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Use of statistical tools to evaluate the reductive dechlorination of high levels of TCE in microcosm studies

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ABSTRACT

A large, multi-laboratory microcosm study was performed to select amendments for supporting reductive dechlorination of high levels of trichloroethylene (TCE) found at an industrial site in the United Kingdom (UK) containing dense non-aqueous phase liquid (DNAPL) TCE. The study was designed as a fractional factorial experiment involving 177 bottles distributed between four industrial laboratories and was used to assess the impact of six electron donors, bioaugmentation, addition of supplemental nutrients, and two TCE levels (0.57 and 1.90 mM or 75 and 250 mg/L in the aqueous phase) on TCE dechlorination. Performance was assessed based on the concentration changes of TCE and reductive dechlorination degradation products. The chemical data was evaluated using analysis of variance (ANOVA) and survival analysis techniques to determine both main effects and important interactions for all the experimental variables during the 203-day study. The statistically based design and analysis provided powerful tools that aided decision-making for field application of this technology. The analysis showed that emulsified vegetable oil (EVO), lactate, and methanol were the most effective electron donors, promoting rapid and complete dechlorination of TCE to ethene. Bioaugmentation and nutrient addition also had a statistically significant positive impact on TCE dechlorination. In addition, the microbial community was measured using phospholipid fatty acid analysis (PLFA) for quantification of total biomass and characterization of the community structure and quantitative polymerase chain reaction (qPCR) for enumeration of *Dehalococcoides* organisms (Dhc) and the vinyl chloride

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reductase (*vcrA*) gene. The highest increase in levels of total biomass and Dhc was observed in the EVO microcosms, which correlated well with the dechlorination results.

1. Introduction

Enhanced anaerobic biodegradation involves the addition of carbon substrates to the subsurface to stimulate anaerobic bacteria capable of reductively dechlorinating chlorinated solvents like tetrachloroethene (PCE) and trichloroethene (TCE) to the non-toxic end products ethene and ethane. In these processes, chlorinated compounds act as an electron acceptor for the dechlorinating microorganisms, while an electron donor is required to provide energy (Freedman and Gossett, 1989; McCarty, 1994). Hydrogen is generally considered to be the direct electron donor for reductive dechlorination, but is typically produced from the anaerobic fermentation of carbon substrates (Maymo-Gatell et al., 1995). A wide variety of electron donors are commercially available, including soluble donors like sugars, organic acids, and alcohols or insoluble or slow release donors like lactic acid polymers, emulsified vegetable oil (EVO), chitin, and wood chips (AFCEE et al., 2004). These donors ferment via different pathways and at different rates, producing varying levels of hydrogen, and affecting the ability of dechlorinating bacteria to grow and compete with other hydrogen consuming microorganisms (Fennell et al., 1997; Yang and McCarty, 1998).

Dechlorinating bacteria also require basic nutrients like nitrogen, phosphorus, trace minerals, and a circumneutral pH. Nutrients necessary for microbial growth are often present in sufficient quantities in soil and groundwater, but can be limiting in some cases. A diverse microbial population is required to accomplish both the electron donor fermentation and chlorinated solvent dechlorination reactions. Bacteria that can ferment electron donors and those that can dechlorinate TCE to cis-1,2-dichloroethene (cDCE) are present at most chlorinated solvent sites, although the *Dehalococcoides* (Dhc) group bacteria, which dechlorinate cDCE to vinyl chloride (VC) and ethene, are not present at all sites or may be heterogeneously distributed across individual sites (Fennell et al., 2001; Hendrickson et al., 2002). If Dhc are absent they can be added via bioaugmentation and will grow and proliferate in the subsurface under favorable conditions (Ellis et al., 2000; Harkness et al., 1999).

Other site specific variables that may impact the efficacy of enhanced bioremediation include environmental factors like temperature, pH, and/or the presence of inhibitory co-contaminants such as nitrate, carbon tetrachloride, chloroform, and 1,1,1-trichloroethane (Adamson and Parkin, 2000; Chappelle et al., 1996; Holliger et al., 1993; Nelson et al., 2002; Zhuang and Pavlostathis, 1995). Finally, high levels of chlorinated ethenes themselves may also be inhibitory to reductive dechlorination (Yang and McCarty, 2000a).

Laboratory microcosm studies are a common and usually cost-effective approach to evaluate whether enhanced bioremediation is an appropriate remedial approach for a specific site prior to field implementation (Morse et al., 1998). In addition to evaluating electron donors and determining if nutrients or bacterial populations are limiting, microcosm studies can also assess the impact of environmental factors, microbial distribution, co-contaminants, or concentration of target compounds without the expense and risk associated with field trials. However, testing effects individually is highly inefficient and may miss important interactions between variables, while testing all variables of interest simultaneously can require a large number of bottles, especially when replicates and controls are included. For example, one widely cited protocol for microcosm studies (Morse et al., 1998) recommends thirty bottles per location to evaluate three potential substrates or combinations and the effect of yeast extract and vitamin B_{12} addition with abiotic and unamended controls, all run in triplicate. This problem can be managed by using statistical tools to design microcosm studies and analyze the results. In this case, a fractional factorial experimental design can reduce the number of combinations of the participating variables while still enabling assessment of the effects and interactions that are of primary interest.

As an example of this approach, an extensive microcosm study was performed as part of project SABRE (Source Area BioREmediation), a public/private consortium of twelve companies, two government agencies, and three research institutions whose charter was to determine if enhanced anaerobic bioremediation can result in effective and quantifiable treatment of chlorinated solvent DNAPL source areas. The focus of this 4-year, $5.7 million dollar research and development project was a field site in the United Kingdom (UK) containing a dense non-aqueous phase liquid (DNAPL) source area with groundwater concentrations ranging from 150 to 1100 milligrams per liter (mg/L) TCE.

There were several unique features of the SABRE microcosm study. The program sought to demonstrate this technology in the field on a source area containing TCE DNAPL. Whereas direct biodegradation of the DNAPL itself is unlikely, enhancement in dissolution of the DNAPL phase has been demonstrated in several laboratory studies using PCE (Adamson et al., 2003, 2004; Carr et al., 2000; Cope and Hughes, 2001; Yang and McCarty, 2000b). These results have been reasonably replicated by modeling the dissolution process based on first principles utilizing dechlorination rate constants produced in the laboratory (Seagren et al., 1993, 1994; Sleep et al., 2002). PCE has a relatively low solubility (ca. 150 mg/L) and at this concentration, DNAPL dissolution is relatively slow and toxicity thresholds for the dechlorinating bacteria are generally not exceeded. TCE has a much higher solubility (ca. 1100 mg/L), so dissolution is faster and toxicity issues more pronounced. For these reasons, fewer DNAPL studies have been performed with TCE.

As biodegradation in proximity to DNAPL has been recognized, interest has grown in use of electron donors that can partition into the DNAPL phase (Cápiro et al., 2011; Yang and McCarty, 2002). Donors in this category include EVO and longer chain alcohols. If these donors can be effectively introduced into the subsurface and mixed with the DNAPL, they will slowly dissolve into the water phase where they can be fermented and provide a long-term source of hydrogen at the DNAPL water interface for the dechlorination