vegetable oil fermentation serves as the electron donor. Dechlorinating bacteria in the subsurface catalyze the sequential reactions, deriving energy in a process called dehalorespiration. For reductive dechlorination of CVOCs to proceed, sufficient hydrogen must be generated to meet the stoichiometric demand of target compounds (PCE, TCE, and daughter products) and non-target species (e.g., other electron acceptors) in the subsurface. At many sites, abundant electron acceptors can limit the availability of hydrogen to support contaminant biodegradation. Therefore, substrates that release higher hydrogen yields over extended periods of time are more favorable. As a way to compare the different products, fermentation reactions can be written as if the substrate is metabolized to bicarbonate, hydrogen cation, and dihydrogen gas. Table 1 shows the theoretical hydrogen yield for a variety of bioremediation amendments on a molar and mass basis.
Table 1: Hydrogen Yield of Bioremediation Substrates

<table>
<thead>
<tr>
<th>Bioremediation Product</th>
<th>Chemical Formula</th>
<th>MW</th>
<th>Percent Composition (% by weight)</th>
<th>H₂ (mol) per substrate (mol)¹</th>
<th>H₂ (g) per substrate (g)</th>
<th>H₂ (g) per product (kg)³</th>
<th>H₂ (g) per product (lb)³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl Lactate</td>
<td>C₅H₁₀O₃</td>
<td>118.2</td>
<td>98</td>
<td>2</td>
<td>12</td>
<td>0.21</td>
<td>0.20</td>
</tr>
<tr>
<td>Sodium Lactate</td>
<td>NaC₃H₅O₃</td>
<td>112.1</td>
<td>60</td>
<td>40</td>
<td>6</td>
<td>12.12</td>
<td>0.11</td>
</tr>
<tr>
<td>Ethanol</td>
<td>C₂H₆O</td>
<td>46.1</td>
<td>80</td>
<td>20</td>
<td>6</td>
<td>12.12</td>
<td>0.26</td>
</tr>
<tr>
<td>Molasses</td>
<td>C₁₂H₂₂O₁₁</td>
<td>342.3</td>
<td>60</td>
<td>40</td>
<td>24</td>
<td>48.48</td>
<td>0.14</td>
</tr>
<tr>
<td>Glycerol</td>
<td>C₃H₈O₃</td>
<td>92.1</td>
<td>75</td>
<td>--</td>
<td>7</td>
<td>14.14</td>
<td>0.12</td>
</tr>
<tr>
<td>CAP 18® Anaerobic</td>
<td>Proprietary blend</td>
<td>~280</td>
<td>100</td>
<td>0</td>
<td>50</td>
<td>101.36</td>
<td>0.36</td>
</tr>
<tr>
<td>Bioremediation Product</td>
<td>Emulsified Vegetable Oil (60%)</td>
<td>C₁₈H₃₂O₂</td>
<td>280.5</td>
<td>60</td>
<td>10</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Emulsified Vegetable Oil (40%)</td>
<td>C₁₈H₃₂O₂</td>
<td>280.5</td>
<td>40</td>
<td>10</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Emulsified Vegetable Oil (35%) + Ethyl Lactate (35%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Calculated from the reaction of substrate and water to bicarbonate, hydrogen ion, and hydrogen gas
²General formulations for competitor bioremediation products
³Calculated from % composition (by weight)

Ethyl Lactate:
\[ \text{C}_5\text{H}_{10}\text{O}_3 + 12\text{H}_2\text{O} \rightarrow 12\text{H}_2 + 5\text{HCO}_3^- + 5\text{H}^+ \]

Sodium Lactate:
\[ \text{NaC}_3\text{H}_5\text{O}_3 + 6\text{H}_2\text{O} \rightarrow 6\text{H}_2 + 3\text{HCO}_3^- + \text{Na}^+ + 2\text{H}^+ \]

Ethanol:
\[ \text{C}_2\text{H}_6\text{O} + 5\text{H}_2\text{O} \rightarrow 6\text{H}_2 + 2\text{HCO}_3^- + 2\text{H}^+ \]

Molasses:
\[ \text{C}_{12}\text{H}_{22}\text{O}_{11} + 25\text{H}_2\text{O} \rightarrow 24\text{H}_2 + 12\text{HCO}_3^- + 12\text{H}^+ \]

Glycerol:
\[ \text{C}_3\text{H}_8\text{O}_3 + 6\text{H}_2\text{O} \rightarrow 7\text{H}_2 + 3\text{HCO}_3^- + 3\text{H}^+ \]

Soybean Oil (Linoleic Acid):
\[ \text{C}_{18}\text{H}_{32}\text{O}_2 + 52\text{H}_2\text{O} \rightarrow 50\text{H}_2 + 18\text{HCO}_3^- + 18\text{H}^+ \]
CASE STUDY USING CAP 18® ANAEROBIC BIOREMEDIATION PRODUCT

Burns & McDonnell was selected by the Kansas Department of Health & Environment (KDHE) to perform environmental services for the former Cinderella Cleaners and Stickel Cleaners facilities located in Manhattan, Kansas listed under the State of Kansas Dry Cleaning Facility Release Trust Fund (DFRTF) program. Historical chlorinated solvent releases associated with dry cleaning activities from these two facilities have been identified and characterized at the site and the primary contaminants of concern (COCs) are PCE, TCE, cDCE and VC. Baseline groundwater sampling conducted prior to groundwater remediation in July 2009 showed maximum contaminant concentrations in groundwater as follows: PCE 11,000 ug/L, TCE 1,770 ug/L, cDCE 700 ug/L, and VC 957 ug/L. The comimgled contaminant plume extends approximately 4,000 feet east/northeast of the site toward the City of Manhattan public water supply (PWS) wells #12 and #13.

At the request of KDHE, Burns & McDonnell designed an innovative approach to remediate the groundwater contamination near the source area and reduce migration of the contaminant plume down gradient of the site. In 2009, a Corrective Action Plan was prepared and an enhanced anaerobic bioremediation (EAB) groundwater treatment was successfully implemented. CAP 18® anaerobic bioremediation product was selected as the electron donor substrate due to its compatibility with high groundwater seepage velocities, low cost, and extended longevity and reactivity in the subsurface. EAB treatment at the Site consisted of the injection of a non-emulsified vegetable oil substrate, which creates an anaerobic environment in the aquifer. The product consists of triacylglycerols, which are made up of fatty acids and glycerol. Once injected into the subsurface the triacylglycerols slowly hydrolize releasing free fatty acids and glycerol. The fatty acids, which consist of large hydrogen-rich molecules, are digested by microorganisms via beta oxidation (or other processes). CAP 18 offers many advantages over other bioremediation products including:

- CAP 18 has viscosity similar to vegetable oil, and can be injected via monitoring wells or temporary points using standard grout pumps or diaphragm pumps.
- The product is not diluted with water, so 100% of the product contributes hydrogen to support bioremediation. Normalized to the cost of hydrogen produced, the product is less expensive than other soluble or insoluble substrates.
- The product degrades slowly and provides a long-term hydrogen source that lasts for years. Unlike more soluble or less viscous amendments, frequent re-injection or recirculation systems are not necessary.
- CAP 18 is a metabolically diverse substrate composed of C18 fatty acids which produces a wide range of compounds for microbial hydrogen production that is ideal for diverse aquifer conditions.
- CAP 18 is a concentrated hydrogen source, providing fuel to establish optimal groundwater conditions and overcome competitive demand.
- The product contains natural compounds that inhibit microbial reduction of acetate to methane and compared to other substrates yields efficient hydrogen utilization for contaminant destruction rather than for methane production.
- CAP 18 is a proprietary blend that is an easy to inject amendment that will not travel vertically following emplacement. The specific gravity of the product is very close to that of water (0.92 g/mL), and the combination of viscosity and interfacial tension prevents upward migration of the product in saturated soils.
ENHANCED ANAEROBIC BIOREMEDIATION CORRECTIVE ACTION DESIGN AND IMPLEMENTATION

At the Cinderella-Stickel site, CAP 18® anaerobic bioremediation product was distributed throughout the vertical treatment interval in the form of five substrate distribution curtains, oriented perpendicular to the direction of groundwater flow at the site. The vertical target treatment interval extended from the static groundwater surface (approximately 20 feet below ground surface [bgs]) to the top of bedrock (approximately 60 feet bgs). Approximately 168 gallons of CAP 18 was injected at a total of 59 injection points completed within the five injection curtains. A total of approximately 9,900 gallons of CAP 18 was injected into the subsurface throughout the field implementation (see Figures 2 and 3).

Figure 2: Cinderella-Stickel Cleaners EAB Target Treatment Area
Each curtain spanned the width of the groundwater plume and was completed using direct-push injection techniques. A generalized overview of a curtain injection strategy is provided below in Figure 3.

The EAB injection strategy at the Cinderella-Stickel site consisted of five injection curtains at the two site areas: three curtains downgradient of the former Cinderella cleaners and two curtains down gradient of the former Stickel cleaners. Injection wells were spaced 15 feet apart, and each curtain was spaced 50-80 feet apart, with a total linear footage of 570 feet for the five injection curtains. Each point was injected at five-foot intervals, with a varying injection volume of CAP 18® anaerobic bioremediation product at each interval depending on the seepage velocity for each interval’s lithology. The dosage amounts for each injection interval were:

- Injection depths of 20-35 feet bgs: approximately one gallon per 3-5 foot interval
- Injection depths of 35-45 feet bgs: approximately eight gallons per 3-5 foot interval
- Injection depths of 46-60 feet bgs: approximately 35 gallons per 3-5 foot interval

Sixty feet bgs represents top of bedrock in this area. Approximately 1,278 pounds (approximately 168 gallons) of CAP 18 were injected at each injection point.

**PERFORMANCE MONITORING RESULTS**

Since the conclusion of EAB injection activities at the site, groundwater sampling has been conducted at eight monitoring wells to provide data used in assessing performance of the EAB corrective action. Sampling is conducted on a semiannual basis using low-flow sampling techniques. Wells are sampled and analyzed for EAB performance indicator parameters as well as known COCs. Concentrations of the presumptive parent compound for CVOC groundwater impacts at the site (PCE) have significantly decreased in all EAB performance monitoring wells since the completion of CAP 18 injection activities.

As of September 2012, PCE concentration reductions in the monitoring wells range from 72% to 100%, with six of the eight wells reporting reductions of 93% or greater. TCE concentrations have also decreased in seven of the eight monitoring wells since substrate injection while cDCE and VC concentrations have increased in some wells as a normal function of the dechlorination process.
While concentrations of degradation products (cDCE and VC) have increased at some wells, the formation of ethene, a terminal end product of reductive dechlorination, confirms that the treatment process is completely converting CVOCs into inert compounds. Ethene has been detected at six of the eight monitoring wells and at every monitoring well exhibiting an increase in cDCE or VC concentrations. Consequently, the remaining cDCE or VC impacts are expected to attenuate over time as the dechlorination process continues. The CVOC concentration trends for select monitoring wells are illustrated and discussed below.

As shown in Figure 4, PCE and TCE concentrations increased to 9000 ug/L following the EAB injection and decreased rapidly to a low of < 2.0 ug/L at the latest sampling event in September 2012. Followed by subsequent increases in both cDCE and VC increased as expected, due to PCE and TCE dechlorination, but subsequently decreased to concentrations below baseline levels. Ethene analysis for groundwater samples began in March 2011. MW-8D ethene concentrations increased from March 2011 through April 2012, confirming complete dechlorination of targeted CVOCs.

In Figure 5, the observed TCE and PCE concentrations remained relatively stable following the July 2009 EAB injection event followed by a sharp decline and continue to remain consistently low following the September 2012 monitoring event. TCE concentrations followed a similar trend while cDCE concentrations spiked before the PCE and TCE decreasing trends began. Following the initial spike, cDCE decreased to levels slightly above baseline. Following the spikes and subsequent declines in PCE, TCE, and cDCE concentrations, vinyl chloride levels increased but show a decreasing trend during the September 2012 sampling event.
As previously mentioned, this increase in VC is expected during the dechlorination process and is considered temporary with ethene detections in MW-12D confirming that complete dechlorination of targeted CVOCs is occurring.

Figure 5: MW-12D CVOC Degradation
Figure 6 illustrates that PCE and TCE concentrations decreased following the July 2009 EAB injection event followed by a spike during the October 2011 sampling event before declining to consistently low levels after the September 2012 monitoring event. While cDCE concentrations increased from 600 to >1400 ug/L which is evidence of sequential dechlorination this was followed by a continued and gradual decline. As with the other monitoring wells discussed previously, ethene detections in MW-20D continue to confirm that complete dechlorination of the targeted CVOCs is ongoing.
EAB PERFORMANCE ASSESSMENT

Figure 7 below illustrates the PCE reductions and performance assessment results for the Cinderella-Stickel EAB site with removal efficiencies ranging from 72% to 100%.

![Figure 7: PCE Performance Assessment Results and Removal Efficiencies](image)
GEOCHEMICAL ASSESSMENT

EAB performance is often tracked by measuring a variety of geochemical parameters including: nitrate, sulfate, methane, pH, dissolved oxygen (DO), oxidation-reduction potential (ORP), total organic carbon (TOC), iron and ferrous iron. Post-EAB performance highlights of a few of these parameters are discussed below.

Complete dehalogenation to ethene at many field sites can be hindered by a number of factors including development of a low groundwater pH. To overcome this, some EAB substrates are amended with buffers in order to reach near neutral conditions. The slow release characteristics of CAP 18® anaerobic bioremediation product limit the amount of acidity generated which assists in maintaining favorable pH for dechlorinating microorganisms. The pH values measured before and following EAB curtain installation are provided below (Figure 8).

![Figure 8: pH Values following EAB at the Cinderella-Stickel Site, Manhattan, KS](image-url)
Following initial pH decreases at the site, the latest sampling event recorded near neutral values ranging from 6.6 to 7 which helps promote sustained microbial activity. In addition, TOC data can provide information on the transport of organic carbon in groundwater occurring downgradient from the CAP 18® anaerobic bioremediation product curtains (Figure 9).

![Figure 9: Total Organic Carbon Values following EAB at the Cinderella-Stickel Site, Manhattan, KS](image)

Figure 9 illustrates that elevated TOC levels (above baseline values) were measured in all wells; however, TOC levels would be expected to decline over time (as noted in MW-12 and MW-20D) as microbial growth and activity increases and the substrate is consumed.
CONCLUSIONS

This case study presents the results of a large-scale EAB field implementation of CAP 18® anaerobic bioremediation product for cleanup of a chlorinated solvent plume in Manhattan, KS. Field activities began with the baseline sampling occurring in July 2009 with continued monitoring of CVOC degradation planned through 2013. The following provides a summary of the major conclusions drawn from the three years of data presented above:

The Cinderella-Stickel site in Manhattan, KS continues to show significant reductions in CVOC over three years with ongoing ethene generation.

The TOC concentration data suggests that substrate is still being released three years following a single application, and that bioremediation activity is ongoing.

A result of the increased substrate utilization efficiency, very high TOC concentrations are not required to support reductive dechlorination.

A large pH shift was not observed during the barrier lifetime, despite degrading >12,000 ug/L of PCE.

The slow-release characteristics of CAP 18 minimized large pH fluctuations that are commonly observed from soluble amendment injections, thus eliminating the need to co-inject costly buffers.

Complete anaerobic reductive dechlorination of PCE to ethene was stimulated through the application of CAP18.

Anaerobic reductive dechlorination was observed to distances of almost 60 ft from the CAP18 curtains.

The injection of non-emulsified vegetable oil into the target treatment areas at depths up to 60 feet was easily accomplished with direct push tooling.

The corrective action costs associated with the EAB site treatment was approximately $250,000.