FINAL

PROTOCOL FOR \textit{in situ} BIOREMEDIATION OF CHLORINATED SOLVENTS USING EDIBLE OIL

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Prepared for:

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6.2.2.2 Methane, Ethane, Ethene

Methanogenesis is characterized by coupled enzymatic reactions. Excess hydrogen released during substrate fermentation can be used to reduce carbon dioxide to methane. In addition, acetate may also be fermented forming carbon dioxide and methane. Methanogenesis generally occurs after oxygen, nitrate, Fe(III), and sulfate have been depleted in the treatment zone. The presence of methane in groundwater is indicative of strongly reducing conditions because methanogenic bacteria are obligate anaerobes. The presence of methane above background concentrations in areas with chlorinated solvents is an indication that the groundwater geochemical conditions are favorable for anaerobic reductive dechlorination. The fastest and most complete reductive dechlorination of CAHs typically occurs under methanogenic conditions.

Ethane and ethene are the desired end-products of the reductive dechlorination process for chlorinated ethenes and chlorinated ethanes. These light hydrocarbon gasses can be detected in groundwater at low concentrations, but tend not to accumulate as a result of diffusion into a volatile phase (gas) or as a result of further biodegradation to carbon dioxide (CO₂) and water. The presence of elevated levels of ethene and ethane are favorable indicators that the biodegradation pathway for sequential reductive dechlorination is complete.

6.2.2.3 pH and Alkalinity

There is often a positive correlation between zones of microbial activity and increased alkalinity. Increases in alkalinity result from dissolution of carbonate mineral by carbon dioxide produced during substrate biodegradation and by reduction of iron oxide minerals. Alkalinity is important in the maintenance of groundwater pH because it buffers the groundwater system against acids generated during both aerobic and anaerobic biodegradation. A pH close to neutral (i.e., 6 to 8) is the most conducive to the proliferation of healthy, diverse microbial populations. Low pH conditions (<5) are detrimental to sulfate-reducing and methanogenic bacteria. Fermentative organisms that favor lower pH conditions will compete with both sulfate reducing and methanogenic bacteria in this environment. This can result in the formation of undesirable low-molecular-weight organic byproducts of fermentation, such as ketones, alcohols, and aldehydes. Lowering of pH is more prevalent where excessive amounts of organic substrate have been applied.

Aquifer systems with lower buffering capacities are more susceptible to a decrease in pH due to biological activity. Alkalinity is an indicator of the buffering capacity of an aquifer system. From a practical standpoint, alkalinity greater than 300 mg/L are generally sufficient to buffer against adverse pH changes. Alkalinity less than 100 to 200 mg/L is a cause for concern, and pH should be monitored carefully. In such cases, pH buffering may be used during implementation to raise and/or neutralize pH against further decreases.

Buffering may be implemented using compounds such as sodium bicarbonate (NaHCO₃), quicklime (CaO), dolomitic quicklime (CaO/MgO), caustic hydroxide (NaOH), and magnesium hydroxide (Mg(OH)). It is more cost effective to add these materials during the initial injection, rather than re-mobilizing to the site for an additional injection of buffering agent. Commercial emulsified oil products are now available in buffering formulations or with buffering mixtures that can be added when diluting and mixing the concentrated product.
6.2.2.4 Chloride

Chloride is released to groundwater during biodegradation of CAHs. This results in chloride concentrations in the contaminant plume that are elevated relative to background concentrations. As a consequence, elevated chloride concentrations can serve as a useful indicator of reductive dechlorination. However, if substantial levels of chloride are present in the background groundwater, it may be difficult to detect small increases in chloride concentration.

Chloride ions generally do not enter into oxidation-reduction reactions, do not form important solute complexes with other ions unless the chloride concentration is extremely high, do not form salts of low solubility, are not significantly adsorbed on mineral surfaces, and play few vital biochemical roles (Hem, 1985). Thus, physical processes control the migration of chloride ions in the subsurface and chloride behaves as a conservative indicator of biological activity.

6.2.2.5 Oxidation-Reduction Potential

The ORP of groundwater is a measure of electron activity (Eh) and is an indicator of the relative tendency of a solution to accept or transfer electrons. The redox of groundwater generally ranges from -400 mV to +800 mV. Oxidation-reduction reactions in groundwater containing organic compounds (natural or anthropogenic) are usually biologically mediated, and therefore, the redox of a groundwater system depends upon and influences rates of biodegradation. Knowledge of the redox of groundwater is important because some biological processes operate only within a prescribed range of redox conditions. Redox measurements can be used to provide real-time data on the location of areas undergoing anaerobic biodegradation. Due to instability, redox measurements should be taken in the field during well purging and immediately before sample acquisition using a direct-reading meter.

6.2.2.6 Dissolved Hydrogen

Concentrations of dissolved hydrogen have been used to evaluate redox processes, and thus the efficiency of reductive dechlorination, in groundwater systems (Lovley and Goodwin, 1988; Lovley et al., 1994; Chapelle et al., 1995). The concentration of hydrogen can be used to identify groundwater where reductive dechlorination may be occurring. Hydrogen is continuously produced in anoxic groundwater systems by fermentative microorganisms that decompose natural and anthropogenic organic matter. This hydrogen is then consumed by respiratory microorganisms that use nitrate, Fe(III), sulfate, or CO2 as terminal electron acceptors. This continuous cycling of hydrogen is called interspecies hydrogen transfer.

Significantly, nitrate-, Fe(III)-, sulfate- and CO2-reducing (methanogenic) microorganisms exhibit different efficiencies in utilizing the hydrogen that is being continually produced. Nitrate reducers are highly efficient users of hydrogen and maintain very low steady-state hydrogen concentrations. Fe(III) reducers are slightly less efficient and thus maintain somewhat higher hydrogen concentrations. Sulfate reducers and methanogenic bacteria are progressively less efficient and maintain even higher hydrogen concentrations. Because each TEAP has a characteristic hydrogen concentration associated with it, hydrogen concentrations can be an indicator of predominant redox processes. These characteristic ranges are given in Table 6.3. If hydrogen concentrations are very low, reductive dechlorination is not efficient.
and Type III behavior is indicated. If hydrogen concentrations are greater than approximately 1.0 nM, rates of reductive dechlorination should have environmental significance.

**Table 6.3**

Range of Hydrogen Concentrations for a Given Terminal Electron-Accepting Process

<table>
<thead>
<tr>
<th>TERMINAL ELECTRON- ACCEPTING PROCESS</th>
<th>DISSOLVED HYDROGEN CONCENTRATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(nmol/L)</td>
</tr>
<tr>
<td>Denitrification and Manganese Reduction</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Iron (III) Reduction</td>
<td>0.2 to 0.8</td>
</tr>
<tr>
<td>Sulfate Reduction</td>
<td>1 to 4</td>
</tr>
<tr>
<td>Methanogenesis</td>
<td>5 to 20</td>
</tr>
<tr>
<td><strong>Optimum for Anaerobic Reductive Dechlorination</strong></td>
<td>2 to 11</td>
</tr>
</tbody>
</table>

Adapted from Lovley et al., 1994; Chapelle et al., 1995; and Yang and McCarty, 1998 per AFCEE et al., 2004

* In gas phase in equilibrium with water containing dissolved hydrogen.

Redox measurements are based on the concept of thermodynamic equilibrium and, within the constraints of that assumption, can be used to evaluate redox processes in groundwater systems. The hydrogen method is based on the ecological concept of interspecies hydrogen transfer by microorganisms and, within the constraints of that assumption, can also be used to evaluate redox processes. These methods, therefore, are fundamentally different.

A direct comparison of these methods (Chapelle et al., 1995) has shown that redox measurements were effective in delineating oxic from anoxic groundwater, but that redox measurements could not distinguish between nitrate-reducing, Fe(III)-reducing, sulfate-reducing, or methanogenic zones in an aquifer. In contrast, the hydrogen method could readily distinguish between different anaerobic zones. For those sites where distinguishing between different anaerobic processes is important information, hydrogen measurements are an available technology for making such distinctions. At sites where concentrations of redox sensitive parameters such as dissolved oxygen, Fe(II), sulfide, and methane are sufficient to identify operative redox processes, hydrogen concentrations are not always required to identify redox zones.

In practice, it is preferable to interpret hydrogen concentrations in the context of electron acceptor (oxygen, nitrate, Fe(III), sulfate) availability and the presence of the final products (Fe(II), hydrogen sulfide, methane) of microbial metabolism (Chapelle et al., 1995). For example, if sulfate concentrations in groundwater are less than 0.5 mg/L, methane concentrations are greater than 0.5 mg/L, and hydrogen concentrations are in the 5-20 nM range, it can be concluded with a high degree of certainty that methanogenesis is the predominant redox process in the aquifer. Similar logic can be applied to identifying denitrification (presence of nitrate, hydrogen <0.1 nM); Fe(III) reduction (production of Fe(II), hydrogen 0.2 to 0.8 nM); and sulfate reduction (presence of sulfate, production of sulfide, hydrogen 1-4 nM).
Methods for measuring hydrogen in groundwater are commercially available. Hydrogen measurements can be useful, especially on sites where other parameters may not offer clear-cut indications of conditions favorable for enhanced reductive dechlorination. However, their use is limited, often due to cost of sample collection and analysis.

6.3 BIODEGRADATION RATE CONSTANT CALCULATIONS

If biodegradation has been stimulated by addition of edible oil, then an increase in biodegradation rates should be observed. Biodegradation rate constants should be estimated prior to substrate addition (if possible) and during performance monitoring. Biodegradation rate constant estimates can be calculated by many methods. The reader is referred to such documents as USEPA (1998) and Newell et al. (2003) for a detailed discussion of biodegradation rate constant estimation.

While monitoring contaminant biodegradation rates can be very useful, accurate estimation of biodegradation rate constants can be difficult due to partitioning of chlorinated solvents between the sediment, injected oil and aqueous phases. Monitoring well sample protocols tend to preferentially extract sample volume from zones of higher permeability. However, groundwater sampling results will also be influenced by the slow diffusion of contaminants out of lower permeability zones.

In contaminant source areas, there are no generally accepted methods for estimating overall average contaminant biodegradation rates. The point decay approach described by Newell et al. (2003) can be used to estimate rates of contaminant decline in individual monitor wells. However, these rates may not be representative of the entire treatment zone. Pure edible oil and oil-in-water emulsions are preferentially transported through the higher permeability (K) zones. As a consequence, biodegradation rates may be greater in the higher K zones than low K zones. Contaminant concentrations often decline very rapidly in monitor wells (which preferentially sample the high K zones), even though some contaminants remain in the lower permeability layers.

For a strongly heterogeneous site in the North Carolina Piedmont, Solutions-IES (2005) reported that TCE was reduced from approximately 1,000 µg/L to below detection within 50 days of emulsion injection. However, cis-1,2-DCE, VC, and ethene continued to be produced for over 12 twelve months indicating additional TCE was slowly diffusing out of lower permeability zones and being degraded. Slow diffusion of contaminants out of low K zones is not a problem as long as some oil remains to support contaminant biodegradation. However, if the oil is depleted before both the high and low K zones are remediated, additional oil injections may be necessary to maintain biodegradation rates.

In barrier systems, mass transfer between high and low K zones is less of an issue, and degradation rates can be calculated once geochemical and microbiological conditions stabilize. To be considered ‘stable’, important indicators of biogeochemical conditions (pH, ORP, DO, sulfate, methane) and contaminant biodegradation (contaminant molar ratios, Cl#) should be reasonably constant over three or more sampling events. Once conditions stabilize, degradation rates can be estimated by adjusting the rate constants in BIOCHLOR (Aziz et al., 2000; 2002) until model simulations approximately match average concentrations (after conditions stabilize) in monitor wells at various locations upgradient and downgradient of the barrier. Typically, degradation rates are assumed equal to background conditions, except in areas directly impacted by edible oil (indicated by DOC > 20 mg/L). Accurate estimates of