

A Systematic Approach to In Situ Bioremediation in Groundwater

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In situ bioremediation (ISB) melds an understanding of microbiology, chemistry, hydrogeology, and engineering into a strategy for planned and controlled microbial degradation of specific contaminants. ISB creates subsurface environmental conditions, typically through reduction oxidation manipulation, which induce the degradation of contaminants 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 type of microorganisms, contaminant, and the geological conditions at the site must be considered. Since in situ conditions are manipulated by engineered means, the most important consideration is the ability to transmit and mix liquids in the subsurface. The Interstate Technology Regulatory Council (ITRC)–ISB Team has recently completed a guidance document that describes a systematic approach to ISB in groundwater. ITRC is a state-led coalition of more than 40 states working together with industry and stakeholders to achieve regulatory acceptance of environmental technologies. © 2003 Wiley Periodicals, Inc.

INTRODUCTION

Groundwater remediation applications involving in situ bioremediation (ISB) submitted to regulatory authorities have not typically been prepared or evaluated using a consistent approach. This has led to inefficient and inconsistent methods of decision making. The ISB Team of the Interstate Technology Regulatory Council (ITRC) has developed a guidance document that provides a systematic approach to evaluate the feasibility and effectiveness of ISB technologies with the expectation that this methodology can be applied at any site for any specific contaminant (ITRC, 2002). ITRC is a state-led, national coalition of personnel from the regulatory and technology programs of some 40 states and the District of Columbia; three federal agencies; and tribal, public, and industry stakeholders. The organization is devoted to lowering regulatory barriers to the acceptance and deployment of innovative, improved, and more cost-effective environmental technologies.

The systematic approach to ISB is embodied in a decision tree for reviewing, planning, evaluating, and approving ISB systems for contaminated groundwater. This approach defines relevant site-specific parameters as well as appropriate ranges of criteria necessary for characterization, testing, design, and monitoring of ISB technologies. Although contaminants and their biodegradation pathways can vary widely, many site characteristics used to assess the feasibility and determine the efficacy of ISB are similar. Once contaminants of concern and their degradation products have been identified at a site and additional information to evaluate the feasibility of implementing ISB has been

tion rates can be enhanced by manipulating subsurface conditions. To do this effectively requires an understanding of the natural capacity of the subsurface for contaminant degradation. The flow diagram shown in Exhibit 1 defines the elements and primary decision points for evaluating MNA and enhanced ISB as remedial options. The flow diagram references sections of the ITRC guidance document (ITRC, 2002) where each element is more thoroughly discussed. This guidance document, among others, may be found at the ITRC Web site (www.itrcweb.org).

What Is In Situ Bioremediation?

Bioremediation is the process by which living organisms act to degrade or destroy contaminants. Typically, it involves the control and manipulation of microbial processes in above-ground reactors or in the subsurface for in situ treatment.

Bioremediation requires an understanding of microbiology, chemistry, hydrogeology, and engineering into a cohesive strategy for controlled microbial degradation of specific classes of organic compounds and in certain instances, inorganic compounds as well. This assemblage of science and engineering requires a rigorous degree of data evaluation to determine the effect and efficiency of bioremediation.

ISB involves the creation of subsurface environmental conditions conducive to the degradation of chemicals (i.e., contaminants of concern) via microbially catalyzed biochemical redox reactions. In other words, certain microbes can be induced to degrade specific chemicals in the subsurface at accelerated rates by optimizing environmental conditions to promote their growth and reproduction (Cookson, 1995). The microbes produce enzymes that are utilized to derive energy, and which are instrumental in the degradation of target contaminants. In order for this chain of events to be realized, however, a number of crucial elements must converge:

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

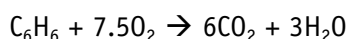
Once converged, microbial activity is accelerated and, in turn, causes “biological” destruction of target chemicals. Thus bioremediation provides for an elegant and cost-effective way to attack chemicals in the environment using naturally occurring microbes.

Microbes and Oxidation-Reduction Reactions: The basic premise of bioremediation is to accelerate microbial activity by providing an adequate substrate (i.e., carbon source) and nutrients (i.e., phosphorus, nitrogen) to create conditions favorable to biodegradation of a target chemical or contaminant. This concept in itself is not new. Sanitary engineers understood the implications of bioremediation as early as the turn of the 20th century when the earliest versions of the common “sewage treatment plant” were first utilized for treatment of raw human excrement (i.e., sewage). These engineers recognized that controlled aeration of sewage would cause a decrease in odor and “offensiveness.” They also observed that the “effluent” from such “treatment” could be easily settled (i.e., clarified) and then discharged to a watercourse without the detrimental effects of the original raw sewage. This was one of the first applications of engineered bioremediation systems to enhance environmental conditions.

What these engineers discovered was that microbial conditions could be optimized through an engineered approach that resulted in biodegradation of the obnoxious

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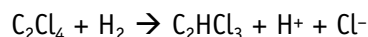
organic matter and also produced positive impacts on the overall environment. But to accomplish such an effect, a more precise understanding of microbes and microbial processes needed to be developed. Microbes can “use” a variety of organic chemicals for their own growth and propagation. These organic chemicals may serve various functions, but their primary use may be as either a carbon source for growth or as a source of “electrons” for energy. Microbes extract energy by catalyzing energy-yielding biochemical reactions, thus enzymes produced by the microbe can cleave chemical bonds and assist in the transfer of electrons from a chemical compound. These types of reactions are termed oxidation-reduction reactions, where one chemical (the electron donor) is oxidized (i.e., electrons are lost) and another chemical (an electron acceptor) is reduced. For instance, a hydrocarbon compound, such as benzene, can serve as an electron donor, while oxygen can serve as an electron acceptor. This is the basis of the classic aerobic respiration process. Here microbes “eat” benzene and “breathe” oxygen:



During the anaerobic degradation of organic contaminants, nitrate, sulfate, iron, manganese, or carbon dioxide can function as electron acceptors.

In this case, one would expect to observe decreases in concentrations of benzene and dissolved oxygen and a simultaneous increase in carbon dioxide in groundwater. Such resultant biochemical signatures can be monitored.

At the other end of the spectrum are the anaerobic processes, which occur in the absence of oxygen. During the anaerobic degradation of organic contaminants, nitrate, sulfate, iron, manganese, or carbon dioxide can function as electron acceptors. In some special cases, organic chemicals can play the role of electron acceptor. For example, anaerobic hydrogen-oxidizing bacteria can dechlorinate tetrachloroethylene (PCE) to trichloroethylene (TCE) with the release of a proton (H^+) and chloride ion:



The biochemical signature of this reaction in groundwater includes a decrease in parent PCE compound concentration with a concomitant increase in degradation product (TCE) and chloride ion. Depending on the specific subsurface environment, a decrease in pH may not be observed, particularly in the case of carbonate-rich formations that tend to rapidly neutralize the released H^+ . Under extremely reducing conditions, one would expect to observe the complete reductive dechlorination of PCE to ethene.

Hydrogeologic Environment: The hydrogeologic environment largely controls contaminant distribution in the subsurface. Some important hydrogeologic factors influencing subsurface transport and fate of contaminants include:

- contaminant distribution and concentrations in source area(s),
- groundwater flow direction and velocity,
- dispersion,
- retardation (e.g., sorption), and
- biogeochemical conditions (biodegradation).

Once a contaminant reaches the water table and dissolves into groundwater, advection and dispersion play major roles in its subsequent distribution in the subsurface. Advection is the movement of contaminants carried by groundwater in the direction of flow and is controlled by the linear velocity of the groundwater. That is, dissolved con-

taminants generally move in proportion to the groundwater velocity. Dispersion is the “spreading out” of a contaminant plume and includes both molecular diffusion and mechanical mixing. Many factors affect dispersion, including pore size distributions, pore geometry, and the fabric of the subsurface matrix (stratification, etc.).

Moreover, since ISB requires manipulation of subsurface conditions by engineered means, one of the most important feasibility considerations is the ability to transmit liquids in the subsurface. For systems that circulate liquids in the subsurface, hydraulic conductivity is an important parameter. Generally, target zone hydraulic conductivity values on the order of 10^{-4} cm/sec or greater are adequate for transmission of liquids (i.e., bioremediation amendments).

As can be seen from the above synopsis, the underpinning of ISB is based on application of diverse scientific and engineering disciplines. It requires simultaneous evaluation of subsurface hydrogeology, contaminant interactions, and biogeochemistry. It necessitates the ability to scientifically understand, predict, monitor, and engineer the in situ co-location of contaminants, substrate, nutrients, and microbial processes to achieve bioremediation. ISB entails establishing optimal subsurface conditions, utilizing injected substrates and nutrients, to enhance natural biodegradation, the ultimate result of which is accelerated destruction of the target contaminants.

SITE CHARACTERIZATION

Understanding the site background, history of contamination, and contaminant characteristics is required prior to remediation at any contaminated site. The site concep-

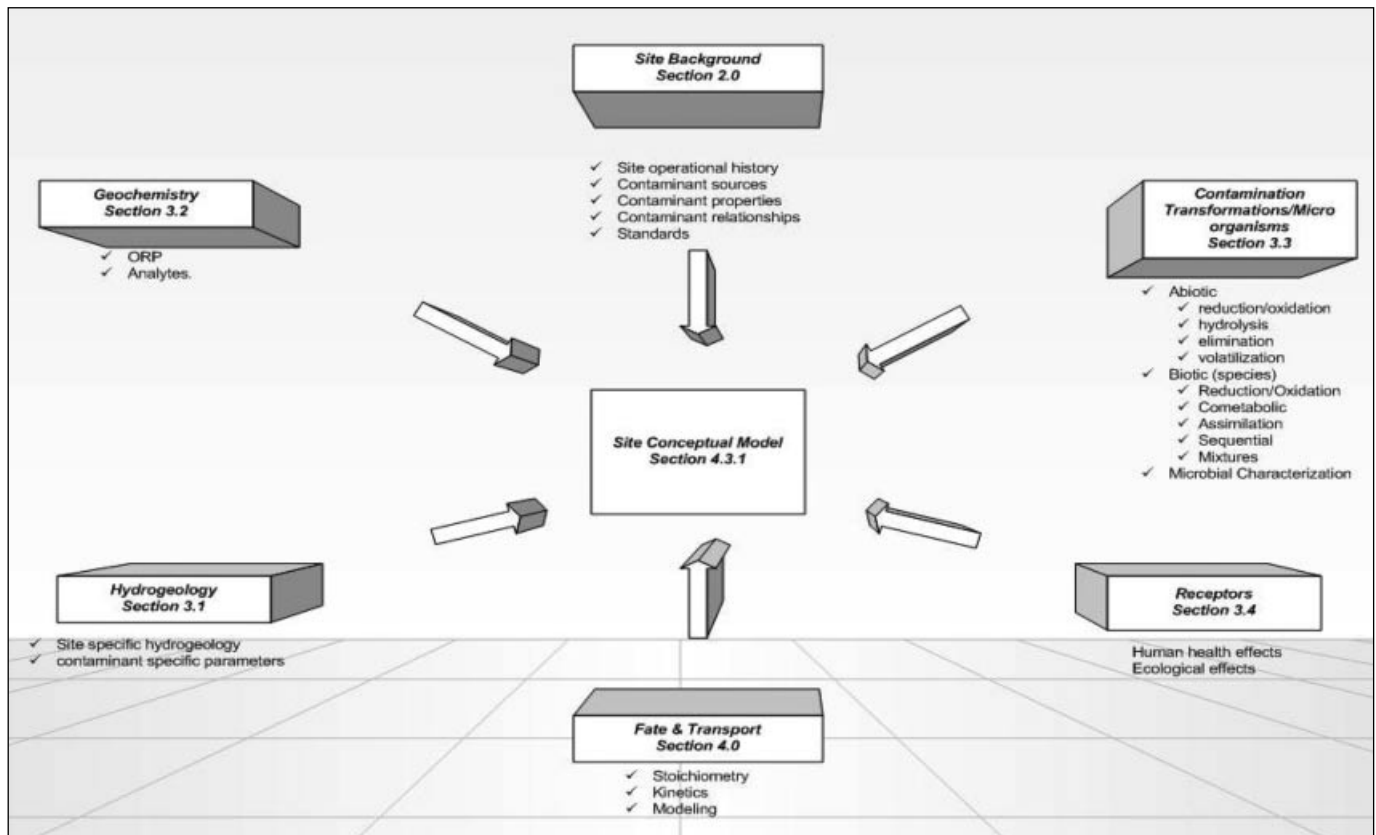


Exhibit 2. Site characterization block diagram.

tual model block diagram in Exhibit 2 displays the elements of site characterization used to develop the site conceptual model. The conceptual model is a representation of the physical, chemical, biochemical, and hydrologic characteristics of the subsurface as visualized from the site investigation. The subsurface system is represented in terms of hydrostratigraphic units, hydrologic boundaries, matrix type, groundwater flow, contaminant concentrations and distribution, and processes that affect the fate and transport of contaminants. Information and data specific to the site, contaminants, and relevant biogeochemical reactions must be understood in order to evaluate whether the contaminated site is amenable to ISB. Even if a contaminant is known to be biodegradable, site conditions govern the overall effectiveness of biodegradation in meeting remediation goals.

The major elements of an ISB-specific site characterization include review of existing site data, identification of data needs for hydrogeologic, geochemical, source area, and plume characterization, and development and implementation of work plans for collecting this data. Once this information is obtained, it is integrated into a site conceptual model. Based on this model, an initial decision on whether to further consider ISB can be made. A more detailed discussion can be found in ASTM (1996).

When considering the application of ISB, it is important to have an overall understanding of the site and its characterization. Several interrelated factors determine whether ISB is applicable at a specific site. These factors include:

- hydrogeology,
- geochemistry,
- contaminant transformations, and
- receptors.

Some of these factors and their interrelationships are considered in more detail below.

Hydrogeology

Hydrogeologic site characterization provides a basis for predicting how fluids and solutes move through the subsurface. Only the aspects relevant to ISB are discussed here. These include parameters that control the transport and distribution of contaminants and amendments added to enhance biodegradation in the subsurface. Hydrogeologic characterization parameters important to ISB include:

- lithology,
- hydraulic conductivity (k),
- effective porosity (n_e),
- hydraulic gradient (i),
- groundwater flow velocity (v),
- depth to water,
- dispersion, and
- dilution.

Contaminant-specific parameters that determine the rate of transport of COCs in groundwater include:

The major elements of an ISB-specific site characterization include review of existing site data, identification of data needs for hydrogeologic, geochemical, source area, and plume characterization, and development and implementation of work plans for collecting this data.

- octanol-water partitioning coefficient (K_{ow}),
- organic carbon partitioning coefficient (K_{oc}),
- soil-water distribution or partition coefficient (K_d), and
- retardation factor (R_f).

The distribution or partition coefficient describes the equilibrium distribution of a chemical between solids and groundwater. This is usually described as a sorption isotherm, which relates the concentration of the chemical sorbed onto the soil to the concentration in solution at equilibrium. It is expressed as

$$K_d = \frac{C_s}{C_w}$$

where K_d = distribution coefficient (cm^3/g), C_s = sorbed concentration ($\text{g}/\text{g}\text{-soil}$), and C_w = dissolved concentration ($\text{g}/\text{cm}^3\text{-solution}$). For hydrophobic organic chemicals, K_d is also related to the organic carbon partitioning coefficient of the chemical through the organic carbon fraction of the subsurface matrix through

$$K_d = K_{oc} \times f_{oc}$$

where K_{oc} = organic carbon partitioning coefficient, and f_{oc} = fraction of organic carbon in the matrix.

Retardation is the process by which the movement of a reactive chemical through an aquifer is slowed or impeded due to sorption. Retardation is expressed in terms of the retardation factor:

$$R_f = 1 + \frac{P_b \times K_d}{n_e}$$

where P_b = bulk density of the matrix (g/cm^3), and n_e = effective porosity (cm^3/cm^3). The retardation factor represents the velocity of transport of the chemical relative to the velocity of groundwater flow. The transport velocity of the chemical in groundwater, v_c , can be derived from R_f by

$$v_c = \frac{v}{R_f}$$

where v = groundwater velocity, and v_c = velocity of the chemical in groundwater. This is an important consideration for ISB systems because a contaminant that is strongly retarded may have limited availability for ISB.

Geochemistry

Essentially all reactions responsible for contaminant biodegradation are microbially mediated redox reactions. As such, the oxidation-reduction potential (ORP), measured in millivolts (mV), can be used as an indirect indicator of the in situ redox conditions (i.e., which terminal electron-accepting processes or TEAPs are active), and hence to infer whether conditions are suitable for a particular contaminant transformation reaction to occur. Indigenous bacteria capable of mediating contaminant redox transformations are present in most aquifers. The most oxidizing electron acceptor in groundwater is usually dissolved oxygen. Given sufficient electron donors, aerobic bacteria can flourish. A wide range of studies have

The distribution or partition coefficient describes the equilibrium distribution of a chemical between solids and groundwater.

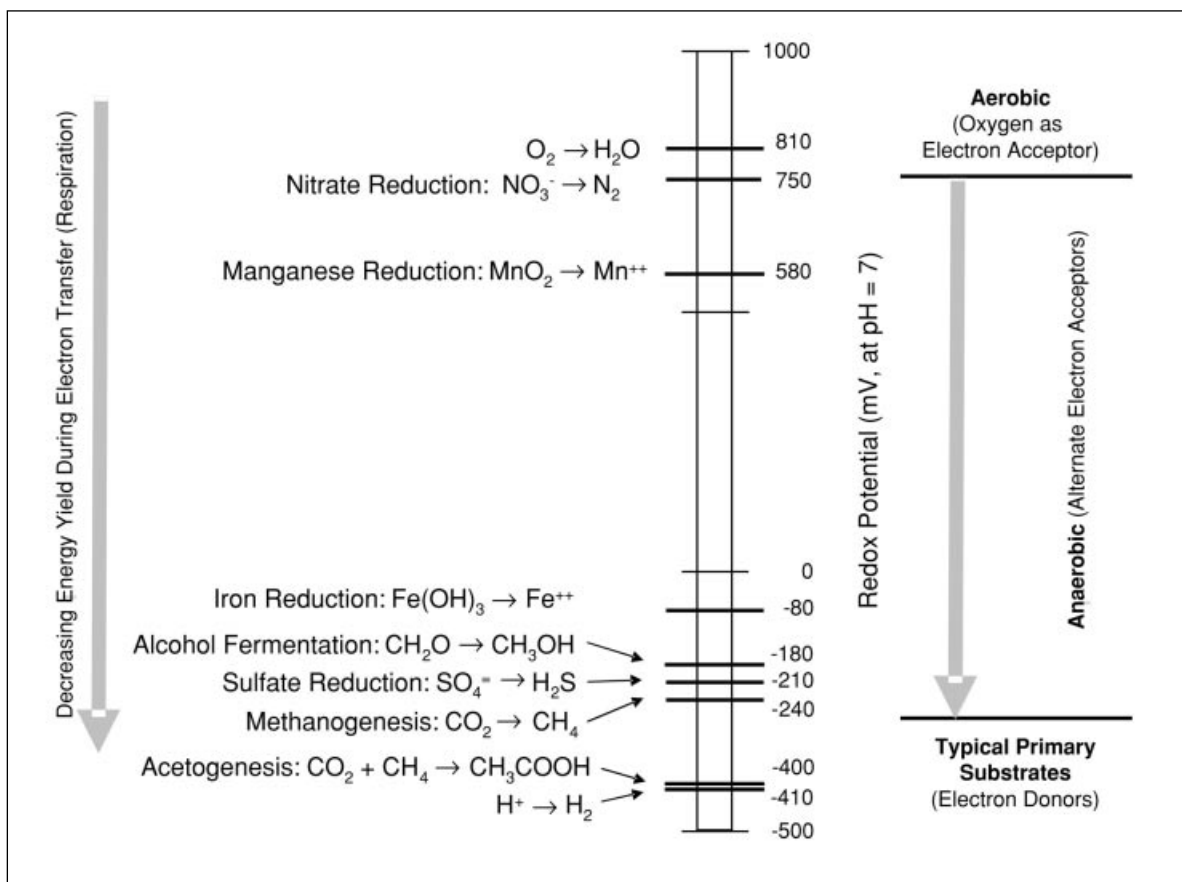


Exhibit 3. Equilibrium potentials of redox couples of commonly monitored chemical species.

shown that petroleum hydrocarbons (electron donors) are readily biodegraded in the subsurface if sufficient dissolved oxygen is present or supplied. Other contaminants have been shown to be biotransformed and/or become immobilized under aerobic conditions as well.

Biodegradation of contaminants by anaerobic bacteria proceeds in the absence of dissolved oxygen. Under anaerobic conditions, specific contaminants can act either as electron donors or electron acceptors. Exhibit 3 shows the equilibrium potentials of some relevant redox reactions on the redox potential (Eh) scale. Exhibit 4 shows half reaction potentials for ORP of halogenated aliphatic compounds. At Eh values greater than the equilibrium potential of a given redox couple, the reduced species is thermodynamically less stable than the oxidized species, and its oxidation is favored. Conversely, at Eh levels below the equilibrium potential, reduction of the oxidized species is favored. This tool can be useful, in conjunction with ORP measurements, to infer the distribution of active electron accepting processes in the subsurface. The equilibrium potentials were calculated for a pH of 7. Because many redox equilibria are pH-dependent, they should be evaluated for site-specific pH conditions. In some cases, depending on pH, the relative positions of specific redox equilibrium potentials may be reversed.

Care must be exercised when interpreting ORP measurements. ORP measurements made in the field may be affected by several factors, including the transient presence of oxygen as well as lack of attainment of equilibrium of all redox couples with the ORP electrode. In theory, the measured potential is related to the relative concentrations of the reduced (subscript *red*) and oxidized (subscript *ox*) species of a redox couple through the Nernst equation:

$$E = E_0 - \left(\frac{RT}{zF} \right) \ln \left(\frac{a_{\text{red}}}{a_{\text{ox}}} \right)$$

where E is the measured potential, E_0 is the standard equilibrium potential of the redox couple, R is the gas constant, T is absolute temperature in K, z is the charge number of the electrode reaction (which is the number of electrons transferred), and F is the Faraday constant ($96,500 \text{ C mole}^{-1}$). Conventionally, ORP is expressed relative to the standard hydrogen electrode, and is denoted E_h . E_h is calculated as follows:

$$E_h = E + E_{\text{ref}}$$

where E_h = redox potential referred to hydrogen scale, E = measured reduction-oxidation potential relative to reference electrode, and E_{ref} = reduction-oxidation potential of the reference electrode relative to the standard hydrogen electrode, all in mV.

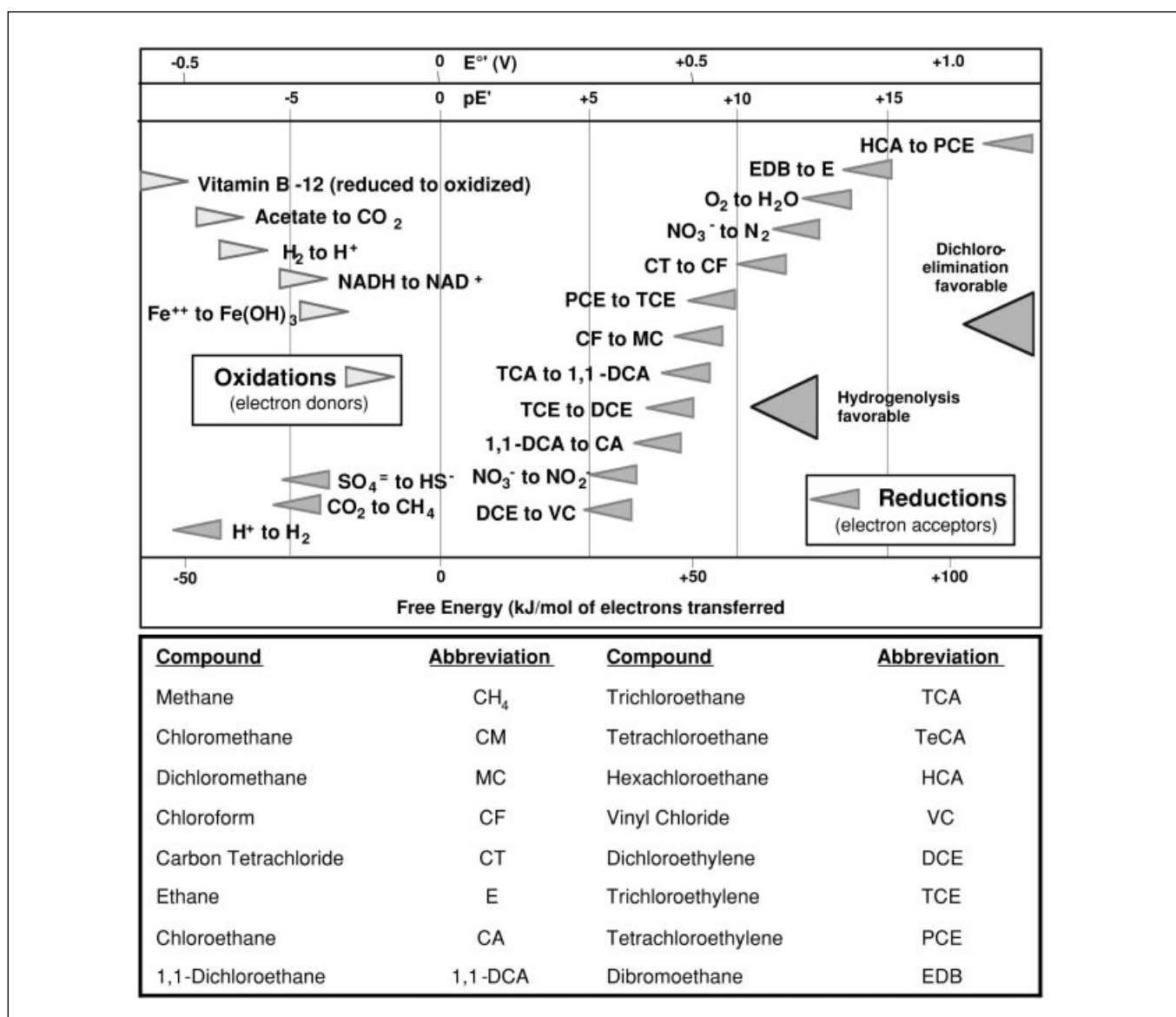


Exhibit 4. Half reaction potentials for ORP of halogenated aliphatic compounds: potential electron acceptors and electron donors.

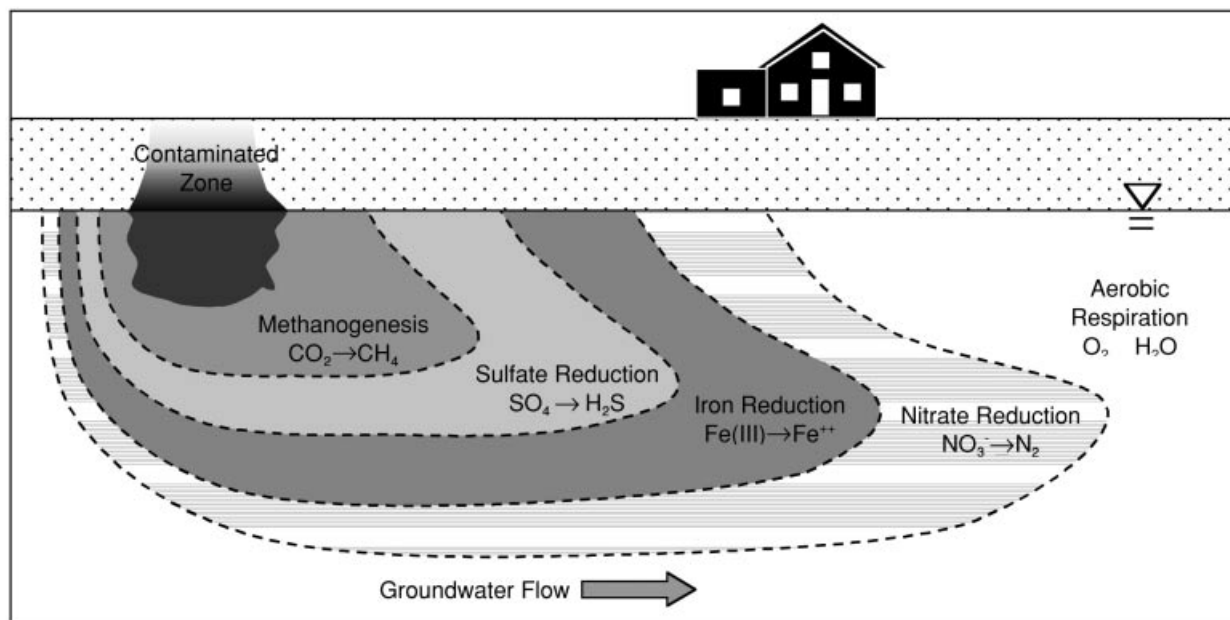


Exhibit 5. Idealized sequence of terminal electron acceptor processes.

Many chlorinated hydrocarbons that are commonly associated with groundwater contamination have been observed to dechlorinate in one or more of the redox environments associated with the terminal electron-accepting processes (TEAPs) shown in Exhibit 5. Both direct halorespirers and bacteria involved in the various TEAPs can reduce chlorinated solvents. The stimulation of these bacteria under reducing conditions is essential to anaerobic dechlorination reactions. Starting with fully chlorinated compounds (i.e., carbon tetrachloride and tetrachloroethylene, or PCE), dechlorination occurs as a stepwise process. During each step of the transformation, one chloride ion is released and one hydrogen is gained. The electrons released in the process are believed to be a source of energy for the bacteria. The reductive dechlorination of PCE and carbon tetrachloride can occur over a large Eh range, encompassing both denitrification and methanogenesis. Vinyl chloride (VC), on the contrary, is anaerobically reduced in a more limited range at or below the Eh of sulfate reduction. VC reductive dechlorination is reportedly dependent on the availability of H_2 , which acts as an electron donor for respiration (Newell et al., 1998). On the other hand, VC has been reported to undergo direct or cometabolic degradation under aerobic conditions. It is therefore not considered persistent.

Denitrification and manganese reduction can occur in slightly aerobic as well as anaerobic groundwater. At slightly lower Eh values than those for denitrification, manganese oxides are reduced to more soluble Mn^{++} . At still lower Eh, ferric iron is reduced to much more soluble Fe^{++} . Thus, high groundwater concentrations of dissolved iron (Fe^{++}) would be suggestive of anaerobic iron-reducing conditions. This could be further confirmed by field-measured Eh and a lack of measurable dissolved oxygen. Even more reducing conditions are characterized by sulfate reduction to hydrogen sulfide (H_2S), which possesses a pungent rotten-egg odor. Very low Eh values may be indicative of methanogenesis, in which carbon dioxide (CO_2) is reduced to methane (CH_4). Such conditions are common in groundwater beneath landfills, where high levels of organic carbon support fermentation of complex organic matter to

hydrogen gas (H₂), alcohols, and short chain organic acids. Fermentation products fuel methanogenic reactions and other anaerobic processes.

There are several analytes, in addition to contaminants of concern and their breakdown products, that are useful in characterizing and evaluating ISB systems. These analytes are listed and discussed in Exhibit 6.

Contaminant Transformations

Transformation of groundwater contaminants can be defined as a change in contaminant state, including physical changes, such as transformation from a liquid to a gas, or chemical changes. Transformation can occur by both abiotic and biologically medi-

Analyte	Analytical Method	Holding Time	Sample Volume	Use
Alkalinity	EPA 310.1 (field)	14 days	100 ml	CO ₂ and HCO ₃ ⁻ are produced by microbial respiration; an increase in alkalinity may indicate microbial activity; organic acid production (acetogenesis) also lowers the pH which increases carbonate solubility.
Chloride	EPA 325.3	28 days	100 ml	A conservative tracer; for chlorinated contaminants, an increase may indicate reductive dechlorination.
Dissolved Oxygen	Field ¹	-	-	A major electron acceptor, high levels (>2 mg/l) indicate aerobic conditions, which persist until it is depleted.
Manganese (dissolved)	EPA 6010B EPA 200.7 (field) ²	180 days ³	250 ml	An increase in dissolved manganese (Mn(II)) relative to background, may indicate anaerobic Mn(IV)-reducing conditions.
Iron (dissolved)	EPA 6010B EPA 200.7 (field) ²	180 days ³	250 ml	An increase in dissolved iron (Fe(II)) relative to background, may indicate anaerobic Fe(III)-reducing conditions.
Nitrate/nitrite (total)	EPA 353.2 (field)	28 days ³	500 ml	A decrease in nitrate, relative to background, may indicate anaerobic nitrate-reducing conditions.
pH	Field	-	-	Optimum range for ISB is 5 to 9.
Phosphate as P (soluble)	EPA 365.1	28 days ³	100 ml	Nutrient needed for microbial growth; may need to be added as amendment to promote biodegradation.
Oxidation-Reduction Potential (ORP)	Field ¹	-	-	Measurement of reducing or oxidizing environment may be indicative of a real or potential biological activity. Note that ORP values may be difficult to measure accurately.
Sulfate	EPA 375.4 (field)	28 days	100 ml	A decrease in sulfate, relative to background, may indicate anaerobic sulfate-reducing conditions; may be accompanied by an increase in sulfide.
Methane	GC-0019	14 days	40 ml	An increase in methane, relative to background, indicates reducing conditions, possibly methanogenesis (microbial production using carbon dioxide as an electron acceptor).
Total organic carbon	EPA 415.1	28 days ³	100 ml	TOC may provide electron donors for biodegradation, thereby reducing the amount of electron donor amendment required; may affect retardation of contaminants due to sorption.

¹ Obtaining meaningful measurements in the field may be difficult, and may provide conflicting results.

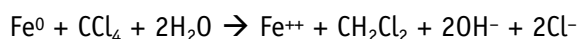
² EPA 6010B is used for RCRA projects; EPA 200.7 is used for NPDES (CWA) projects

Holding time is based on the proper preservative added to the sample.

Exhibit 6. Useful analytes for bioremediation evaluations.

ated processes. Natural attenuation of most groundwater contaminants is predominately due to biotransformation processes rather than abiotic reactions. This is so because many abiotic redox reactions are very slow in nature, and many contaminated systems are in a state of redox disequilibrium. Bacteria can make use of the chemical energy stored in the thermodynamically unstable compounds by facilitating otherwise sluggish redox reactions.

Abiotic transformations may include reduction-oxidation reactions, hydrolysis reactions, elimination reactions, and volatilization. An example of an abiotic reduction-oxidation reaction is the reduction of carbon tetrachloride by zero-valent iron to produce methylene chloride:

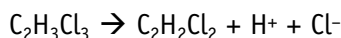


Hydrolysis of carbon tetrachloride is very slow under natural conditions, with a half-life of approximately 41 years:



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Elimination reactions are nucleophilic reactions that can result in transformation of alkanes to alkenes. An example of an elimination reaction is the dehydrohalogenation of chlorinated solvents, such as the transformation of 1,1,1-trichloroethane to 1,1-dichloroethene:



In this reaction, a chlorine ion is removed from one carbon atom, and a hydrogen ion is removed from the other carbon atom, with the formation of an alkene bond between the two carbons.

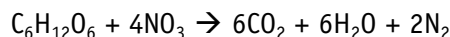
Biotic Transformations

Several types of biotic transformation reactions are important to ISB. These are described below.

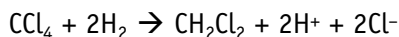
Reduction and Oxidation Reactions: Microorganisms can gain energy for growth by coupling reduction-oxidation reactions via electron transport systems. Groundwater contaminants can serve as electron donors or electron acceptors in these biocatalyzed reactions. For example, ammonia can serve as an electron donor for the reduction of oxygen by nitrifying bacteria to produce nitrite and water:



In the above reaction, oxygen serves as the electron acceptor. Oxygen is an excellent electron acceptor for biologically mediated electron transfer because its reduction produces a relatively high energy yield. Under anaerobic conditions, alternative electron acceptors, including nitrate, nitrite, Mn(IV), Fe(III), sulfate, and CO₂, can be used by specific groups of microorganisms. The use of these alternative acceptors in electron transfer bioprocesses is termed anaerobic respiration. In the case of nitrate, denitrifying bacteria couples the oxidation of organic matter with the reduction of nitrate:

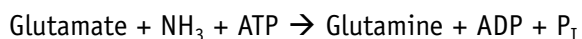


In anaerobic environments, hydrogen can serve as an electron donor for the reduction of contaminants. Halorespiration refers to biological reduction of organic solvents to produce energy for growth. In this process, hydrogen is oxidized while the chlorinated solvent is reduced:



Cometabolism: Cometabolism refers to a process whereby a compound is degraded by an enzyme or cofactor that is produced by microorganisms for other purposes, and is considered to be a fortuitous reaction. For example, bacteria produce metalloenzymes, such as cytochrome P450 and iron (II) porphorins that are capable of dechlorinating carbon tetrachloride. The cometabolic process does not benefit the microorganism producing the enzyme or cofactor. Another example, one of the most important inducible enzymes for cometabolism of chlorinated compounds, is the oxygenases, including various mono- and dioxygenase enzymes produced by bacteria. Cometabolic transformation kinetics, however, are complex and not well understood.

Assimilation: Assimilation refers to the incorporation of substances into biomass. Microorganisms require sources of carbon, hydrogen, oxygen, and nitrogen, as well as minor and trace nutrients. In some cases, groundwater contaminants can be converted into biomass by microorganisms. For example, microorganisms can use ammonia (NH_4^+), nitrate, or nitrite as sources of nitrogen for growth. Although assimilation processes may involve redox reactions, these processes are different from dissimilatory redox processes because the latter produce energy for the growth of microorganisms. In contrast, assimilatory reactions (anabolic reactions) often require energy. For example, the assimilation of ammonia via the glutamine synthesis reaction utilizes ATP (energy):



Sequential Transformations: Transformation of contaminants in groundwater is often sequential with various intermediates (or degradation products) appearing before the contaminant is completely mineralized. For example, the generally accepted sequence for the mineralization of nitrate is:



The degradation pathways of sequential transformations vary depending on the contaminant. For example, ISB for carbon tetrachloride can be accomplished by numerous bacteria via numerous pathways as shown in Exhibit 7. Carbon tetrachloride reduction may occur either through a biological sequential reduction or a direct biological mineralization process. A significant pathway is a sequential two-electron reductive pathway whereby a chloride ion is removed in each reductive process until methane remains.

The detection of intermediates of sequential transformations is often used as an indicator of contaminant degradation by natural or enhanced bioremediation. In the case of groundwater contaminated with carbon tetrachloride, the presence of chloroform and methylene chloride may indicate that contaminant degradation has occurred. The reaction rates for the various steps in a sequential transformation may be considerably

Cometabolism refers to a process whereby a compound is degraded by an enzyme or cofactor that is produced by microorganisms for other purposes, and is considered to be a fortuitous reaction.

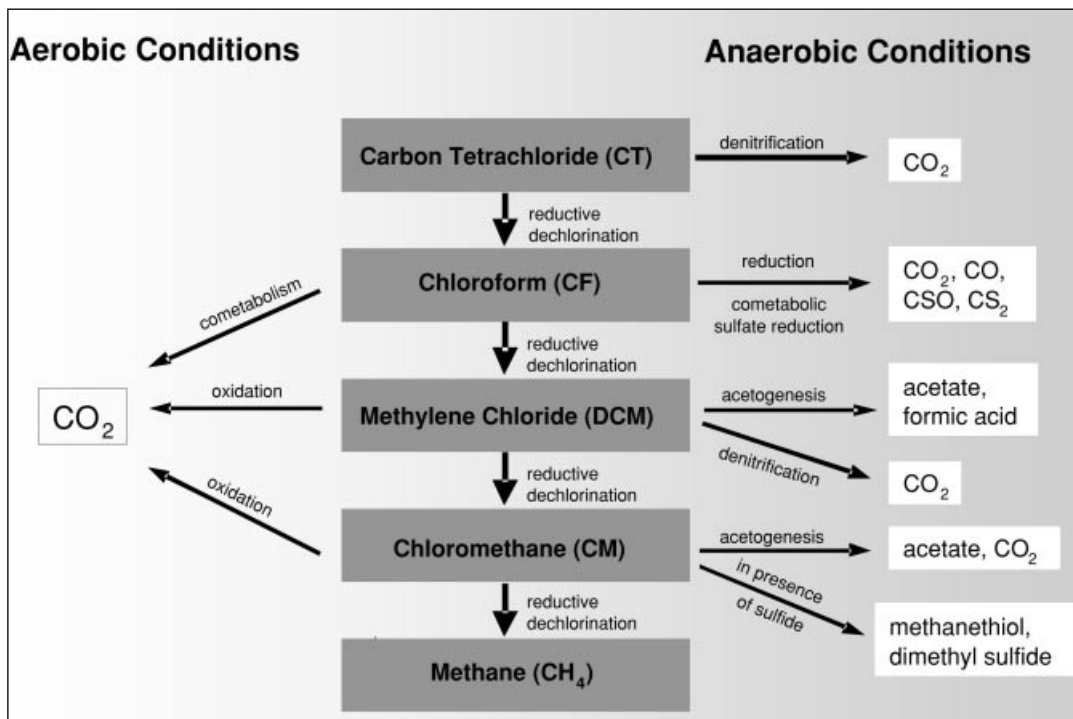


Exhibit 7. Carbon tetrachloride degradation pathways.

different. Thus, an intermediate compound in a transformation sequence that is formed rapidly (i.e., its parent degrades rapidly) but itself degrades slowly, can accumulate during sequential degradation.

Contaminant Mixtures: Mixtures of multiple contaminants may display more complex transformations than single contaminants. For instance, a mixture of an organic carbon source (e.g., petroleum hydrocarbons) with ammonium and nitrate in groundwater could lead to creation of anaerobic conditions, which would increase the rate of nitrate reduction, but decrease the rate of ammonia oxidation.

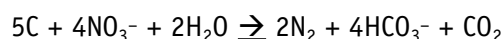
SITE CONCEPTUAL MODEL DEVELOPMENT FOR ISB

In developing an ISB strategy for a contaminated site, understanding of both stoichiometry and kinetics of site-specific contaminant biodegradation reactions is needed, in addition to the site characterization information previously outlined. Since most bioremediation processes are metabolic, chemical reactions should follow a well-defined stoichiometry. An understanding of the relevant reactions and their stoichiometries gives a clear scientific basis for ISB system design and operation.

Stoichiometry

As applied to ISB, stoichiometry defines the relative amounts of electron donors and electron acceptors that react in microbially mediated reactions. Hence this relationship defines the minimum necessary amount of amendment required to achieve bioremediation goals. Stoichiometric equations are used to design the operation of an ISB system by defining minimum donor concentrations that will react with the target contaminant. The stoichiometric equation of a chemical reaction is a statement of the rel-

ative number of molecules or moles of reactants and products that participate in the reaction, for example:



In this generic reaction, 5 moles of carbon react with 4 moles of nitrate and 2 moles of water to produce 2 moles of nitrogen gas plus other products as noted. Thus, on a purely stoichiometric basis, 5/4 moles of carbon are required for the denitrification of each mole of nitrate to nitrogen gas. In the ambient subsurface environment, however, numerous other “sinks” exist that can react with (or compete for) the carbon. In field applications of ISB, the presence of such competing reactants increases the required carbon amendment rate in excess of the theoretical.

Many different carbon amendments can be used to stimulate denitrification in groundwater. Amendments such as ethanol, acetate, and sugar are most commonly used although some regulated compounds such as toluene have also been shown to be effective. Stoichiometric formulations for some commonly used carbon amendments in denitrification are listed below.

- Methanol $\text{NO}_3^- + 5/6 \text{CH}_3\text{OH} \rightarrow 1/2 \text{N}_2 + 5/6 \text{CO}_2 + 7/6 \text{H}_2\text{O} + \text{OH}^-$
- Acetate $\text{NO}_3^- + 5/8 \text{CH}_3\text{COO}^- \rightarrow 1/2 \text{N}_2 + 5/4 \text{CO}_2 + 1/8 \text{H}_2\text{O} + 13/8 \text{OH}^-$
- Ethanol $\text{NO}_3^- + 5/12 \text{CH}_3\text{CH}_2\text{OH} \rightarrow 1/2 \text{N}_2 + 5/6 \text{CO}_2 + 3/4 \text{H}_2\text{O} + \text{OH}^-$
- Acetone $\text{NO}_3^- + 5/16 \text{CH}_3\text{COCH}_3 \rightarrow 1/2 \text{N}_2 + 15/16 \text{CO}_2 + 7/16 \text{H}_2\text{O} + \text{OH}^-$
- Sugar (sucrose) $\text{NO}_3^- + 5/48 \text{C}_{12}\text{H}_{22}\text{O}_{11} \rightarrow 1/2 \text{N}_2 + 5/4 \text{CO}_2 + 31/48 \text{H}_2\text{O} + \text{OH}^-$

Many different carbon amendments can be used to stimulate denitrification in groundwater.

Each reaction shows the moles of amendment consumed in the overall reduction of one mole of nitrate to nitrite to $-1/2$ mole of nitrogen gas. When selecting a carbon source as an ISB amendment, keep in mind that lower carbon content sources will require higher amendment rates than higher carbon content sources.

Material Balance (Chemical, Electron): Measuring material balances of a treatment process confirms reactions are taking place and their stoichiometry. Since bioremediation reactions are stoichiometrically defined, it is possible to accurately estimate the amount of required amendment. Mass balance is checked during ISB by monitoring changes in concentrations of amendments and contaminants. A good mass balance may help identify problems with adequate amendment distribution. Poor amendment mixing and distribution can cause problems as shown at an ISB test site in New Mexico (Nuttall, et. al., 2002). If previously unknown side reactions are occurring, these will also be indicated, in part, by the mass balance.

Kinetics

Chemical kinetics is the study of reaction rates and mechanisms by which one chemical species is converted to another. Reaction rate refers to the change in amount (moles) of a reactant (consumed) or product (produced) per unit time. This information can be used to estimate how long it will take to reduce a given concentration of contaminant

to a target cleanup level. Knowledge of the reaction kinetics, including rates as well as mechanisms, of contaminant biotransformation is crucial for ensuring an ISB system design will meet operating and economic constraints. If biotransformation reactions are extremely slow, it is necessary to know this as early as possible in the ISB project. To identify what amendments may be needed to enhance reaction rates, it is necessary to know if biodegradation reactions are limited by availability of one or more reactant species, for example the availability of oxygen during aerobic bioremediation.

The mathematical form of the rate equation is derived in part from an understanding of the reaction mechanism. Most often the rate equation needed for ISB design is obtained by fitting a mathematical function to experimental data. A physically based kinetic rate equation is desirable for designing and operating an ISB system, because the coefficients determined are physically meaningful reaction parameters.

Such rate equations can provide essential “reactive” input terms to flow and transport models that are used for system design and optimization. The models provide estimates of electron donor and acceptor degradation (temporal and spatial), which, in turn, affects the system design flow rates, donor/nutrient input rates, residence time in the subsurface flow field, and overall system layout (i.e., flow field size).

Biodegradation Half-Life: Reaction half-life, $t_{1/2}$, is defined as the time it takes for the reactant (contaminant) concentration to be reduced to half of its initial value. By experimentally measuring the reaction half-life as a function of initial concentration, reaction order and the specific reaction rate can be determined. Half-lives can also be estimated by measuring the reduction in concentration along a single flow line over a known period of time. These methods are only approximate because they generally do not account for heterogeneities in the aquifer, dilution, dispersion, and abiotic reactions.

Reaction Order and Forms: Buscheck et al. (1993) developed a simplified technique to quantify intrinsic bioremediation of chlorinated solvent plumes in groundwater that was applied to several field sites. Their evaluation included derivation of apparent first-order biodegradation rates from temporal (concentration versus time) and spatial (concentration versus distance) trends in contaminant concentrations. This method assumes that sorption sites in the aquifer are saturated with respect to contaminants and the plume is at steady-state (i.e., neither growing nor shrinking with time). A first-order differential equation is used to derive the various estimates of biodegradation rates as follows:

$$\frac{dC}{dt} = -k_1 C$$

where t is time, k_1 is the first-order decay rate (per unit time), and C is the dissolved contaminant concentration. The solutions for this differential equation for temporal trends is:

$$C(t) = C_0 e^{-k_1 t}$$

where $C(t)$ is concentration as a function of time t , C_0 is the concentration at $t = 0$, and the solution for spatial trends (assuming a constant pore water velocity) is:

$$C(x) = C_0 e^{-\frac{k_1 x}{v}}$$

where $C(x)$ is concentration as a function of distance x , C_0 is the concentration at $x=0$, and v is the pore water velocity. Furthermore, the biodegradation half-life ($t_{1/2}$) of the contaminant is given by:

Most often the rate equation needed for ISB design is obtained by fitting a mathematical function to experimental data.

$$t_{1/2} = \frac{\ln 2}{k_1}$$

In bioremediation, kinetics are often treated as pseudo-first-order processes. A more detailed discussion of cell growth kinetics and rate equation forms is provided in Bailey and Ollis (1986).

Modeling

Fate and transport modeling is generally conducted to predict transport of a given contaminant under a set of environmental conditions. A model is a tool that represents an approximation of a field condition. Development of both a conceptual and groundwater computer model is a critical task in designing an enhanced ISB system. Development of a representative site conceptual model is a critical step in helping to define achievable remedial objectives, select the appropriate computer (groundwater flow and transport) model, and design cost-effective data collection activities. The refinement of a site conceptual model is a necessary step to design a remedial system for the site. A conceptual groundwater model is a simplified depiction of the groundwater flow system as visualized from the site investigation results. Fate and transport models interpret the movement of the contaminant plume according to information describing the hydrostratigraphic units, boundary conditions, matrix composition, water movement, contaminant distribution, and biotic and abiotic processes that affect the fate and transport of contaminants. The final conceptual model is usually different from the initial version, because preparation of a conceptual model is an evolutionary process. The conceptual model provides the basis on which to select the proper groundwater computer model to design an ISB system that will meet the remedial objectives.

The site conceptual model helps reveal key mechanisms governing groundwater flow and the fate of solute species being transported in the subsurface. It tests assumptions and simplifications required to reflect the real situation and validates the framework of the model itself. A conceptual model can be described with mass-balance summaries, geological cross-sections, and three-dimensional diagrams delineating site conditions. Note that during the development of any site conceptual model, sufficient monitoring points must be in place to adequately define the spatial (vertical and horizontal) extent of the contaminant plume. Furthermore, a sampling frequency of these points should also be established to determine temporal variations in contaminant concentration and, more specifically, natural attenuation mechanisms as they relate to ISB.

The site conceptual model helps reveal key mechanisms governing groundwater flow and the fate of solute species being transported in the subsurface.

FEASIBILITY

Good site characterization data and a representative conceptual model are necessary but not sufficient to establish the suitability for implementation of ISB remediation technologies (ITRC, 1998). There are certain general limitations to ISB that affect feasibility. If MNA is considered as a stand-alone remedy, then the time to achieve remedial goals may be a limiting factor. On the other hand, if enhanced ISB is the chosen remediation technology, then the mixing and distribution of amendments is critical. For established ISB applications (e.g., petroleum hydrocarbons), laboratory and field testing may not be necessary. For innovative ISB technologies, both laboratory treatability studies and field pilot testing are recommended.

Monitored Natural Attenuation

Evaluation of MNA as a remedial option requires an understanding of the physical and biogeochemical conditions at a contaminated site, and quantification of relevant biogeochemical reactions to determine whether the naturally occurring contaminant degradation processes can achieve remediation goals. USEPA (1998) and ITRC (1999) are excellent resources that address this topic and provide additional information on both MNA and ISB. If MNA is chosen as the final remedy, no treatment system is engineered or installed. However, the length of time over which monitoring is to be conducted must be considered with respect to potential future impacts (other wells, aquifers, surface water bodies, or the vadose zone). In some cases, MNA may be an appropriate final remedial option, in combination with and following enhanced ISB. Collecting evidence to demonstrate the feasibility of MNA is not a cursory effort. USEPA requires a rigorous technical assessment via multiple-lines-of-evidence (USEPA, 1999). MNA is typically approved for stable or shrinking plumes. Expanding plumes typically require additional remedial action (e.g., ISB) to accelerate the degradation process.

In some cases, MNA may be an appropriate final remedial option, in combination with and following enhanced ISB.

Enhanced ISB Systems

Enhanced ISB systems typically introduce amendments into the contaminated subsurface to promote microbial activity that results in the destruction of the contaminant of concern. Enhanced ISB systems can be deployed for source reduction, dissolve-phase contaminant reduction, or as a biological barrier to contain the contaminant plume. Depending on the contaminant(s), site conditions, and remediation goals, enhanced ISB processes can be designed based on reduction or oxidation of the contaminant, either directly or cometabolically, or, depending on its transformation sequence, by a combination of reactions. ITRC (1998) and USEPA (2000) provide helpful descriptions of engineered systems with associated references.

Treatability Testing

Laboratory treatability studies are used to determine what types of biodegradation reactions occur naturally in samples of the contaminated media and whether reaction rates can be enhanced under controlled conditions. These studies are recommended to provide specific information on contaminant degradation, the types of biodegradation reactions that occur naturally at the site, and can assist in identifying kinds of amendments needed to accelerate these reactions. These studies can also be used to evaluate the effectiveness of different amendments and to optimize the amendment rate. Two types of treatability studies are commonly employed: microcosm batch studies and soil column studies. Both types of tests use groundwater samples and/or aquifer material from the contaminated site to which amendments are added (electron donors, electron acceptors, and/or nutrients), and the extent and rate of biodegradation is evaluated over time.

The Reductive Anaerobic Biological In Situ Treatment Technology (RABITT) Technical Protocol (ESTCP, 2001) provides detailed guidance on developing laboratory treatability tests, including information used to design the injection formulation and enhancement strategy. The results can be used to develop comprehensive, site-specific contaminant transport/fate models, allowing quantitative estimates of dynamic response

to alternative enhancement strategies. Furthermore, the RABITT protocol provides a phased approach to evaluate a site for reductive ISB, including site characterization, pilot-scale field demonstrations, and full-scale deployment.

A detailed discussion of laboratory treatability tests can also be found in ITRC (1998), which states: “the need for laboratory treatability studies at every enhanced ISB site may diminish as these technologies become more accepted and understood. However, for now, they provide an important portion of the evidence to judge the effectiveness of the proposed degradation mechanisms.”

Applicability

Results from laboratory treatability studies are used to determine whether ISB is a viable remediation alternative. If the treatability results show that ISB does not occur, or the process is incomplete or too slow under the various conditions tested, then ISB is most likely not a viable remediation alternative for the site in question. One thing to keep in mind is that treatability studies are usually conducted on a limited number of samples, and the validity of decisions regarding applicability depends crucially on how representative these samples are of the site on the whole. If the treatability results show that ISB does occur, then a pilot-scale field demonstration project is recommended prior to a full-scale ISB remediation deployment.

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Pilot-Scale Field Demonstration

A pilot-scale field demonstration for ISB is typically necessary to test the design in a small and relatively low-cost field application. This will also allow for modification of any design parameters (i.e., injection methods and rates, specific amendments, etc.) to accommodate site-specific circumstances and conditions prior to full-scale implementation. Prior to implementation of the pilot-scale field demonstration, goals and objectives of the test should be clearly defined. These can include achievement of predefined cleanup criteria, cleanup within an acceptable time frame, minimal adverse impacts on site conditions (physical, biogeochemical, etc.), and keeping treatment costs within projected budgets. Aspects of pilot-scale field demonstration implementation of ISB may include:

- permitting and regulatory acceptance
- preliminary site selection
- focused hydrogeologic study
- engineering design
- pilot system operation, monitoring, and maintenance
- performance evaluation

Each ISB application is site-specific, and a pilot-scale field demonstration may proceed without a laboratory treatability study if site conditions demonstrate the applicability of ISB technologies. For specific contaminants for which mature ISB technologies exist (i.e., petroleum hydrocarbons), pilot-scale field demonstration may not be necessary prior to full-scale implementation. Exhibit 8 shows a schematic of a pilot-scale field demonstration system, developed by the Remediation Technology Development Forum for the Dover Air Force Base ISB pilot test. Exhibit 9 shows a larger pilot-scale field

demonstration for an in situ biodenitrification system in Albuquerque, New Mexico. Contaminated water is extracted from four extraction wells at a rate of 1.25 gpm per well, mixed with substrate (sodium acetate and trimetaphosphate), and the amended water reinjected into the center injection well.

Biofouling

Biofouling is attributed to the growth of microbial populations (biomass) and perhaps more directly to the generation and accumulation of extracellular polysaccharides. These slimy biofilms are important for the colonization of microorganisms on surfaces or within porous media and can contribute significantly to permeability reduction of a formation or injection well. The increase of biomass during in situ remediation presents a challenge to the system design and operation. A portion of the carbon substrate is used for bacterial cell growth. If unchecked, eventually bacterial growth can reduce circulation and injection of the amendment and may lead to plugging of the formation or injection well. Various operating strategies have been devised to reduce or minimize this potentially undesirable side effect. These include pulsed amendment addition (pulsing alternately with amendment and amendment-free water), addition of oxidizers (i.e., hydrogen peroxide) and/or acids, use

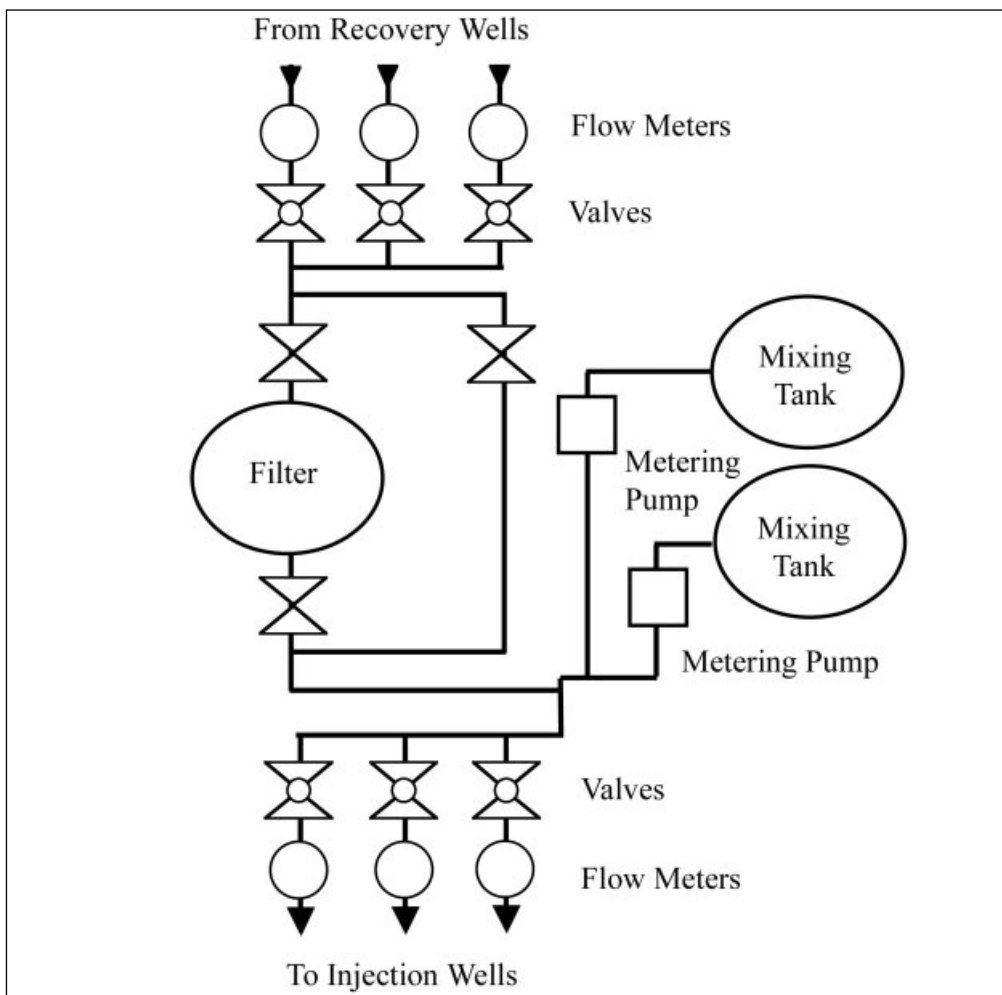


Exhibit 8. Field demonstration system schematic example.

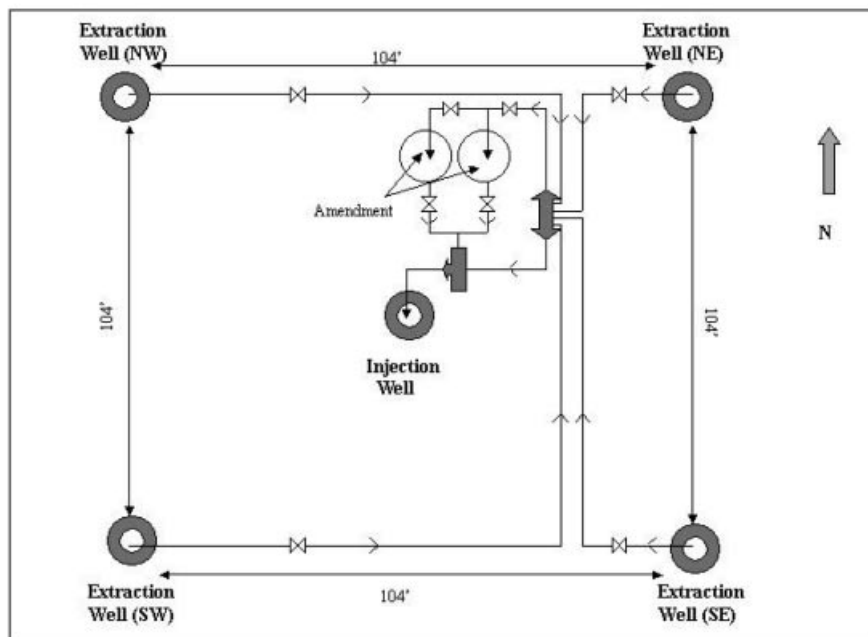


Exhibit 9. In situ bioremediation pilot-scale field-demonstration, Albuquerque, New Mexico.

of CO₂ “freeze” injection, manual “scrubbing” of injection or extraction well screens, and injection of biocides. No one approach is a clear winner. However, biofouling is an issue that must be considered in any ISB system design and operation.

Delivery and Mixing of Amendments

The two key challenges to enhancing ISB are delivery and mixing of bioremediation amendments. Biodegradation of contaminants requires the presence of contaminant-degrading bacteria, plus appropriate concentrations of electron acceptors, electron donors, and/or microbial nutrients such as nitrogen and phosphorus. In some situations, the contaminant itself may serve as the electron donor or electron acceptor. If availability of required component is limited, the biodegradation process may slow or even stop. Consequently, the focus of a successful ISB system design is an effective delivery process that will produce adequate distribution of amendments in the subsurface treatment target area.

Ex Situ Mixing: One approach is to mix the amendments above ground and then inject the mixture into the subsurface. This approach assures good mixing of amendments but requires an effective distribution system to deliver mixed amendments to the subsurface. In relatively permeable formations, conventional wells or infiltration trenches can serve as delivery systems. Pneumatic or hydraulic fracturing enhancements have been shown to be effective at increasing amendment delivery effectiveness into fine-grained silt and clay formations. Recirculation systems also can take advantage of ex situ mixing of amendments prior to reinjection.

In Situ Mixing: Subsurface mixing of amendments occurs primarily by the physical processes of fluid flow and diffusion of dissolved components from high to low concentration areas. These processes are increasingly restricted as the soil particles become smaller. Increasing the contact surfaces between high and low concentration zones can increase the rate of mixing by diffusion. Pneumatic and hydraulic fracturing under cer-

tain circumstances can also be used to speed distribution and mixing of subsurface amendments, especially in fine-grained formations. Pulsed injection of fluids into the subsurface can also enhance mixing.

FULL-SCALE IMPLEMENTATION

Prior to deciding on full-scale implementation, ISB system goals, including cleanup levels, time constraints, and cost, should be clearly defined. If a pilot-scale field demonstration shows that ISB is an effective technology for attaining remediation goals within an acceptable time frame, approval of a full-scale ISB project is greatly simplified. That is to say, permits are easily modified from prior pilot-scale field demonstration permits, limited additional site characterization data may be needed, and full-scale design does not typically require considerably more engineering than the pilot-scale field demonstration.

Economics

An ITRC document entitled *Cost and Performance Reporting for In Situ Bioremediation Technologies* (http://www.itrcweb.org/isb_5.pdf) describes a reporting methodology for obtaining comparable information regarding costs and performance associated with different types of ISB technologies, and includes an easy-to-use reporting template. Interested readers are encouraged to use this template to document the economics of ISB systems.

The Federal Remediation Technologies Roundtable (FRTR) has developed a guidance document for evaluating the cost and performance of remediation technologies (<http://costperformance.org/pdf/guide.pdf>). This document, entitled *Guide to Documenting Cost and Performance Information for Remediation Projects*, may be useful in determining the performance measures for any site-specific ISB system.

The FRTR has also compiled case studies from specific sites that have deployed enhanced ISB. These reports provide a cost and performance value for each site, along with site information and cleanup authority. To search the list of the numerous case studies, visit the FRTR Web site (www.frtr.gov).

The paper by Quinton et al. (1997) is another excellent reference for economic analysis. A consistent method for documenting cost and performance for a template site is presented, and comparative costs of various remediation technologies are developed and summarized as total present value cost, cost per pound of contaminant removed, and cost per 1,000 gallons of water treated using a discounted cash flow analysis.

Site-Specific Issues

Evaluation of site-specific data and information plays an important role in the decision to deploy a full-scale ISB system. To the extent possible, available site characterization data should be used at this stage of the ISB application. In most cases, some additional site-specific site characterization data may be needed to locate the ISB implementation target area and to decide upon the appropriate type of delivery system and amendments.

Risks and Liabilities

Risks and liabilities are always a major issue for any remediation system. Perceived risk involved with implementing ISB systems may stem from lack of knowledge of some of the

Subsurface mixing of amendments occurs primarily by the physical processes of fluid flow and diffusion of dissolved components from high to low concentration areas.

parties involved. There may also be concerns from responsible parties that contingency plans based on traditional remediation systems may need to be prepared and implemented. The concern that ISB has not yet been universally accepted as a viable remediation technology is another perceived risk, along with public concerns, and the risk of plume migration onto other properties during the course of in situ treatment. All of these concerns should be addressed if ISB systems are to be deployed. Risks and liabilities vary between sites and should be identified and resolved prior to selecting an ISB remediation system.

Performance Monitoring

A good understanding of the specific contaminant's transformation reactions, and their stoichiometry and kinetics, is essential for developing an appropriate monitoring plan. An ISB monitoring plan should not be solely based on contaminant concentration reduction. Degradation products, as well as other byproducts of the ISB process (electron donors and acceptors, CO₂ production, and other geochemical parameters) must be quantified to demonstrate that the ISB system is remediating the contaminant plume and to identify any possible need for modification of the system. In addition, monitoring should track amendment additions, flow rates, injection rates, and maintenance and operations activities. In general, monitoring programs are system-specific and site-dependent.

A good understanding of the specific contaminant's transformation reactions, and their stoichiometry and kinetics, is essential for developing an appropriate monitoring plan.

CAVEATS

Any remediation technology has certain limitations, and ISB is no different. If data collected during the site characterization and feasibility assessment (hydrology, geology, biogeochemistry) does not support an ISB system, then other technologies must be considered. Frequently, land use may be a limiting factor in selection of a remediation technology, and land use conditions may solely dictate the remediation technology used. For instance, if a plume is threatening a receptor, then a permeable reactive barrier or plume containment may be implemented as a first response action. However, ISB systems may be deployed as part of a treatment train. If access to properties is restricted, ISB may not be the remediation technology of choice. An economic evaluation of all remediation technologies appropriate for the contaminated site is essential. Also, public understanding of ISB systems is critical prior to implementation. If stakeholders of a contaminated site do not accept or support ISB systems, then the success of regulatory approval and deployment is substantially diminished.

REGULATORY CONCERNS

There are specific regulatory issues related to ISB. ISB is still considered by many as an emerging technology and, thus, current regulations have either not been amended to deal with these specific issues or the interpretation of the regulations are opposed to ISB. A major regulatory issue is the reinjection of contaminated water or the injection of amended water into the subsurface. Reinjection under the Resource Conservation Recovery Act (RCRA) 3020(b) states: "...contaminated groundwater must be treated to substantially reduce hazardous constituents prior to reinjection." EPA's Office of Solid Waste issued a memorandum that stated reinjection of treated groundwater to promote *in situ* treatment is allowed under 3020(b) [emphasis added] as long as certain conditions are met. Specifically, the groundwater must be treated prior to

reinjection; the treatment be intended to substantially reduce the hazardous constituents in the groundwater—either before or after reinjection; the cleanup must be protective of human health and environment; and the injection must be part of a response action under CERCLA Section 104 or 106 or a RCRA corrective action intended to clean up the contamination. Furthermore, established through the Safe Drinking Water Act, the Underground Injection Control (UIC) Program describes requirements intended to protect the nation's drinking water sources when fluids are injected into the subsurface. For non-CERCLA and RCRA sites, a UIC permit is typically required for an enhanced ISB system.

Site Closure Criteria

A major regulatory issue is the reinjection of contaminated water or the injection of amended water into the subsurface.

Site closure criteria are always a significant issue. Understandably, a site may be closed when regulatory standards have been met for a specified period of time, and ISB may meet those standards in a shorter time frame than other technologies. However, since enhanced ISB remediation systems normally result in changes to subsurface geochemical conditions, (e.g., alkalinity, dissolved oxygen, perhaps dissolved metals, or specific ions), site closure may not be granted until conditions and relevant water quality parameters return to levels that either meet applicable standards or are not detrimental to human health or the environment.

An example of closure criteria is taken from the Pennsylvania Department of Environmental Protection's Act 2 Regulations for confirming site cleanup for groundwater. (Pa Code 25, Ch. 250):

Groundwater cleanup may be demonstrated by showing no statistically significant exceedance of the risk-based cleanup standard at the point of compliance via quarterly sampling/analysis for two years (a lesser period of time may be proposed if groundwater flow regime so warrants and relevant statistical tests are utilized and met). At this stage, a request for site closure (i.e., conformance with Act 2) will be made and confirmed.

Another example of closure criteria taken from the New Mexico Environment Department's Water Quality Control Regulation (NMAC 20.6.2.4103.D) addresses the completeness of subsurface and surface water abatement:

Abatement shall not be considered complete until a minimum of eight (8) consecutive quarterly samples from all compliance sampling stations approved by the secretary meet the abatement standards.

Furthermore, if ISB is deployed in conjunction with other technologies (treatment train), closure may only occur after all remedial actions have met the closure criteria. The bottom line for closure is that the site meets regulatory standards. If a risk-based approach is approved, scheduled monitoring of the site may be required even after the ISB system has been shut down.

ACKNOWLEDGMENTS

The team leader of the ISB team would like to acknowledge the time, effort, and dedication of each of the team members during the development of the ITRC document that is summarized in this article. ITRC would like to acknowledge the funding and support received by its partners.

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ITRC is a state-led coalition of more than 40 states working together with industry and stakeholders to achieve regulatory acceptance of environmental technologies. In Situ Bioremediation Team is a sub group of the ITRC.
