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 feasi-

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bility 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 (U.S. 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 chlorinated solvent, PAH, PCB, dioxin, MTBE, metals, and radionuclides. 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 led 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 led to a paucity of peer-reviewed data, miss application of terminology, and confusion as to what some terms mean. More importantly it has also led 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, also known as monitored natural attenuation.

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 nonaqueous 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.

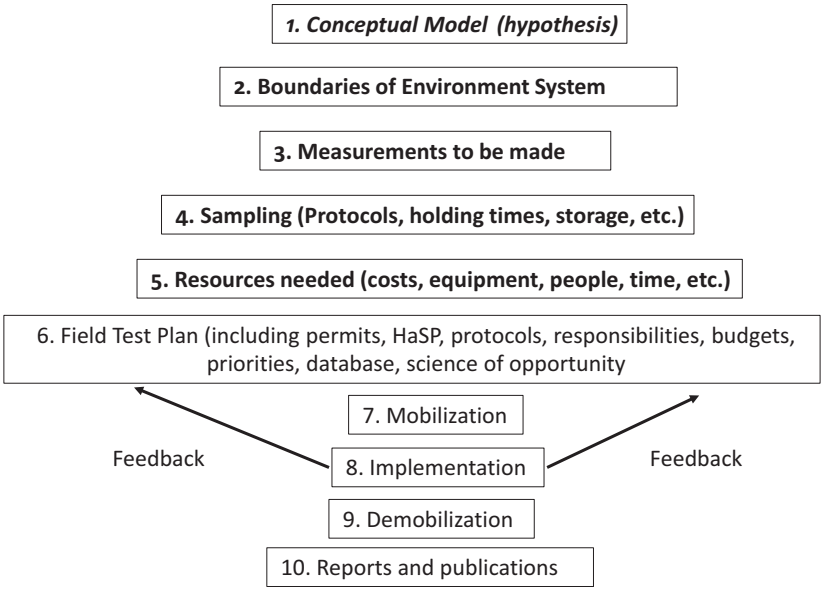
Biomobilization – A biological treatment process that involves making the contaminant more mobile in the environment. No biodegradation of the contaminant but usually requires removal of the contaminant.

Permeable Reactive Barriers (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 careful planning of the overall project including the characterization and monitoring that is done before and during the field deployment. The overall planning of the remediation needs to take into account a number of steps from conceptual model to demobilization and report writing (Table 1, Hazen and Sayler 2016). 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 (EPA 2013). For example, characteristics of the aquifer will have a profound

Table 1 In situ groundwater field plan



impact on the remediation strategy (Table 2). 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. 1) and can be the most limiting part of the environment. Fortunately, new advances in geophysics and hydraulic push technology (Geoprobe) have 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 baseline characterization of the microbiology is essential to establish that the right 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 3 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). The in situ sampling including push/pull studies are usually the best sort of information for rates of biodegradation, effective porosity, and colloidal borescope measurements of groundwater flow rates and vectors (Paradis et al. 2016, 2018). 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.

Table 2 Aquifer and contaminant characteristics

Aquifer Profile

Site Characteristics	Impact on Remediation Program
a) soil type	a) level of difficulty
- homogeniety	
- permeability	
- chemistry	
b) aquifer type and use	b) remediation goals, urgency, level of difficulty, treatment strategy
- confined, perched	
- drinking water, agriculture, etc.	c) urgency
c) groundwater flow	d) duration
d) sustainable pumping rate	e) level of difficulty
e) water table location	
- current depth to water	
- depth to water	
- water table fluctuation (seasonal and extreme)	f) level of difficulty, treatment strategy
f) recharge	
- location	
- seasonal rainfall	

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

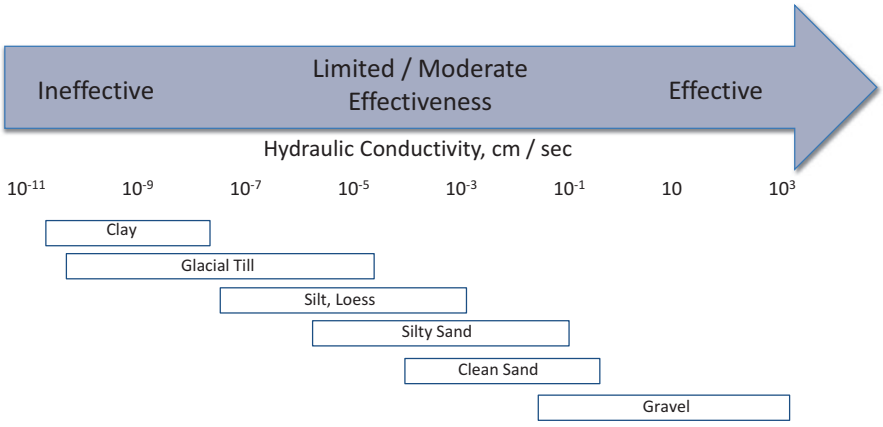


Fig. 1 Hydraulic conductivity

Table 3 Bioremediation characterization and monitoring parameters

Measurements	Parameter
Biomass	
Viable Counts	plate counts, Most Probable Number (MPN), enrichments, BIOLOG™
Direct Counts	Acridine Orange Direct Count (AODC), Fluorescien 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™, phosphatase, dehydrogenase, Iodophenyl-Nitrophenyl, 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, thymidine
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, heavy metals
Toxicity	Microtox™, Mutatox™

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 conductivity for a formation is generally considered to be 10^{-4} cm/sec (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 is 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.

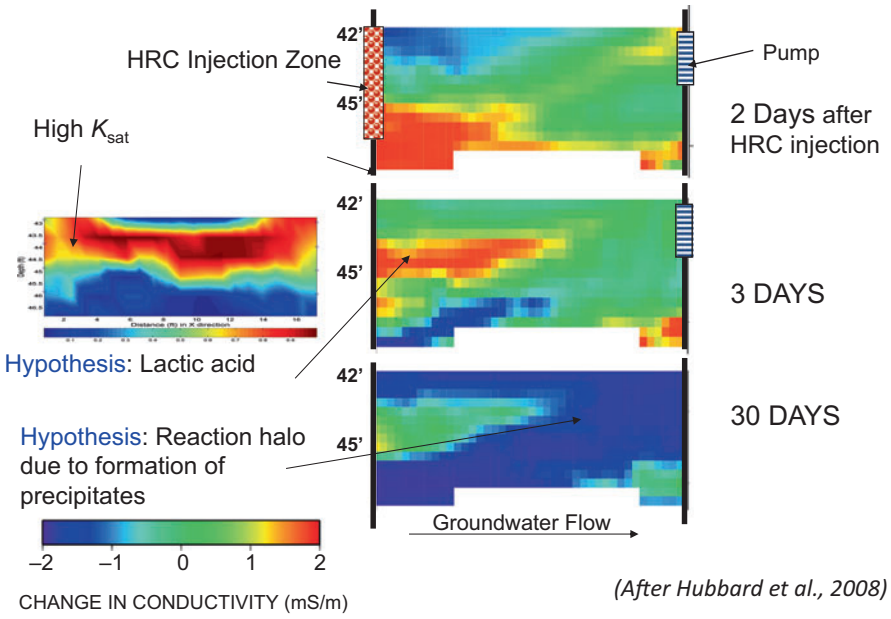


Fig. 2 Geophysical measurements of polylactate injection for groundwater bioremediation

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. 2). These measurements can potentially save time, expense, and increase our resolution of biogeochemical changes, hydrology, contaminant inventory, and amendment injection pathway (Hubbard et al. 2008; Faybishenko 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 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

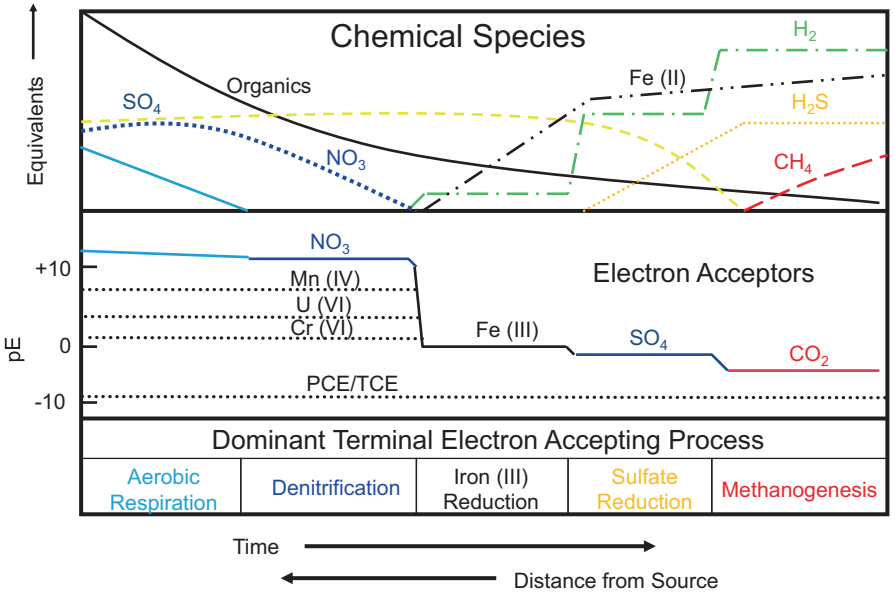


Fig. 3 Critical biogeochemistry involving terminal electron acceptors and their hierarchical redox potential relationships

showed significant anaerobic reductive dechlorination processes occurring in an aquifer whose bulk groundwater was aerobic (>2 mg/L 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 thorough 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 (Fig. 3). 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. Failure to deplete these electron donors for chlorinated solvents will result in a dichloroethylene or vinyl chloride stall (frequently a fatal flaw in the conceptual model and remediation plan). 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 (Hazen and Tabak 2005; 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, 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 (Table 4). 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 (USEPA 1988). Recent studies have demonstrated that microbial community structures at any given sampling can be used to predict ambient geochemistry and suggest bacteria that are stimulated by the amendment or hampered by the amendments (Smith et al. 2015). Using the Structured Learning in Microbial Ecology (SLiME) model (Smillie et al. 2011) and analyzing over 100 wells for 58 biogeochemical parameters in a contaminated water shed, 36 geochemical parameters were accurately predicted from the 16S rDNA community structure (Fig. 4). In addition, two OTUs (*Brevundimonas* and *Sediminibacterium*) were found to have geochemical drivers that would be important for in situ groundwater bioremediation (Fig. 5) (Smith et al. 2015). 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

Table 4 Biostimulation versus bioaugmentation strategy requirements

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

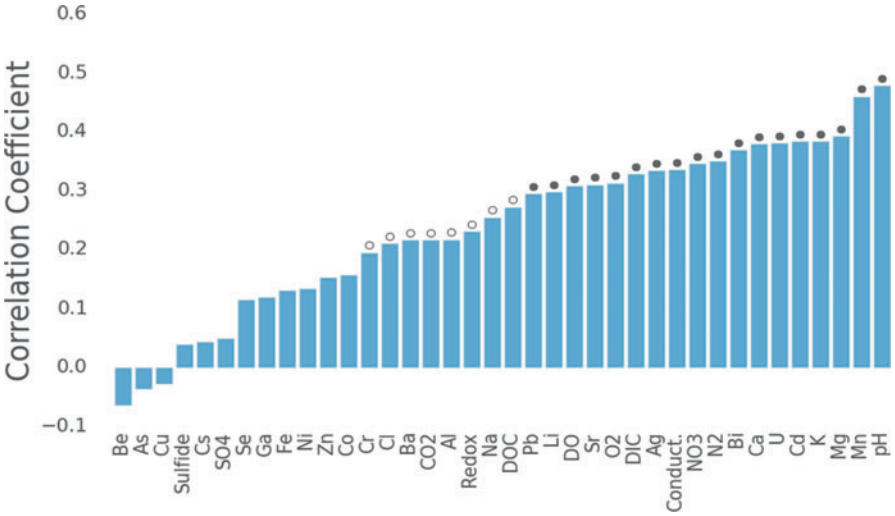


Fig. 4 Bacterial 16S sequence data can be used to predict quantitative values for a variety of geochemical measurements. Correlation coefficient (Kendall's tau) between true and predicted values. Eighteen of these correlations are highly significant ($p < 0.0001$, indicated by ●), 8 are significant ($p < 0.01$, indicated by o), and 12 of these correlations are not significant (Smith et al. 2015)

peroxide via infiltration galleries, tilling, sparging, or venting has proven to be extremely effective in bioremediating petroleum contaminants and a variety of other organic compounds that are not particularly recalcitrant (Thomas and Ward

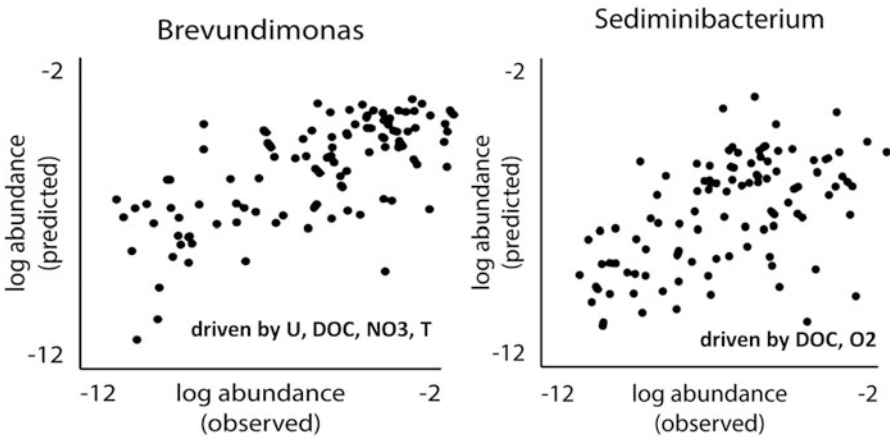


Fig. 5 Geochemical drivers for OTUs can be predicted using SLiME

1992). However, if the environment has been anaerobic for 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 (EPA 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 (EPA 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 clean-up 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 don't 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/sec 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/sec 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 than biostimulation in repeated field trials over the last two decades. Indeed, there is only one bacterium that has demonstrated that it can perform better than 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 μm) 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, which 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 monitored natural attenuation (MNA) 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 MNA 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 clean-up represents and our need to use more risk-based clean-up goals for the thousands of new contaminated sites identified every year. MNA 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 (EPA 2017). 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 (Martin and Imbrigotta 1994; Wilson et al. 1994; Major et al. 1994).

The coupling of intrinsic bioremediation or MNA 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 clean-up 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. Afterward, 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 groundwater 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 (1997) 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). Other models that are in use these days are BIOSCREEN (http://www.epa.gov/water-research/bioscreen-natural-attenuation-decision-support-system), BIOCHLOR (https://www.epa.gov/water-research/biochlor-natural-attenuation-decision-support-system), REMChlor (http://www.epa.gov/water-research/remediation-evaluation-model-chlorinated-solvents-remchlor), REMFuel (http://www.epa.gov/water-research/remediation-evaluation-model-fuel-hydrocarbons-remfuel), and Matrix Diffusion Toolkit (http://www.gsi-net.com/en/software/free-software/matrix-diffusion-toolkit.html). 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. Monitored 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 and 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 clean-up. Much more modeling at all scales (Lee and Swartz 2007) using a systems biology approach is needed to find the fastest, most efficient, and lowest life cycle cost solution for contaminated groundwater.

References

Alfoldi L (1988) Groundwater microbiology, and problems and biological treatment-state-of-the-art report. Water Sci Technol 20:1–31

Baker KH, Herson DS (1990) In situ bioremediation of contaminated aquifers and subsurface soils. Geomicrobiol J 8:133–146

Bekins BA, Godsy EM, Goerlitz DF (1993) Modeling steady-state methanogenic degradation of phenols in groundwater. J Contam Hydrol 14:279–294

Borden RC, Bedient PB (1986) Transport of dissolved hydrocarbons influenced by reaeration and oxygen limited biodegradation 1. Theoretical development. Water Resour Res 22:1973–1982

Chiang CY, Salanitro JP, Chai EY, Colthart JD, Klein CL (1989) Aerobic biodegradation o benzene, toluene, and xylene in sandy aquifer, and data analysis and computer modeling. Ground Water 27:823–834

Choi NC, Choi JW, Kim SB, Park SJ, Kim DJ (2009) Two-dimensional modelling of benzene transport and biodegradation in a laboratory-scale aquifer. Environ Technol 30:53–62

Cusack F, Singh S, McCarthy C, Grieco J, Derocco M, Nguyen D, Lappinscott H, Costerton JW (1992) Enhanced oil-recovery – 3-dimensional sandpack simulation of ultramicrobacteria resuscitation in reservoir formation. J Gen Microbiol 138:647–655

Deflaun MF, Marshall BM, Kulle EP, Levy SB (1994) Tn5 insertion mutants of *Pseudomonas fluorescens* defective in adhesion to soil and seeds. Appl Environ Microbiol 60:2637–2642

Enzien MV, Picardal F, Hazen TC, Arnold RG, Fliermans CB (1994) Reductive Dechlorination of trichloroethylene and tetrachloroethylene under aerobic conditions in a sediment column. Appl Environ Microbiol 60:2200–2204

Faybishenko B, Hazen TC, Long PE, Brodie EL, Conrad ME, Hubbard SS, Christensen JN, Joyner D, Borglin SE, Chakraborty R, Williams KH, Peterson JE, Chen JS, Brown ST, Tokunaga TK, Wan JM, Firestone M, Newcomer DR, Resch CT, Cantrell KJ, Willett A, Koenigsberg S (2008) In situ long-term reductive bioimmobilization of Cr(VI) in groundwater using hydrogen release compound. Environ Sci Technol 42:8478–8485

Fredrickson JK, Hicks RJ, Li SW, Brockman FJ (1988) Plasmid incidence in bacteria from deep subsurface sediments. Appl Environ Microbiol 54:2916–2923

Ginn TR, Wood BD, Nelson KE, Scheibe TD, Murphy EM, Clement TP (2002) Processes in microbial transport in the natural subsurface. Adv. Water Resour 25:1017–1042

Harvey RW, George LH (1987) Growth determination for unattached bacteria in a contaminated aquifer. Appl Environ Microbiol 53:2992–2996

Harvey RW, Smith RL, George LH (1984) Effect of organic contamination upon microbial distributions and heterotrophic uptake in a cape cod, Mass. Aquifer. Appl Environ Microbiol 48:1197–1202

Hazen TC, Lombard KH, Looney BB, Enzien MV, Dougherty JM, Fliermans CB, Wear J, Eddy-Dilek CA (1994) Summary of in situ bioremediation demonstration (methane biostimulation) via horizontal wells at the Savannah River site integrated demonstration project. In: Gee GW, Wing NR (eds) Proceedings thirty-third Hanford symposium on health and the environment in-situ remediation: scientific basis for current and future technologies. Battelle Press, Columbus, pp 135–150

Hazen TC, Jimenez L, De Victoria GL, Fliermans CB (1991) Comparison of bacteria from deep subsurface sediment and adjacent groundwater. Microb Ecol 22:293–304

Hazen TC, Sayler GS (2016) Environmental systems microbiology of contaminated environments. In: Yates M, Nakatsu C, Miller R (eds) Manual of environmental microbiology, 4th edn. ASM Press, Washington, DC

Hazen TC, Tabak HH (2005) Developments in bioremediation of soils and sediments polluted with metals and radionuclides: 2. Field research on bioremediation of metals and radionuclides. Rev Environ Sci Biotechnol 4:157–183

Hazen TC (1997) Bioremediation. In: Haldeman PA (ed) Microbiology of the terrestrial subsurface. CRC Press, Boca Raton, pp 247–266

Horvath RS (1972) Microbial co-metabolism and the degradation of organic compounds in nature. Bacteriol Rev 36:146–155

Hubbard SS, Williams K, Conrad ME, Faybishenko B, Peterson J, Chen JS, Long P, Hazen TC (2008) Geophysical monitoring of hydrological and biogeochemical transformations associated with Cr(VI) bioremediation. Environ Sci Technol 42:3757–3765

Johnson WP, Zhang P, Fuller ME, Scheibe TD, Mailloux BJ, Onstott TC, DeFlaun MF, Hubbard SS, Radtke J, Kovacik WP, Holben W (2001) Ferrographic tracking of bacterial transport in the field at the Narrow Channel focus area, Oyster, VA. Environ Sci Technol 35:182–191

Keet BA (1995) Bioslurping state of the art. In: Hinchee RE, Kittel JA, Reisinger HJ (eds) Applied bioremediation of petroleum hydrocarbons. Battelle Press, Columbus, pp 329–334

Konikow LF, Bredeheoft JD (1978) Computer model of two dimensional solute transport and dispersion in ground water. Automated data processing and computations. Techniques of Water Resources Investigations of the U.S. Geological Survey, Washington, DC

Lee ES, Schwartz FW (2007) Characterization and optimization of long-term controlled release system for groundwater remediation: a generalized modeling approach. Chemosphere 69:247–253

Litchfield CD (1993) In situ bioremediation: basis and practices. In: Levin MA, Gealt MA (eds) Biotreatment of industrial and hazardous waste. McGraw-Hill, New York, pp 167–196

Loffler FE, Sun Q, Li J, Tiedje JM (2000) 16S rRNA gene-based detection of Tetrachloroethene-dechlorinating *Desulfuromonas* and *Dehalococcoides* species. Appl Environ Microbiol 66:1369–3445

Major D, Cox E, Edwards E, Hare PW (1994) The complete dechlorination of trichloroethene to ethene under natural conditions in a shallow bedrock aquifer located in New York state. In: Proceedings of the EPA Symposium on Intrinsic Bioremediation of Ground Water, August 30–September 1, Denver, CO. U.S. Environmental Protection Agency, EPA/540/R-94/515

Martin M, Imbrigiotta TE (1994) Contamination of ground water with trichloroethylene at the building 24 site at Picatinny arsenal, New Jersey. In: Proceedings of the EPA symposium on intrinsic bioremediation of ground water, august 30-September 1, Denver, CO. U.S. Environmental Protection Agency, EPA/540/R-94/515

Molz FJ, Widdowson MA, Benefield LD (1986) Simulation of microbial growth dynamics coupled to nutrient and oxygen transport in porous media. Water Resour Res 22:1207–1216

National Research Council (U.S.). Water Science and Technology Board (1993) In situ bioremediation: when does it work? National Academy Press, Washington, DC

Nelson MJK, Compeau G, Maziarz T, Mahaffey WR (1994) Laboratory treatability testing for assessment of field applicability. In: Flathman PE, Jerger DE, Exner JH (eds) Bioremediation field experience. Lewis Publishers, Boca Raton, pp 59–80

Paradis CJ, Jagadamma S, Watson DB, McKay LD, Hazen TC, Park M, Istok JD (2016) In situ mobility of uranium in the presence of nitrate following sulfate-reducing conditions. J Contam Hydrol 187:55–64

Paradis CJ, McKay LD, Perfect E, Istok JD, Hazen TC (2018) Push-pull tests for estimating effective porosity: expanded analytical solution and in situ application. Hydrogeol J 26:381–393

Paul EA, Clark FG (1989) Soil microbiology and biochemistry. Academic, San Diego

Plaza G, Ulfig K, Hazen TC, Brigmon RL (2001) Use of molecular techniques in bioremediation. Acta Microbiol Pol 50:205–218

Raymond RL, Jamison VW, Hudson JO (1977) Beneficial stimulation of bacterial activity in groundwater containing petroleum hydrocarbons. Am Inst Chem Eng Symp Ser 73:390–404

Rifai HS, Bedient PB, Borden RC, Haasbeek JF (1987) BIOPLUME II computer model of two-dimensional contaminant transport under the influence of oxygen limited biodegradation in groundwater User's manual version 1.0 Houston. Rice University, National Center for ground water research

Rifai HS, Bedient PB, Wilson JT, Miller KM, Armstrong JM (1988) Biodegradation modeling at an aviation fuel spill. ASCE J Environ Eng 114:1007–1029

Smillie CS, Smith MB, Friedman J, Cordero OX, David LA, Alm EJ (2011) Ecology drives a global network of gene exchange connecting the human microbiome. Nature 480:241–244

Smith MB, Rocha AM, Smillie CS, Olesen SW, Paradis C, Wu L, Campbell JH, Fortney JL, Mehlhorn TL, Lowe KA, Earles JE, Phillips J, Techtmann SM, Joyner DC, Elias DA, Bailey KL, Hurt RA Jr, Preheim SP, Sanders MC, Yang J, Mueller MA, Brooks S, Watson DB, Zhang P, He Z, Dubinsky EA, Adams PD, Arkin AP, Fields MW, Zhou J, Alm EJ, Hazen TC (2015) Natural bacterial communities serve as quantitative geochemical biosensors. MBio 6: e00326–e00315

Thomas JM, Ward CH (1989) In situ biorestoration of organic contaminants in the subsurface. Environ Sci Technol 23:760–766

Thomas JM, Ward CH (1992) Subsurface microbial ecology and bioremediation. J Hazard Mater 32:179–194

Travis BJ, Rosenberg ND (1997) Modeling in situ bioremediation of TCE at Savannah River: effects of product toxicity and microbial interactions on TCE degradation. Environ Sci Technol 31:3093–3102

Tremblay D, Tulis D, Kostecki P, Ewald K (1995) Innovation skyrockets at 50,000 LUST sites, EPA study reveal technology use at LUST sites. Soil Groundwater Cleanup 1995:6–13

USEPA (1988) Clean-up of releases from petroleum USTs. Vol. Report-530-UST-88-001 USEPA, Washington, DC

USEPA (1989) Bioremediation of hazardous waste sites workshop. CERI-89-11. Washington, DC

USEPA (2013) Introduction to in situ bioremediation of groundwater. EPA 542-R-13-018

USEPA (2017) How to evaluate alternative cleanup technologies for underground storage tank sites. EPA 510-B-17-003

Widdowson MA, Molz FA, Benefield LD (1987) Development and application of a model for simulating microbial growth dynamics coupled to nutrient and oxygen transport in porous media. In: Proceedings of the Association of Groundwater Scientists and Engineers/international ground water model center, pp 28–51, Holcomb research center institute conference on solving ground water problems with models. Dublin: National Ground Water Association

Widdowson MA, Molz FJ, Benefield LD (1988) A numerical transport model for oxygen- and nitrate-based respiration linked to substrate and nutrient availability in porous media. Water Resour Res 24:1553–1565

Wilson JT, Weaver JW, Kampbel DH (1994) Intrinsic bioremediation of TCE in ground water at an NPL site in St. Joseph, Michigan. Vol. proceedings of the EPA symposium on intrinsic bioremediation of ground water, august 30-September 1, Denver, CO. U.S. Environmental Protection Agency, EPA/540/R-94/515