A Systematic Approach to *In Situ* Bioremediation in Groundwater

Including
Decision Trees for *In Situ* Bioremediation of Nitrates, Carbon Tetrachloride, and Perchlorate

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EXECUTIVE SUMMARY

Applications submitted to regulatory authorities containing *in situ* bioremediation have not been prepared nor evaluated using a consistent and accepted suite of parameters. The ITRC In Situ Bioremediation (ISB) Team prepared this document to provide guidance for the systematic characterization, evaluation, and appropriate design and testing of ISB for any biotreatable contaminant. It serves as guidance for regulators, consultants, responsible parties, and stakeholders when an ISB technology is considered.

The ISB Team is composed of eight state environmental agencies (New Mexico, North Dakota, Missouri, Oklahoma, Colorado, New Hampshire, Kansas, and Virginia), three federal agencies (DOE, DOD, and EPA), 12 companies, two universities, and public stakeholders. ITRC has produced a number of products related to ISB, which are available at ITRC's Web site (www.itrcweb.org).

Bioremediation is the application of biological treatment to the cleanup of contaminants in groundwater. Bioremediation melds an understanding of microbiology, chemistry, hydrogeology, and engineering into a cohesive strategy for planned and controlled microbial degradation of specific classes of organic compounds and in certain instances, inorganic compounds. This assemblage of science and engineering requires a rigorous degree of data evaluation to determine the effect and efficiency of bioremediation.

In situ bioremediation creates subsurface environmental conditions, typically through oxidation-reduction manipulation, which induce the degradation of chemicals (i.e., the target chemical) via microbial catalyzed biochemical reactions. In turn, the microbes produce enzymes that are utilized to derive energy and that are instrumental in the degradation of target chemicals. To accomplish this chain of events, the following aspects must be considered:

- type of microorganisms,
- type of contaminant, and
- geological conditions at the site.

Since *in situ* conditions are manipulated by engineered means, the most important consideration is the ability to transmit and mix liquids in the subsurface.

In response to ITRC's and California's requests for clarification of RCRA, EPA's director of the Office of Solid Waste clarified EPA's policy on the injection of contaminated groundwater by explaining that reinjection of treated groundwater to promote *in situ* treatment is allowed under 3020(b) as long as…the groundwater is treated prior to reinjection; the treatment is intended to substantially reduce the hazardous constituents in the groundwater before or after reinjection; the cleanup must be protective; and the injection must be part of a response action under CERCLA or RCRA. In addition, if the injected fluid contains a hazardous waste, and the fluid is being injected into an aquifer, an exception to the usual prohibition of Class IV Underground Injection Control wells is available for CERCLA and RCRA cleanups. The ISB Team of ITRC concludes from the reviews conducted as part of this project, there are no regulatory barriers preventing the full use of *in situ* bioremediation to remediate nitrate, carbon tetrachloride, and perchlorate.

All indications point to enhanced *in situ* biodenitrification as a reasonable remediation alternative for nitrate- (NO_3) contaminated groundwater. The decision tree in Section 8.0 of this guidance provides the user a process of evaluating the applicability of enhanced *in situ* biodenitrification. The first step is to define site oxidation-reduction potential (ORP) (see Section 3.1.1 and Figure 3-1). ORP measurements are taken to determine which constituent will be the electron acceptor. Nitrate may serve as an electron acceptor at an ORP value of approximately 750 mv, after oxygen has been depleted. If ORP is less than 750 mv, it is likely that NO_3 will not be present in groundwater since it has already denitrified to nitrogen (N_2) gas. If the dissolved oxygen concentration is above 2.0 mg/L, it needs to be reduced, which can be achieved by adding additional carbon to the treatment area.

Dechlorination and cometabolism are two major reductive pathways for ISB of carbon tetrachloride (CT). The cometabolic pathway occurs either through reductive dechlorination or denitrification. The first decision tree in Section 9.0 of this guide describes reductive dechlorination through direct or cometabolic reduction, while the second decision tree describes a reductive denitrification-cometabolic pathway. The key to the dechorination pathway is the recognition of degradation products and the ability to carry this reduction to completion so neither contaminants nor degradation products are above site closure criteria. Treatability tests can determine if the necessary halorespirers are present to affect degradation.

The reductive cometabolic dechlorination pathway yields degradation products through cometabolic processes and not by serving as electron acceptors. They are produced fortuitously when biologically produced enzymes or cofactors degrade carbon tetrachloride during the microbial consumption of an alternate carbon source. The reductive cometabolic denitrification pathway yields little to no degradation products.

Section 10.0 provides a decision pathway assessing the reductive pathway for *in situ* bioremediation of perchlorate (ClO_4) in groundwater. More than 30 different strains of perchlorate-reducing microbes have been isolated from diverse environments. In this reductive process, bacteria utilize the perchlorate ion as a terminal electron acceptor. Perchlorate is ultimately completely converted into chloride and oxygen through the anaerobic reduction process. The perchlorate-to-chlorate step is thought to be the rate-limiting step, being considerably slower than the other steps. Buildup of toxic intermediates, specifically chlorite, does not occur as the chlorite-to-chloride step proceeds at a rate on the order of 1000 times that of the accepted rate-limiting step.

Contaminants and breakdown products differ; however, many characteristics of a site used to determine the efficacy of ISB are similar. Once a site has been characterized and the contaminants of concern and daughter products have been defined, engineered approaches can be designed, pilot tested, and deployed. Flow diagrams define the primary decision points and characteristics evaluating ISB either through monitored natural attenuation (MNA) or enhanced ISB.