13 In Situ: Groundwater Bioremediation

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Abstract: In situ groundwater bioremediation of hydrocarbons has been used for more than 40 years. Most strategies involve biostimulation; however, recently bioaugmentation have been used for dehalorespiration. Aquifer and contaminant profiles are critical to determining the feasibility and strategy for in situ groundwater bioremediation. Hydraulic conductivity and redox conditions, including concentrations of terminal electron acceptors are critical to determine the feasibility and strategy for potential bioremediation applications. Conceptual models followed by characterization and subsequent numerical models are critical for efficient and cost effective bioremediation. Critical research needs in this area include better modeling and integration of remediation strategies with natural attenuation.

1 Introduction

A patent for in situ bioremediation of groundwater contaminated with gasoline by stimulating indigenous bacteria via nutrient injection into the terrestrial subsurface was issued to Dick Raymond in 1974 (US Patent 3,846,290). He successfully demonstrated this technology and began commercial applications in 1972 (Raymond et al., 1977). Clearly in situ groundwater bioremediation has been used successfully for more than 50 years and much is understood about where it is applicable, especially for petroleum contaminants. The really new bioremediation applications that have been done in the last 20 years are in the area of solvent, PAH, PCB, dioxin, MTBE, and metals. Bioremediation has been around for a long time, only its application breadth in terms of types of contaminants and environments has increased in the last 20 years. This explosive proliferation of new applications and environments in the last 20 years, especially by companies trying to establish themselves with a proprietary edge, has lead to a large number of terms, many of which are highly redundant, in what they try to uniquely describe. Also, the bioremediation field applications that have been reported, frequently lack comprehensive field data, especially in the terrestrial subsurface. Though bioremediation has been used at a large number of sites these applications were nearly all done by companies trying to do the study for (1) clients, who usually wanted to remain confidential, (2) the least possible cost to the client and the vendor, and (3) protecting the vendors proprietary edge for their product. This has lead to a paucity of peer-reviewed data, miss application of terminology, and confusion as to what some terms mean. More importantly it has also lead to many "failures" of in situ groundwater bioremediation due to a lack of fundamental understanding of requirements, and limitations, in terms of hydrology, geology, and biogeochemistry at various scales.

2 Terminology

Biological Treatment – Any treatment process that involves organisms or their products, e.g., enzymes.

Biotransformation – A biological treatment process that involves changing the contaminant, e.g., valence states of metals, chemical structure, etc.

Intrinsic Bioremediation – Unmanipulated, unstimulated, unenhanced biological remediation of an environment; i.e., biological natural attenuation of contaminants in the environment.

Engineered Bioremediation – Any type of manipulated or stimulated or enhanced biological remediation of an environment.

Biostimulation – The addition of organic or inorganic compounds to cause indigenous organisms to effect remediation of the environment, e.g., fertilizer.

Bioaugmentation – The addition of organisms to effect remediation of the environment, e.g., contaminant-degrading bacteria injection into an aquifer.

Biosparging – Injection of air or specific gases below ground, usually into saturated sediments (aquifer material) to increase biological rates of remediation.

Bioslurping – This treatment combines soil vapor extraction with removal of light non-aqueous phase liquid contaminants from the surface of the groundwater table, thereby enhancing biological treatment of the unsaturated zone and the groundwater, especially the capillary fringe zone where hydrocarbons tend to smear.

Biofilters – Normally used to refer to treatment of gases by passing through a support material containing organisms, e.g., soil, compost, trickle filter. Sometimes used to refer to treatment of groundwater via passage through a biologically active area in the subsurface.

Biocurtain – The process of creating a subsurface area of high biological activity to contain or remediate, usually in aquifer material.

Bioremoval – A biological treatment involving uptake of the contaminant from the environment by an organism or its agent.

Bioimmobilization – A biological treatment process that involves sequestering the contaminant in the environment. No biodegradation of the contaminant, e.g., metal bioreduction.

Biomobilization – A biological treatment process that involves making the contaminant more mobile in the environment. No biodegradation of the contaminant, but usually requires removal of the contaminant.

Permeable Reactive Barrier (PRBs) – are often referred to as iron filing walls, reactive barriers, funnel and gate systems, or passive treatment walls. They are constructed underground to intercept groundwater flows and to provide preferential flow paths through bioreactive materials, e.g., as groundwater moves through the bioreactive materials, contaminants are treated and transformed into harmless by-products.

3 Characterization and Monitoring Feasibility

The success of any bioremediation application will be highly dependent on the characterization and monitoring that is done before and during the field deployment. For any field remediation, the first step is to form a conceptual model of the contaminant plume in the environment and how that environment effects that plume. The uncertainties in this conceptual model provide the drivers for the characterization and monitoring needs. For example, characteristics of the aquifer will have a profound impact on the remediation strategy (*Fig. 1*). The largest part of the expense of any remediation project is the characterization and monitoring. Hydraulic conductivities can have a severe effect on your ability to deliver nutrients to the subsurface (*Fig. 2*) and can be the most limiting part of the environment. Fortunately, new advances in geophysics and hydraulic push technology (Geoprobe) has enabled us to characterize sites in a fraction of the time and cost. Once we have established the hydrology and basic geochemistry at the site and used that data to refine our conceptual model, a base line characterization of the microbiology is essential to establish that the right

Site characteristics	Impact on remediation program
Aquifer profile	
a) Soil type - Homogeniety - Permeability - Chemistry	a) Level of difficulty
b) Aquifer type and use - Confined, perched - Drinking water, agriculture, etc.	b) Remediation goals, urgency, level of difficulty, treatment strategy
c) Groundwater flow	c) Urgency
d) Sustainable pumping rate	d) Duration
e) Water table location - Current depth to water - Depth to water - Water table fluctuation (seasonal and extreme)	e) Level of difficulty
f) Recharge - Location - Seasonal rainfall	f) Level of difficulty, treatment strategy
Contamination profile	
a) Number and types (classes or specific compounds)	a) Treatment strategy, level of difficulty
b) Quantity	b) Difficulty
c) Solubility	c) Treatment strategy, level of difficulty
d) Volatility	d) Treatment strategy
e) Biodegradability	e) Treatment strategy
f) Toxicity	f) Urgency, remediation goal

Figure 1
Aquifer and contaminant characteristics.

microorganisms are present, that they can be stimulated, and that no undesirable reactions with the stimulants or daughter products from the stimulation will occur. This usually requires some treatability and soil compatibility studies and monitoring of microbial community structure and function to establish the base conditions prior to stimulation (Plaza et al., 2001). For example, some metals like arsenic actually increase solubility under the same redox potentials that precipitate Cr and U. *Table 1* provides an example list of the types of measurements that should be performed from either treatability slurries, soil columns or in situ sampling (Hazen, 1997). This data and the refined conceptual model provide the functional design criteria for the remediation and can be used to develop a numerical model to predict the remediation rates, stability, and legacy management needs, e.g., monitoring, especially if the remediation is an immobilization strategy.

Bioremediation strategies will be limited most by our ability to deliver the stimulus to the environment. The permeability of the formation must be sufficient to allow perfusion of the nutrients and/or microorganisms through the formation. The minimum average hydraulic

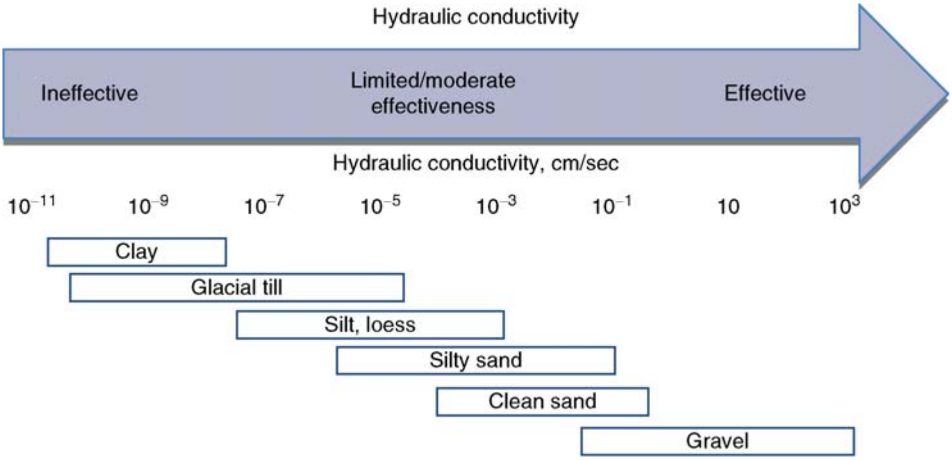

Figure 2
Hydraulic conductivity.

conductivity for a formation is generally considered to be 10^{-4} cm s^{-1} (Thomas and Ward, 1989). Additionally, the stimulants required must be compatible with the environment. For example, hydrogen peroxide is an excellent source of oxygen, but it can cause precipitation of metals in soils, and such dense microbial growth around the injection site that all soil pores are plugged. It is also toxic to bacteria at high concentrations, >100 ppm (Thomas and Ward, 1989). Ammonia also can be problematic, because it adsorbs rapidly to clays, causes pH changes in poorly buffered environments, and can cause clays to swell, decreasing permeability around the injection point. It is generally accepted that soil bacteria need a C:N:P ratio of 30:5:1 for unrestricted growth (Paul and Clark, 1989). The actual injection ratio used is usually slightly higher (a ratio of 100:10:2) (Litchfield, 1993), since these nutrients must be bioavailable, a condition that is much more difficult to measure and control in the terrestrial subsurface. It may also be necessary to remove light nonaqueous phase liquid (LNAPL) contaminants that are floating on the water table or smearing the capillary fringe zone, hence bioslurping (Keet, 1995). This strategy greatly increases the biostimulation response time by lowering the highest concentration of contaminant the organisms are forced to transform.

Recent advances in geophysics are now enabling us to determine aquifer heterogeneity, hydraulic conductivity, amendment movement in the subsurface, changes in biogeochemistry, and real-time monitoring of changes (*Fig. 3*). These measurements can potentially save time, expense, and increase our resolution of biogeochemical changes, hydrology, contaminant inventory, and amendment injection pathway (Faybishenko et al., 2008; Hubbard et al., 2008).

The type of sample used for monitoring and characterization of groundwater can have a significant impact on a bioremediation project. Hazen et al. (1991) demonstrated that deep oligotrophic aquifers have dense attached communities of bacteria that are not reflected in the groundwater from that aquifer. This has serious implications for the in situ bioremediation of deep contaminated aquifers, since monitoring of groundwater is the principal method used to characterize and control biodegradation by indigenous bacteria stimulated by nutrient infiltration. Groundwater monitoring may not indicate community or population numbers, or physiological activity of the sediment attached microbes, the principal biologically active

Table 1

Characterization and monitoring parameters

Measurements	Parameter
Biomass	
Viable counts	Plate counts, Most Probable Number (MPN), enrichments, BIOLOG^{TM}
Direct counts	Acridine Orange Direct Count (AODC), Fluorescine Isothiocyanate (FITC), Direct Fluorescent Antibody (DFA)
Signature compounds	Phospholipid Fatty Acid (PLFA), DNA, RNA,qPCR, phylochips, functional gene arrays
Bioactivity and bioremediation	
Daughter products	Cl, CO_2, CH_4, stable isotopic C, reduced contaminants, stable isotopic fractionation of contaminants
Intermediary metabolites	Epoxides, reduced contaminants
Signature compounds	PLFA, ribosome probes, BIOLOG^{TM}, phosphatase, dehydogenase, IodophenylNitrophenyal, Tetrazolium Chloride (INT), acetylene reduction, recalcitrant contaminants
Electron acceptors	O_2, NO_3, SO_4, Fe(III), CO_2
Conservative tracers	He, CH_4, Cl, Br
Radiolabeled mineralization	^{14}C, ^{3}H – labeled contaminants, acetate, thyamine
Sediment	
Nutrients	PO_4, NO_3, NH_4, O_2, total organics, SO_4
Physical/chemical	Porosity, lithology, cationic exchange, redox potential, pH, temperature, moisture, heavymetals
Toxicity	Microtox^{TM}, Mutatox ^{TM}

component of these aquifers. Harvey et al. (1984) and Harvey and George (1987) have shown that shallow, eutrophic, rapidly moving aquifers, behave quite differently, in that there are no significant differences between groundwater and attached sediment communities. This is reasonable because attachment in such an environment would have no significant advantage, unlike the oligotrophic deep aquifers. Enzien et al. (1994) further underscored the need for careful sampling when they showed significant anaerobic reductive dechlorination processes occurring in an aquifer whose bulk groundwater was aerobic (>2 mg l^{-1} O_2).

The state and fate of contaminants in all environments is highly dependent on the redox or valence state of the environment. The redox potential of the environment will control the direction of chemical equilibria and whether the contaminant is reduced or oxidized. This in turn controls the possible compounds that the contaminant can form and the relative solubility of these metals in the environment. To stimulate microbes to produce conditions that are appropriate for remediation of specific contaminants requires a through knowledge of the geochemistry of that environment. Since electron acceptors vary greatly as to the energy that can be derived from their use in respiration, the most common terminal electron acceptors (TEA) will be utilized in a set order, according to the energy that can be derived

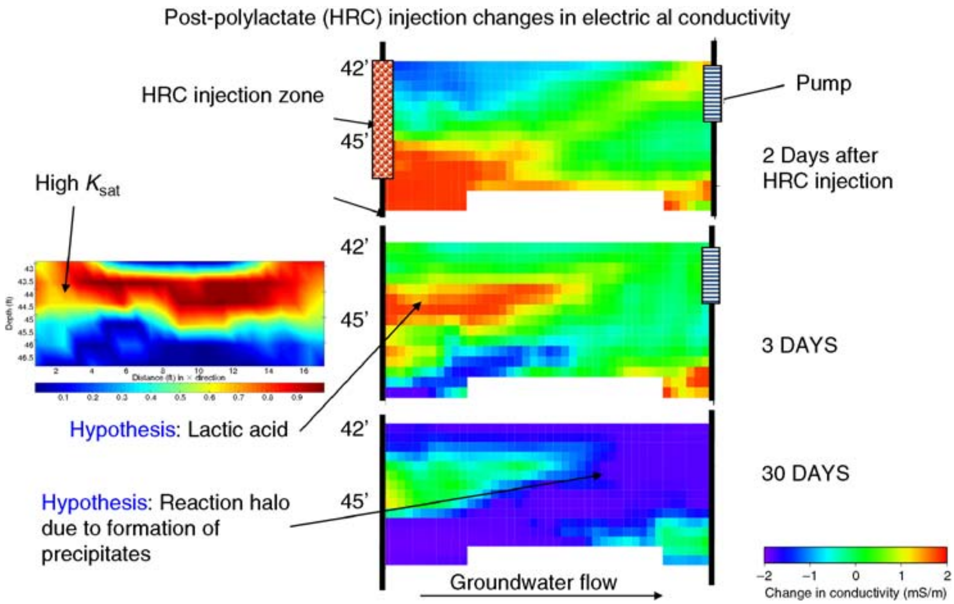

Figure 3

Geophysical measurements of polylactate injection for groundwater bioremediation.

(*Fig. 4*). Thus, oxygen is the preferred TEA and first TEA to be utilized, followed by nitrate, iron (III), sulfate, and carbon dioxide. Since dehalorespiration is not favored until the redox potential is in methanogenic conditions, O_2, NO_3, Fe(III), and SO_4 would have to be depleted first. Indeed, for sites that also have PCE/TCE the iron (III) and the sulfate would have to be depleted before sustained methanogenesis and subsequently dehalorespiration can occur. For field applications, this means that enough electron donor would have to be added to deplete all the oxygen and nitrate present, at a minimum. By monitoring the TEA and their daughter products, it provides an excellent measure of the redox conditions at the site and the potential for degradation of the contaminants of concern (Nelson et al., 1994).

4 Biostimulation and Bioaugmentation of Groundwater

All engineered bioremediation can be characterized as either biostimulation, i.e., the addition of nutrients, or bioaugmentation, i.e., the addition of organisms, or processes that use both. The problems with adding chemical nutrients to sediment and groundwater are fundamentally different from those of adding organisms. Simple infiltration of soil and subsequently groundwater is physically quite different in the two processes (Alfoldi, 1988). Even the smallest bacterium has different adsorption properties from chemicals. For example, clayey soils have very low porosity and may not physically allow bacteria to penetrate. These clays may also bind the microbes that are added, e.g., cationic bridges involving divalent metals and the net negative charge on the surface of the bacteria and the surface of the clay. In some soils,

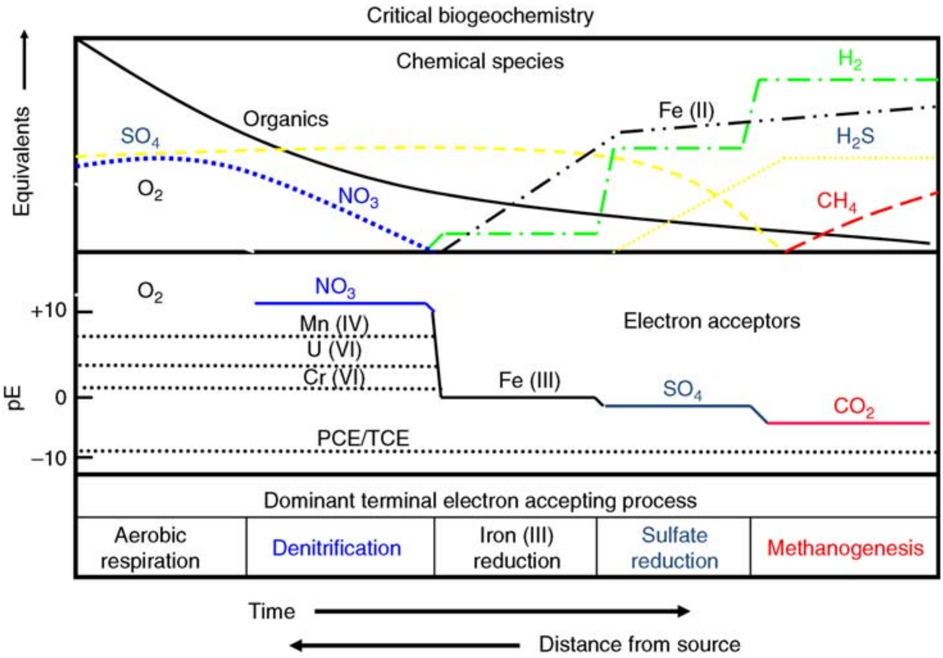

Figure 4

Critical biogeochemistry involving terminal electron acceptors and their heiarchical redox potential relationships.

inorganic chemicals that are injected may precipitate metals, swell clays, change redox potentials, and conductivity, thus having a profound effect on groundwater flow and biogeochemistry of the environment. Indeed, bacterial plugging of subsurface formations has been successfully used for enhanced oil recovery in oil reservoirs (Cusack et al., 1992).

Biostimulation is dependent on the indigenous organisms and thus requires that they be present and that the environment be capable of being altered in a way that will have the desired bioremediation effect (*Fig. 5*). In most terrestrial subsurface environments, the indigenous organisms have been exposed to the contaminant for extended periods of time and have adapted or even naturally selected. Many contaminants, especially organic compounds are naturally occurring or have natural analogs in the environment. Rarely can a terrestrial subsurface environment be found that does not have a number of organisms already present that can degrade or transform any contaminant present. Indeed, even pristine environments have bacteria with an increasing number of plasmids with sediment depth in response to increasing recalcitrance of the organics present (Fredrickson et al., 1988).

Oxygen is quite often limiting since the contaminant can be used as a carbon and energy source by the organisms and the contaminant concentration greatly exceeds the oxygen input needed by the organisms. Introduction of air, oxygen, or hydrogen peroxide via infiltration galleries, tilling, sparging, or venting have proven to be extremely effective in bioremediating petroleum contaminants and a variety of other organic compounds that are not particularly recalcitrant (Thomas and Ward, 1992). However, if the environment has been anaerobic for

Biostimulation requirements

1. Correct microbes must be present
2. Ability to stimulate target microbes
3. Ability to deliver nutrients
4. C:N:P - 30:5:1 for balanced growth (Paul and Clark, 1989) 100:10:2 in field practice (Litchfield, 1993)

Gases: air, oxygen, nitrous oxide, propane, methane, triethyl phosphate, etc.

Liquids: lactic acid, molasses, vegetable oil, acetate, Chitin, hydrogen release compound (HRC®), MRC®, etc.

Solids: bulking agents (saw dust, agricultural byproducts), oxygen release compound (ORC®), etc.

Bioaugmentation advantages

1. "New" spills where microflora has not had time to adapt or grow (vector)
2. Recalcitrant contaminants (GMO)
3. Biomass can not establish or maintain itself (GMO)
4. Biobarrier (ultramicrobacteria, GMO)
5. Controlled environment (GMO)

Pseudomonads (oil spills) – several commercial products

Dehalococcoides ethenogenes (chlorinated solvents) new products from Regenesis, GeoSyntec, and others

Figure 5

Biostimulation versus bioaugmentation strategy requirements.

extended periods of time and the contaminant has a high carbon content, it is likely that denitrification has reduced the overall nitrogen content of the environment making this nutrient limiting. Nitrogen has been successfully introduced into the terrestrial subsurface for biostimulation using ammonia, nitrate, urea, and nitrous oxide (USEPA, 1989). Phosphorus is naturally quite low in most environments and, in terrestrial subsurface environments, even if phosphorus concentrations are high it may be in a mineral form that is biologically unavailable, e.g., apatite. Several inorganic and organic forms of phosphate have been successfully used to biostimulate contaminated environments (USEPA, 1989). In environments where the contaminant is not a good carbon or energy source and other sources of carbon or energy are absent or unavailable, it will be necessary to add an additional source of carbon (Horvath, 1972). An additional source of organic carbon will also be required if the total organic carbon concentration in the environment falls below 1 ppm and the contaminant cleanup levels have still not been met. Methane, methanol, acetate, molasses, sugars, agricultural compost, phenol, and toluene have all been added as secondary carbon supplements to the terrestrial subsurface to stimulate bioremediation (National Research Council, 1993).

Bioaugmentation may provide significant advantages over biostimulation for (1) environments where the indigenous bacteria have not had time to adapt to the contaminant, (2) particularly recalcitrant contaminants that only a very limited number of organisms are capable of transforming or degrading, (3) environments that do not allow a critical biomass to establish and maintain itself, (4) applications where the desired goal is to plug the formation for contaminant containment, e.g., biocurtain, and (5) controlled environments where specific inocula of high rate degraders will greatly enhance the process, e.g., permeable reactive barriers. Like biostimulation, a major factor effecting the use of bioaugmentation in the terrestrial subsurface is hydraulic conductivity. The 10^{-4} cm s^{-1} limit for biostimulation will need to be an order of magnitude higher for bioaugmentation and may need to be higher yet, depending on the size and adherence properties of the organism being applied (Baker and Herson, 1990; Ginn et al., 2002). Studies have shown the less adherent strains of some contaminant-degraders can be produced, allowing better formation penetration (DeFlaun et al., 1994; Johnson et al., 2001). However, the ability to rapidly clog a formation is a significant advantage of bioaugmentation in applications where containment is a primary goal. The oil industry has been using this strategy to plug fluid loss zones and enhance oil recovery for a number of years (Cusack et al., 1992).

A number of novel organisms have been successfully injected into the subsurface for in situ bioremediation of PCBs, chlorinated solvents, PAHs, and creosote (National Research Council, 1993). Bioaugmentation suffers the dilemma of being indistinguishable from biostimulation in many environments, since nutrients are often injected with the organisms and since dead organisms are an excellent source of nutrients for most indigenous organisms. For many applications it is difficult, if not impossible, to determine if the added organisms provided a significant advantage over nutrient stimulation alone. Given the problems and high cost of producing the organisms for inoculation and delivery problems, bioaugmentation applications will probably remain limited. For example, if dehalorespiration was the strategy and the site had a hydraulic conductivity of only 10^{-8} cm s^{-1} with very high nitrate and sulfate levels and high pH it may not be cost effective to use dehalorespiration at this site. These issues also suggest why bioaugmentation has not lived up to its hope. Though bioaugmentation promises "designer biodegraders," it has not proven to be better then biostimulation in repeated field trials over the last 2 decades. Indeed, there is only one bacterium that has demonstrated that it can perform better then biostimulation in situ on most occasions, *Dehalococcoides ethenogenes* for dehalorespiration of chlorinated solvents. Several products are commercially available and have been widely used that are proprietary strains of this organism (e.g., Regenesis and Geosyntec). We suspect the reason that this microbe has been successful is that it is a strict anaerobe, chlorinated solvent dehalorespiration requires established methanogenic redox potentials, and the organism is very small irregular coccus (0.5 m) so it can penetrate the subsurface more easily (Loffler et al., 2000). Patchy distributions of this organism in nature are also common, so bioaugmentation may provide a couple of advantages.

Bioaugmentation may also have a very significant advantage when genetically engineered microorganisms (GEMs) are used. It is possible that a GEM could be constructed with unique combinations of enzymes to facilitate a sequential biotransformation or biodegradation of a contaminant. This would be particularly helpful for contaminants that are extremely recalcitrant, e.g., PCBs, or under limited conditions, e.g., tetrachloroethylene and carbon tetrachloride can only be biodegraded anaerobically. In addition, this GEM could be modified with unique survival or adherence properties that would make it better suited to the environment where it was to be applied.

5 Intrinsic Bioremediation and Modeling

Intrinsic bioremediation is developing rapidly as an important alternative for many contaminated environments. This strategy of natural attenuation by thorough characterization, treatability studies, risk assessment, modeling, and verification monitoring of contaminated environments was first proposed by John Wilson of EPA's Kerr Lab in the early 1990s. Wilson organized the first Symposium on Intrinsic Bioremediation in August, 1994, and development and regulatory acceptance has been exponential ever since. Certainly, much of this rapid deployment of intrinsic bioremediation has been due to the crushing financial burden that environmental cleanup represents and our need to use more risk-based cleanup goals for the thousands of new contaminated sites identified every year. Intrinsic bioremediation as a strategy carries with it a burden of proof of: (1) risk to health and the environment, and (2) a model that will accurately predict the unengineered bioremediation of the environment. Thus applications of intrinsic bioremediation have been confined to environments with few risk receptors, containing contaminants with relatively low toxicity, e.g., petroleum in fairly homogeneous, confined, and predictable subsurface environments. The EPA reported that in 1995 intrinsic bioremediation was already in use at 29,038 leaking underground petroleum storage tank (LUST) sites in 33 states (Tremblay et al., 1995). This represents 28% of the 103,479 LUST sites being remediated in 1995 and an increase of more than 100% since 1993. Intrinsic bioremediation has also been implemented at a creosote-contaminated methanogenic aquifer in Florida (Bekins et al., 1993) and in three TCE-contaminated, reducing aquifers (Major et al., 1994; Martin and Imbrigotta, 1994; Wilson et al., 1994).

The coupling of intrinsic bioremediation to engineered bioremediation could be the best overall solution. Nearly all engineered bioremediation projects could substantially reduce costs by stopping the biostimulation or bioaugmentation process early and allowing intrinsic bioremediation to finish the cleanup process. The only projects that would not benefit from such a strategy would be those where immediate risk to health and the environment demanded an emergency response. Intrinsic bioremediation has the same requirements for treatability, modeling, characterization, and modeling as engineered bioremediation discussed above. The only difference is that a greater emphasis is put on risk assessment, predictive modeling, and verification monitoring. Once an intrinsic bioremediation project has been started, verification monitoring of the predictive model is initially quite rigorous. Afterwards, if the model holds true, monitoring frequency and numbers of parameters gradually decline until the site is cleaned up.

Modeling of the bioremediation process has become increasingly important in determining the fate and effect of contaminants and predicting the outcome of different amendment scenarios. The models will only be as good as the data they receive from the characterization studies and the treatability studies. However, models can also be used to suggest treatability studies that should be performed from a minimum of characterization data. The simple kinetic models using Monod or Michaelis-Menten functions of 15 years ago are completely inadequate for current bioremediation applications in the terrestrial subsurface. One and two-dimensional models of aerobic biodegradation of organic contaminants in ground water did not appear until quite recently (Molz et al., 1986; Widdowson et al., 1987). These models used advective and dispersive transport coupled with an assumption of microcolonies. Widdowson et al. (1988) later added nitrate respiration as an option to their model. Perhaps the best documented and most widely used model for bioremediation has been the BIOPLUME model (Borden and Bedient, 1986). This model, now in its forth version, uses a series of simultaneous equations to simulate growth, decay, and transport of microorganisms, oxygen, and hydrocarbons. Rifai et al. (1987) later modified this model (BIOPLUME II) to incorporate the USGS two-dimensional method of characteristic model (Konikow and Bredehoeft, 1978). The original model was used to simulate PAH biodegradation at a Texas Superfund site

(Borden and Bedient, 1986). BIOPLUME II has been used to model biodegradation of aviation fuel at the US Coast Guard Station at Traverse City, Michigan (Rifai et al., 1988), and to characterize benzene biodegradation over 3 years in another shallow aquifer (Chiang et al., 1989; Choi et al., 2009). Travis and Rosenberg (1994) used a numerical simulation model to successfully predict aerobic bioremediation of chlorinated solvents in the groundwater and vadose zone using methane biostimulation at the US DOE's Savannah River Site near Aiken, South Carolina. Their model also used a series of simultaneous equations for microbial growth, nutrient limitations, and contaminant, microbe, and nutrient transport. The model predicted the amount of TCE that was biodegraded during a 14-month, full-scale demonstration, and was validated by five other methods (Hazen et al., 1994). Models like these are becoming increasingly important as our need to understand the terrestrial subsurface "black box" of bioremediation increases in response to increased emphasis on intrinsic bioremediation as a solution. These types of models, along with rigorous treatability studies, are required for intrinsic bioremediation to be acceptable, particularly as a solution for bioremediation of terrestrial subsurface environments.

6 Research Needs

There are a large number of ex situ and in situ bioremediation methods currently available. Ex situ methods have been around longer and are better understood, and they are easier to contain, monitor, and control. However, in situ bioremediation has several advantages over ex situ techniques. In situ treatment is useful for contaminants that are widely dispersed in the environment, present in dilute concentrations, or otherwise inaccessible (e.g., due to the presence of buildings or structures). This approach can be less costly and less disruptive than ex situ treatments because no pumping or excavation is required. Moreover, exposure of site workers to hazardous contaminants during in situ treatment is minimal. Broadly, bioremediation strategies can be further divided into natural attenuation, biostimulation, and bioaugmentation strategies. Bioaugmentation being the most aggressive, since organisms are added to the contaminated environment. Biostimulation can be aggressive or passive, in that electron donors, electron acceptors, and trace nutrients can be injected into the environment to stimulate indigenous organisms to increase biomass or activity to affect the contaminant. Passive biostimulation techniques include simple infiltration galleries. Natural attenuation relies on the intrinsic bioremediation capabilities of that environment. Environments high in organic carbon and energy sources, low contaminant concentrations, and without significant nutrient deficiencies may be able to degrade or transform the contaminants of concern without any intervention. Ideally, the most cost effective and efficient approach to treat most large contaminant plumes is to use more aggressive approaches, e.g., bioaugmentation or even excavation and removal, at the source, grading into natural attenuation at the leading edge, or over time as the contaminant concentration declines. There are only a few bioaugmentation candidates for in situ groundwater bioremediation (*Dehalococcoides ethenogenes*); however, it is technically possible to use bacteriophage as vectors to provide indigenous bacteria with increases or new degradation capacity. The size of bacteriophages and their specificity overcomes the inherent problem particle injection in the subsurface and the minimizing nontarget effects. Much more research is needed in this area. Rarely is a single remediation approach completely effective or cost efficient. Indeed, combining aggressive physical and chemical treatment techniques like chemical oxidation/reduction, thermal desorption with bioremediation can provide advantages to some types of contaminants and allows bioremediation to be an effective polishing or sentinel strategy for the cleanup. Much more modeling at all scales (Lee and Schwartz, 2007) using a systems biology approach is needed to find the fastest, most efficient, and lowest life-cycle cost solution for contaminated groundwater.

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