

ADVANCES IN UNDERSTANDING AND PREDICTING ENHANCED ANAEROBIC CAH BIOREMEDIATION

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ABSTRACT: There have been more than 20 years of bench-scale research on the anaerobic degradation of chlorinated alkenes. The authors now have benefited from seven years and 85 sites of field application experience with our *In Situ* Reactive Zone (IRZ) technology, which seeks to enhance anaerobic CAH bioremediation through the addition of selected electron donors. This abundance of bench-scale and field application experience does not tell us everything we need to know about this technology. We continue to make significant advances in understanding and predicting the performance of this technology at the field-scale. For example:

- Results of carefully designed demonstrations at DOD facilities that incorporate bromide injection as a nonreactive tracer show that the effects of the IRZ technology on dissolved oxygen and redox potential propagate through the formation more rapidly and widely than would be predicted by the tracer or total organic carbon results. This result can have important implications for the interpretation of process monitoring data at IRZ sites.
- Technologies similar to IRZ have traditionally been recommended primarily when methanogenic conditions could be established through carbon source introduction. Thus, sites with high sulfate levels, for example, have been viewed traditionally as poor candidates for this technology, since large carbohydrate doses would be necessary to consume all of the available sulfate. However, new bench-scale work from several groups, coupled with field experience at some commercial sites, suggests that high sulfate concentrations are not necessarily a barrier to successful implementation of this technology. Rather, it suggests that degradation under sulfate reducing conditions is very feasible, and that there are several mechanisms by which sulfur species can directly cause the degradation of chlorinated ethenes *in situ*.
- Results from the application of different carbohydrate donors (including molasses, whey, and glucose) have now allowed us to make some preliminary recommendations regarding the suitability of different donors to given site conditions.

We have gained significant experience in designing effective system modifications to control the low pH conditions that can form during carbohydrate enhanced anaerobic biodegradation.

Thus, this paper should add considerably to the "state-of-the-art" in the stimulation and enhancement of anaerobic biodegradation.

INTRODUCTION

Two decades of work has been done at various scales on the biotransformation of Chlorinated Aliphatic Hydrocarbons (CAHs) under anaerobic conditions (Vogel and McCarty, 1985; Parsons and Lage, 1985; Bouwer, 1993; and references cited therein). We have seven years and 85 sites of field application experience with our *In Situ* Reactive Zone (IRZ) technology. The IRZ technology seeks to enhance anaerobic CAH bioremediation through the addition of selected electron donors such as molasses, whey, glucose, or other carbohydrates (Suthersan, 1996, 2000, 2002). Many similar efforts by others have been reviewed by the Interstate Technology and Regulatory Cooperation Work Group (ITRC, 1998). This abundance of bench-scale and field application experience does not tell us everything we need to know about this technology. We continue to make significant advances in understanding and predicting the performance of this technology at the field-scale. This paper will present some of these advances in a “selected topics” format, drawing on other presentations and literature to support the arguments advanced.

Propagation Rates for Dissolved Gas Effects vs. Reagent. We have long observed anecdotally that changes in dissolved oxygen concentrations and redox potential appear long before the total organic carbon appears, which, in turn, generally precedes effective enhanced biodegradation (as would be expected mechanistically). However, in our commercial practice, we rarely utilize conservative tracers in the injection solution, and, therefore, we cannot readily determine if this effect was due to the expected sorption of the molasses components to the soil matrix. In recent Air Force Center for Environmental Excellence/Environmental Security Technology Certification Program (AFCEE/ESTCP) sponsored demonstration projects at Hanscom Air Force Base (AFB) and Vandenberg AFB, we utilized these tracers at 100 – 200 mg/l in the injected solution.

Dissolved oxygen (DO), oxygen reduction potential (ORP), total organic carbon (TOC), and Bromide (Br^-) tracer data at Vandenberg (a quite aerobic site pretreatment) suggest establishment of anaerobic conditions was rapid in nearby wells. TOC and Br^- have gradually spread to encompass at least four of the nine downgradient monitoring wells. DO and ORP indicate a wider area of reduced conditions – at least seven of the nine downgradient monitoring wells. Figure 4 in Lutes (2002b) clearly shows that the DO and ORP effect preceded and has spread more widely than the Br^- or TOC.

At Hanscom, a DO and ORP effect was very rapid – within weeks after the initial injection at all of the observation wells (Lutes, 2002a; Lutes, 2002b). Uncertain evidence for delivery of TOC, dissolved organic carbon (DOC), and Br^- was seen at two of the closest observation wells for the first 12 weeks of testing. Definitive evidence of increases in these indicators was seen six to seven months after injection began, and a month after we began using a clean water push after injection to reduce the carbon loading and pH drop proximate to the injection well.

Thus, it appears that the DO and ORP effects of the carbon source injection are often transported much more rapidly than the tracer, which is believed to be conservative (to move at the same velocity as the advective groundwater flow). This has a clear implication for systems operation. Since DO and ORP are parameters that can be measured rapidly in the field, project managers at low-cost enhanced bioremediation sites might be tempted to rely on them as primary indicators of the establishment of

appropriate conditions for enhanced treatment. This observation, however, would suggest that TOC or DOC monitoring should be done equally routinely to ensure the presence of an appropriate electron donor.

Role of Sulfur in Enhanced Bioremediation of CAHs. The role of sulfur in determining the effectiveness of enhanced bioremediation of CAHs is complex and multifaceted. Existing guidance documents tend to suggest that anaerobic bioremediation of CAHs proceeds best under methanogenic conditions. Even though they do document that CAH degradation under sulfate reducing conditions is feasible, it appears anecdotally to us that the majority opinion of field practitioners is that sulfate is problematic for CAH degradation. For example, Wiedemeier states, “Concentrations of sulfate greater than 20 mg/l may cause competitive exclusion of dechlorination. However, in many plumes with high concentrations of sulfate, reductive dechlorination still occurs” (Wiedemeier, 1998). The Wiedemeier protocol scores a site more poorly if sulfate exceeds that level, as does Morse (Morse, 1998). The ITRC guidelines state, “abundant electron acceptors such as sulfate, may inhibit reductive biodegradation.” Further, “High sulfate concentrations may prevent methanogenic conditions from developing,” and “the documentation of high sulfate mineral abundance can be used....to explain slow rates of reductive dechlorination.” Sulfate, whether injected or already present, indeed must be reduced in order to reach methanogenic conditions, but there is ample evidence in the literature for dechlorination of a wide variety of CAHs under sulfate reducing conditions (ITRC, 1998; Devlin and Muller, 1999). It has also been postulated that the presence of abundant electron acceptors, including sulfate, can interfere with the enhancement of dehalorespirators and that dehalorespiring organisms compete for electron donors such as hydrogen with both methanogenic and sulfate reducing organisms (Morse, 1998; Suthersan, 2002). Morse writes, “Depletion of electron acceptors should effectively eliminate competition between dechlorinators and such groups as nitrate reducers, iron reducers and sulfate reducers. Competition from Methanogens on the other hand may never be eliminated and must be managed by choice and delivery of electron donor.” Suthersan reviews the most recent literature and argues that “halorespirators can outcompete methanogens and sulfate reducers at any hydrogen concentration,” and, thus, that strategies that limit the generation of hydrogen to favor dehalorespirators, as recently advocated by others, are not necessary (Suthersan, 2002; Drzyzga, 2002).

Generation of hydrogen sulfide is indeed a problem in certain applications, including those under structures and where there is a direct route between the reactive zones and receptors (although this can be controlled with engineered gas capture systems). Generation of other reduced sulfur compounds, such as thiols (also known as mercaptans), is most likely to occur where the hydrogeology is unfavorable for dispersion due to low permeability or lack of gradient leading to conditions where fermentation predominates.

However, sulfur, whether contained in the formulation of a donor such as molasses or present in the system already, offers important advantages, including sulfide production for metals precipitation and potentially aiding biological degradation of CAHs, through a mechanism that has only recently been discussed by a Swiss academic group. Bushman et al., studied the reduction of halogenated methanes in the presence of cysteine and iron porphyrins. Their conclusion is relevant to the potential role of sulfur

in this system. "...in reactions involving PHMs (polyhalogenated methanes) and organic reductants exhibiting mercapto groups (e.g. mercaptojuglone as a model compound for natural organic matter in the presence of Hydrogen sulfide) an alternative initial reaction step may be an X-philic dissociative two-electron transfer.....The proposed reaction mechanism(s), particularly the proposed involvement of R-SH or R-S-S-R groups in the complete dehalogenation of PHMs, may be helpful in the (re)interpretation of microbially mediated dehalogenation reactions of such compounds. In addition, reduction of PHMs in organisms could lead to the formulation of NO₂- or SH- groups present in biologically important molecules... Finally, from a practical engineering point of view, the results of this study offer an interesting perspective for a fast, complete dehalogenation of PHMs, by using a very reactive one-electron donor (i.e. a reactive iron species) in the presence of organic compounds exhibiting reduced sulfur and nitrogen groups. Using such an approach, the formation of the less halogenated volatile compounds, that are orders of magnitudes less reactive than the parent compounds (sic) can be prevented." (Bushman, et al., 1999). Zwiernik and coworkers suggest that inducing and then "starving" sulfate reducers can induce another mechanism for CAH biodegradation (Zwiernik et al., 1998). The authors of this paper write "It seems plausible that the microorganisms responsible for para-dechlorination of PCBs described here and Desulfomonile tiedjei are both sulfate reducers whose growth is stimulated by sulfate additions. Then, following depletion of sulfate they utilize chloroaromatic compounds as electron acceptors resulting in dechlorination." Further, "We propose that the stimulatory effect of FeSO₄ on paradechlorination resulted from an increase in the population of sulfate reducing bacteria." Yet another mechanism is suggested in which sulfur may be beneficial in this application: by forming FeS, which abiotically degrades TCE and PCE (Butler and Hayes, 1999).

ARCADIS has successfully applied enhanced anaerobic bioremediation at sites with up to 500-700 ppm of sulfate. We have reviewed data that suggests natural attenuation at even higher levels (up to 2,000 ppm). Thus, it is clear that CAHs can be effectively treated under sulfate reducing conditions and that sulfur can be directly involved in several processes that enhance this degradation. Therefore, we believe that future guidance documents should be carefully written so as not to leave the nonspecialist practitioner with a too simplistic conclusion such as "my site has high sulfate, that's bad for enhanced anaerobic bioremediation, therefore, I should use an alternate technology." Rather, practitioners should recognize that multiple complex processes occur in these systems, that research on them is ongoing, and that the presence of substantial sulfate concentrations will not necessarily preclude enhancement of CAH bioremediation to cost-effective rates.

The amount of sulfur already in the groundwater system cannot be controlled. However, the amount of sulfur added to the system can be controlled by selection of a low sulfur (i.e., corn syrup less than 1 mg/l sulfate), medium sulfur (fancy, edible-grade molasses, 250 ppm sulfate) or higher sulfur (i.e., blackstrap molasses, 570 ppm sulfate) reagent. [Sulfate concentrations are given for 10% solutions, which is the typical injected concentration, substantial further dilution should be expected in the aquifer.] When using molasses as a carbon source, the amount of additional sulfur provided to the system can be controlled to some extent by the selection of grade among those electron donors commercially available in the food industry.

Suitability of Differing Electron Donors for Differing Conditions. Effective application of reductive dechlorination is governed by many site-specific conditions. The geochemical character of the matrix and groundwater, and hydrologic conditions such as groundwater velocity, influence the efficacy of *in situ* reductive dechlorination. In cases where extensive dissolved constituents of concern (COC) plumes are being treated, it is desirable that carbon supplements be consumed at a rate sufficient to both lower redox conditions and propagate the maximum area of desired treatment from the injection point. The rate of the carbon supplement release and consumption will influence the volume of aquifer being treated with each injection point and should be balanced with site-specific conditions. Excessive application can result in the production of excess levels of unwanted by-products such as methane or acids. Also, an excessive consumption rate can result in inadequate temporal distribution of the carbon substrate, resulting in an increase in frequency of injections and/or an increase in the number of injection points required to cover a given treatment area. Finally, some have argued that a slow steady release over time of hydrogen from degradation of the electron donor is desirable to optimize the biological conditions for CAH degradation (Smatalak 1996), although, as discussed above, this may not be necessary.

Where groundwater velocities are relatively high, the effect of the carbon supplements may be reduced by dilution into a large volume of oxygenated groundwater, and thus a high consumption of substrate may be necessary to reach anaerobic conditions required for treatment. In systems that are naturally aerobic, it may be necessary to use a rapidly acting carbon substrate to initially drive the redox potential down. Additionally, a highly degradable substrate may aid in overcoming the microbial lag phase attributed to anaerobic bacteria. Once reducing conditions are achieved, a more slowly acting carbon substrate may be desirable to minimize the cost of maintaining reducing conditions. Thus, using a mixture or cocktail of fast and slow acting products may be desirable. Where the groundwater velocities are relatively low, the issue of mixing with oxygenated groundwater is less critical. However, stimulated bacterial populations may increase to levels that too rapidly consume simple carbon substrates, making the volume of the treatment area relatively small.

The economic application of soluble carbon substrates thus requires the ability to match the biogeochemical and hydrodynamic character of the aquifer to the biogeochemical character of one or more sources of soluble carbon. Extensive bench scale work has been performed by numerous workers to test several potential carbon sources (ITRC, 1998). The selection of a carbon substrate(s) will be primarily driven by overall reaction rates, which are, in turn, controlled by the site conditions. The goal is to minimize cost by minimizing the number of required injection points, the number of injection events, and reagent cost (Harkness, 2000). The physical characteristics of the substrate (i.e., phase and solubility) may also make certain substrates more suitable than others in particular applications. Examples of candidate carbon substrate products for widespread field application include:

- Hydrogen, (gas, very rapidly used)
- Butyrate, lactate, etc. (soluble, pure compounds, rapidly used)
- Corn syrup (soluble, readily consumed)
- Molasses (soluble, readily consumed)
- Vegetable oils (partially soluble, readily consumed)

- Yeast extract (partially soluble, readily consumed)
- Whey or other milk solids (solid, slow releasing)
- Soluble humates (soluble, slowly consumed)
- Chitin (partially soluble, slow release)
- Organic mulches (partially soluble, slowly-to-readily consumed)
- Hydrogen release compound (HRC)TM (solid, slow releasing)
- Combination of various products

A cost comparison for a variety of different substrates is presented in Table 4.4 of (Suthersan, 2002) (see also Harkness, 2000).

System Modifications to Control pH reduction. The pH of the groundwater system generally decreases during the injection of degradable organic substrates to enhance *in situ* bioremediation. The magnitude of the pH decrease depends on the dose of substrate and the natural buffering capacity of the system (both the groundwater and the aquifer solids). As discussed in Suthersan, pH drops below 4.0 s.u. should generally be avoided (and stricter pH control may be advantageous where enhanced metals precipitation is sought) (Suthersan, 2002). The two sites involved in our current ESTCP/AFCEE demonstration projects (Lutes, 2002a; Lutes, 2002b) exhibit relatively low buffering capacity. In these cases, pH has been effectively controlled by careful carbon dose control and injection of a clean water push following reagent injection to disperse the dose away from the immediate vicinity of the well. Buffer systems can also be introduced in the injection solution to control pH (i.e. phosphates).

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