Biostimulation

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Abstract

Biostimulation is one of the most mature methods of bioremediation of hydrocarbons, yet recent advances in geophysics, stable isotope analyses, and molecular microbiology promise dramatic increases in the depth, breadth, and throughput of biostimulation strategies. Using a systems biology approach we can now understand not only what microbes are present, but their in situ activities to trace nutrients, electron donors, electron acceptors, contaminants, and environmental stressors. Using this knowledge in combination with critical biogeochemistry, hydrology, geology, and toxicology will be enabling to develop conceptual and numerical models for the best biostimulation strategy and better long-term stewardship of the environment.

Introduction

Bioremediation is the use of biological processes to return the environment to its original state. More realistically, the goal of bioremediation is to make the environment less toxic. In the broadest application sense, bioremediation includes use of enzymes, growth stimulants, bacteria, fungi, or plants to degrade, transform, sequester, mobilize, or contain contaminant organics, inorganics, or metals in soil, water, or air (Figure 1). If we accept the "Doctrine of Infallibility", i.e. there is no compound known to man that microorganisms cannot degrade (Alexander, 1965), then bioremediation becomes one of the great solutions for our environmental problems. Unfortunately, while the Doctrine of Infallibility may be absolutely true, the rates of biodegradation or transformation of some compounds is so slow as to be negligible for some bioremediation purposes. In addition, the conditions (environmental or biological) that allow certain biological reactions to take place may not be obtainable in many environments (Fewson, 1988).

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 nutrients 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 (Figure 2). 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).

Our ability to enhance bioremediation of any environment is directly proportional to knowledge of the biogeochemistry of the site. Finding the limiting conditions for the indigenous organisms to carry out the desired remediation is the most critical step. As with surface environments, the parameters that are usually limiting organisms are required nutrients, inorganic and organic (Figure 3). Of these, the most common are

water, oxygen, nitrogen, and phosphorus. In the terrestrial subsurface, water can be limiting but usually is not. 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 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). Even plants, e.g. Poplar trees, have been used to biostimulate remediation of subsurface environments (Schnoor et al., 1995). In this later case the plants act as solar-powered nutrient pumps stimulating rhizosphere microbes to degrade contaminants (Anderson et al., 1993).

Biostimulation 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 oxygen through the formation. The minimum average

hydraulic conductivity for a formation is generally considered to be 10^{-4} cm/sec (Thomas and Ward, 1989). 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 can also be problematic, in that it adsorbs rapidly to clays, causes pH changes in poorly buffered environments, and can cause clays to swell, decreasing permeability around the injection point. Many of these problems can be handled at some sites by excavating the soil or pumping the groundwater to the surface and treating it in a bioreactor, prepared bed, land farm, bioslurry reactor, biopile, or composting (Figure 1). In these cases, the permeability can be controlled or manipulated to allow better stimulation of the biotreatment process. It is generally accepted that soil bacteria need a C:N:P ratio or 30:5:1 for unrestricted growth (Paul and Clark, 1989). Stimulation of soil bacteria can generally be achieved when this nutrient ratio is achieved following amendment addition. The actual injection ratio used is usually slightly higher 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. Slow release electron donors (low solubility) have also been used to control biostimulation for many types of contaminants (Faybishenko et. al., 2008; Hubbard et. al., 2008). It may also be necessary to remove light non-aqueous 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.

Critical Biogeochemistry

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 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.

Characterization and Monitoring

Characterization and monitoring of bioremediation can be as simple as maintaining a fermentor for above ground processes like prepared beds, land farming, bioslurry reactors, composting and bioreactors. The terrestrial subsurface is much more difficult due to its sampling problems, poorly defined interfaces and spatial heterogeneity. For any type of bioremediation, careful consideration and planning must be given to the remediation objectives, sampling, the types of samples, frequency, cost, priority, and background literature for method verification. The microbiology and chemistry may be of less overall importance to the remediation of the site than the hydrology, geology, meteorology, toxicology, and engineering requirements. All of these things must be integrated into the plan for characterization and monitoring of any site. For an example of test plans for bioremediation see Hazen (1991), Lombard and Hazen (1994), and Nelson et al. (1994).

The type of sample used for monitoring and characterization of sediment or ground water can have a significant impact on a bioremediation project. Fortunately, most bioremediation applications are shallow and eutrophic, due to the nature of the

waste mix usually deposited. 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 ground water was aerobic (> 2 mg/L O_2).

Determining the rate and amount of contaminant that is bioremediated in any environment is one of the most difficult measurements. Many of the problems and measurements discussed above for mass balance in treatability studies also apply here. In past years, bioremediation studies have focused on measurement of biodegradation products rather than the organisms, due to the difficulty in measuring organisms. Soil and ground water measurements of microorganisms used to require long incubations or long preparation times and the measurements were usually not specific to contaminant-degraders. Several methods have been used to determine the rate and amount of biodegradation: monitoring of conservative tracers, measurement of byproducts of anaerobic activity, intermediary metabolite formation, electron acceptor concentration, stable isotopic ratios of carbon, and ratio of non-degradable to degradable substances. Helium has been used at a number of sites as a conservative tracer since it is non-reactive, non-biodegradable and moves like oxygen (National Research Council, 1993). By simultaneously injecting He with O_2 at known concentrations and comparing the subsurface ratios over time, the rates of respiration can be calculated. This technique has also been used to measure rates of injected methane consumption (Hazen, 1991). Bromide has been successfully used as a conservative tracer for liquid injection comparisons with nitrate, sulfate and dissolved oxygen (National Research Council, 1993). Byproducts of anaerobic biotransformation in the environment have been used to estimate the amount of biodegradation that has occurred in anaerobic environments, e.g. PCB-containing sediments. These byproducts include methane, sulfides, nitrogen gas, and reduced forms of iron and manganese (Harkness et al., 1993). Measurements of chloride changes have also proven useful in indicating the amount of chlorinated solvents that have been oxidized or reduced (Hazen et al., 1994). Consumption of electron acceptors (O_2, NO_3, Fe(III), or SO_4) has been used for measuring rates of biodegradation and bioactivity at some bioremediation sites (National Research Council, 1993; Smith et al., 1991). Bioventing remediations of petroleum-contaminated sites rely on stable isotopic ratios of carbon, carbon dioxide production, and oxygen consumption

to quantify biodegradation rates in the field (Hinchee et al., 1991; Hoeppel et al., 1991). Mixtures of contaminants, e.g. petroleum hydrocarbons, can have their own internal standards for biodegradation. By comparing concentrations of non-biodegradable components of the contaminant source with concentrations of degradable components from both virgin and weathered sources, the amount of contaminant degraded can be calculated. These measurements have been used on the Exxon Valdez spill cleanup (Glasser, 1994), and at a number of other petroleum-contaminated sites (Breedveld et al., 1995).

Microbial ecologists have continually struggled with methods for measuring what organisms are in the environment, how many organisms are present, and how active are the organisms that are present. For bioremediation, we need to know what contaminant-degraders are present, how many contaminant degraders are present, and how active are the contaminant-degraders that are present. We may also need to know if there are other organisms present that are important in the biogeochemistry and what proportion of the total community the degraders represent.

Plate counts can only provide a measurement of what microbes are present in the sample that will grow on the media used, under the conditions incubated. Given the infinite number of possible media and the infinite number of possible incubations, this leads to an infinite number of possible interpretations. Generally heterotrophic plate counts have been used to show that bacteria densities in the sediment or ground water increase in response to biostimulation (Litchfield, 1993). Using contaminant enrichment media and either plates or most probable number (MPN) extinction dilution techniques, the number of contaminant-degraders can be estimated (National Research Council, 1993). However, there are serious fallacies in the underlying assumptions of many of these assays, e.g. diesel-degraders are determined using minimal media with a diesel-soaked piece of cotton taped to the top of the petri dish. Are the colonies that are seen using the diesel or are they merely tolerant to the volatile components of the diesel fuel? In contrast, MPN assays have been used to conservatively measure methanotroph densities in soil and ground water at chlorinated solvent-contaminated sites by sealing each tube under an air/methane headspace and then scoring positive only those tubes that are turbid and have produced carbon dioxide and used methane (Fogel et al.,

1986). The incubation time for plate count and MPN contaminant-degrader assays is 1-8 weeks, thus negating their use for real-time monitoring and control.

A number of direct count assays have been tried on contaminant degraders including direct fluorescent antibody staining (DFA), acridine orange direct counts (AODC), and fluorescien isothiocyanate (FITC) direct counts. The fluorochrome stains only indicate the total numbers of organisms present in the sample, they do not indicate the type of organism or it's activity. However, these techniques have been used in bioremediation studies to determine changes in the total numbers of organisms (Litchfield, 1993). Increases in total counts have been found when contaminated environments are biostimulated. DFA shows promise but requires an antibody that is specific to the contaminant-degraders that are in that environment. The environment must be checked for organisms that may cross-react with the antibody and for contaminant-degraders that do not react with the antibody. DFA will be most useful in monitoring specific organisms added for bioaugmentation, though it has been used in biostimulation applications (Fliermans et al., 1994). Since the assay time is only hours for these direct techniques, they have significant advantages for real-time monitoring and rapid characterization.

Biological activity at bioremediation sites has been determined a number of ways: INT activity/dehydrogenase, fatty acid analyses, acetate incorporation into lipids, ^{3}H-thymidine incorporation into DNA, BIOLOG™, phosphatase, and acetylene reduction. The INT test has been used in combination with direct counts since INT-formazan crystals can be detected in the cell. Cells with crystals are assumed to be actively respiring since the reaction occurs at the electron transport system of the cell. The assay requires only a 30-minute incubation; however, it can only be used in ground water samples since particles in sediment samples cause too much interference with interpretation of the intracellular crystals. Barbaro et al. (1994) used this technique to measure microbial biostimulation of the Borden Aquifer in Canada.

Phospholipid fatty acid analyses (PLFA) have been used for characterization and monitoring at a number of bioremediation sites. The PLFAs (signature compounds) that an organism has may be unique to that species or even strain, or they may be conserved across physiological groups, families or even kingdom. Certain groups of

fatty acids (cis and trans isomers) may also change in response to the physiological status of the organism. PLFAs have been used at bioremediation sites to provide direct assays for physiological status (cis/trans ratio), total biomass estimates, presence and abundance of particular contaminant-degraders and groups of organisms, e.g. methanotrophs, actinomycetes and anaerobes (Phelps et al., 1989; Heipieper et al., 1995; Ringelberg et al., 1994). PLFAs would seem to be a panacea for characterization and monitoring of bioremediation. Unfortunately, the assays require -70°C sample storage, long extraction times, have a fairly high detection limit (10,000 cells), and require expensive instrumentation. This technique merits careful consideration since it is so versatile and is a direct assay technique.

Radiolabeled acetate and thymidine incorporation into lipids and DNA, respectively, have been used at bioremediation sites to provide measurements of total community metabolic and growth responsiveness (Fliermans et al., 1988; Palumbo et al., 1995). These techniques require incubation, extraction, purification, and radiolabeled substrates, making interpretation of results difficult.

The BIOLOG™ assay has also been adapted to determine the activity of bacteria in groundwater and soil samples to contaminants. The assay consists of a 96 well titer plate with carbon sources and an electron transport system indicator. It can be used to identify isolates and to examine the overall activity of a soil or water sample to a particular substrate. Gorden et al. (1993) adapted the assay to determine activity to different contaminants by using both contaminants and ETS indicator alone and adding contaminants to the plates with substrates to determine co-metabolic activity. The assay provides more rapid screening than other viable count techniques but it suffers from some of the same problems, e.g. incubation conditions, repeatability. It is also difficult to determine if the contaminants are being transformed or tolerated.

Phosphatase and dehydrogenase enzyme assays have also been used to access bioactivity in soil and ground water during bioremediation of terrestrial subsurface sites. Acid and alkaline phosphatase have been linked to changes in ambient phosphate concentrations and bioactivity at contaminated sites caused by biostimulation (Lanza and Dougherty, 1991). The incubation, extraction and interference caused by pH differences in samples make results difficult to interpret.

Acetylene reduction has been used to indicate nitrogenase activity in a few bioremediation studies; however, the importance of nitrogen fixation for most bioremediation is probably insignificant, unless the site is oligotrophic (Hazen et al., 1994).

Nucleic acid probes provide, at least theoretically, one of the best ways to characterize and monitor organisms in the environment (Hazen et al., 2009; Brodie et. al., 2006). Since many contaminants, especially the more recalcitrant ones, are degraded by only a few enzymes, it is possible to produce DNA or even RNA probes that will indicate the amount of that gene in the environment. This tells us if the functional group that can degrade or transform the contaminant is present and its relative abundance. Since probes have also been found for species, families, and even kingdoms, this allows soil and ground water communities to be monitored. Recently, conserved regions in ribosomes have also been found, allowing samples to be probed for the relative abundance of ribosomes and, hence, the bioactivity of the total community (Fields et. al., 2006). Bowman et al. (1993) demonstrated that probes for methanotrophs indicated their presence in soil at TCE-contaminated sites in South Carolina and Tennessee. Brockman et al. (1995) and Hazen et al. (2009) also showed that methane/air injection at the South Carolina site increased the methanotroph probe signal in sediment near the injection point in the aquifer. The probe signal increases for methanotrophs coincided with increases in the MPN counts for methanotrophs. Thus, sediment and groundwater can be directly extracted and probed with DNA and RNA for bioremediation characterization and monitoring. Sequencing throughput has increased exponentially over the last 10 years. Indeed, facilities like the Joint Genome Institute can now sequence the average bacterial genome before the first coffee break in the morning. More than 700 microbes have now been sequenced but we expect to double that in the next year. We can now sequence whole communities without ever culturing a single organism and determine the biogeochemical relationships and syntrophy of entire communities. Tyson et al. (2004) showed that the metagemone from Iron Mt., California with a pH of 0.7 and a temperature of 42°C harbored an intricate relationship between iron and sulfate reducers, e.g. *Ferroplasma* spp. and *Leptospirillum* spp. This has enabled a whole new area of ecogenomics, the study of genomes in an

environmental context. In addition to metagenome analyses of the DNA sequence we can also use techniques to look at specific components of the genome in highly conserved regions like 16s ribosomal DNA to get specific identifications of species and look at evolutionary relationships between species. A number of other techniques for examining DNA from the environment have also been used over the past several years, one of the most popular being T-RFLP, terminal restriction fragment length polymorphism, which cuts the DNA and then examines the pattern of the fragments as an index of community structure change (Chivian et al, 2008; Lin et al., 2006). DNA codes for RNA, which codes for proteins, which produce metabolites, which lead to the physiology of the cell, the consortia, the community, and the ecosystem (Figure 5). Thus with recent analytical advances and our increasing understanding of cell structure and metabolism we are taking increasing advantage of other components in the cell to determine environmental relationships and biogeochemistry. As the sequence for different microbes has been annotated, it has enabled us to study the up and down regulation of genes being expressed, i.e. transcriptomics. Using microarrays that detect mRNA we can determine what genes are being turned on or off to provide code for protein production (Wu et. al., 2008). Using these functional gene arrays we can determine which biodegradation pathways are active, the dominant terminal electron acceptor process, and if particular daughter products could be formed (He et. al., 2007). We can also use real-time PCR techniques to amplify sequences that being expressed so we can see changes in expression of specific genes, depending on the primer being used. These techniques are now being used to determine if TCE degraders are present and active in environments where bioremediation is being considered or underway (Loffler et al. 2000; Hazen et. al. 2009). Through techniques like Multiple Displacement Amplification, we can now sequence metagenomes with fewer than 5 cells in a sample (Albulencia et. al., 2006)

In the same way that genomes have been used in an ecological context to elucidate new understanding, we are now beginning to use proteins. Recent studies by Ram et al. (2005) demonstrated that metaproteome analyses, i.e. determining all the proteins that are in an environment could determine the relative abundance of a particular protein, again in the Iron Mt., California site. Thus the study of proteins, i.e.,

proteomics, could enable more specific determinations of type of enzymatic reactions that the cells is currently capable of carrying out, not just which genes have been turned on or off to express the code for a particular protein. These studies are starting to show the effects that environmental stressors can have on bacteria and the pathways that bacteria use in stress response (Hazen and Stahl, 2006).

Are Treatment Trains the Final Solution? The coupling of intrinsic bioremediation (natural attenuation) to engineered bioremediation has been proposed but rarely tried (Wilson et. al., 1994). Nearly all engineered bioremediation projects could substantially reduce costs by stopping the biostimulation 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. Afterwards, if the model holds true, monitoring frequency and numbers of parameters gradually decline until the site is cleaned up.

Research Needs

Biostimulation is the most mature technologies available for bioremediation of hydrocarbon contaminants. However, using a systems biology approach to understand, model, predict, monitor, and control bioremediation processes promises to provide faster, better, and cheaper environmental cleanup and stewardship (Figure 6). This will require large research investments to make the links for lab to the field and back at all scales. New molecular techniques promise better and rapid, nearly real-time, assessments of microbiological presence and activity as it relates to the overall biogeochemistry. Further, bioinformatics and environmental modeling in particular need significant research investments in order for us to realize the full potential for these new systems biology techniques and approaches in biostimulation.

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Figure Legends.

Figure 1. Bioremediation Technologies used for bioremediation.

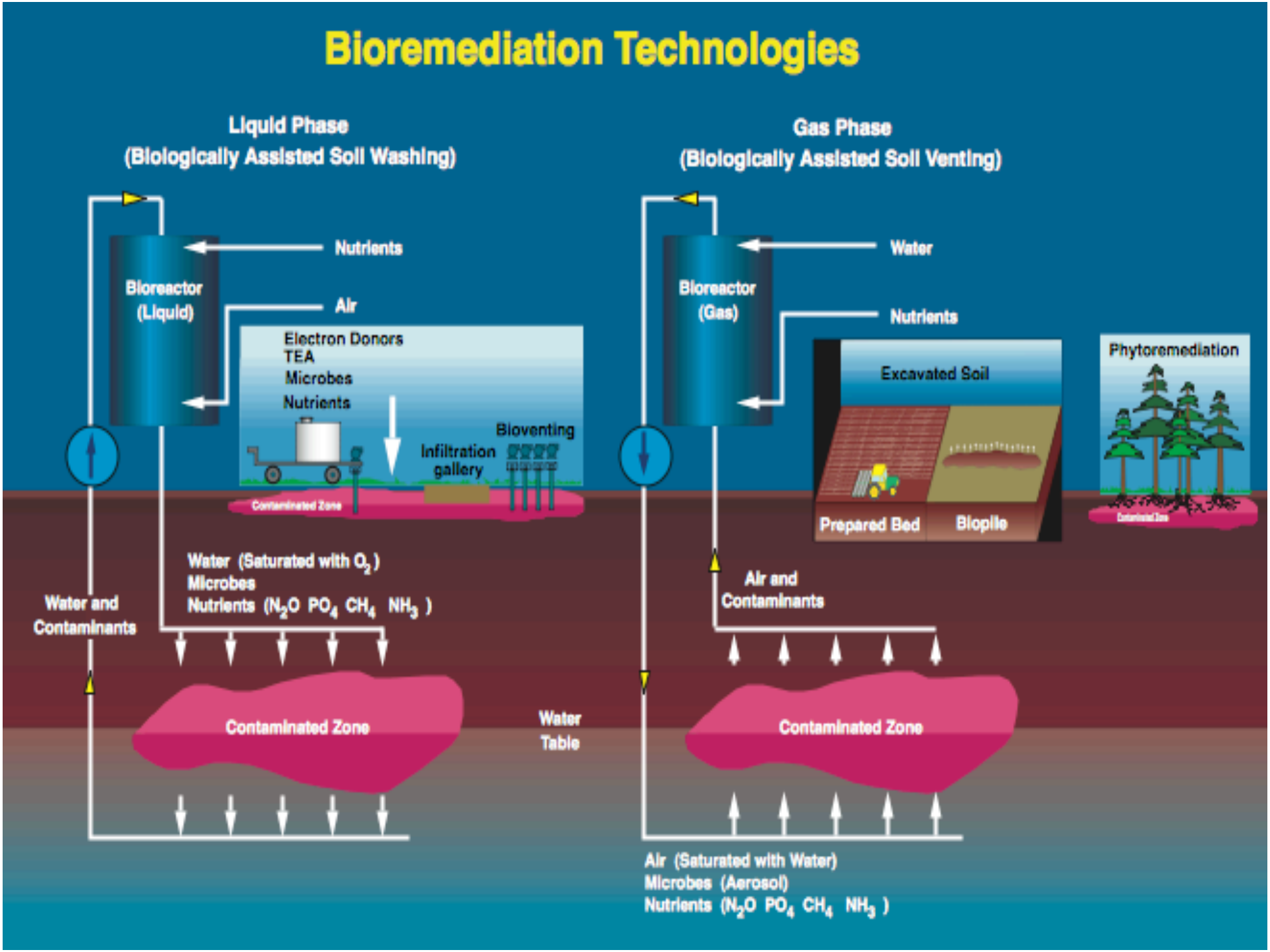
Figure 2. Biostimulation Requirements and examples of ‘stimulants’ that are used.

Figure 3. Factors that Affect Biodegradation.

Figure 4. Critical Biogeochemistry involving terminal electron acceptors and their heiarchical redox potential relationships.

Figure 5. High Density Microarray Analysis for a polylactate biostimulated site in Washington State (http:// esd.lbl.gov/ERT/hanford100h/)

Figure 6. Systems Biology Approach to Bioremediation (after M. Fields, Montana State University, see also http://vimss.lbl.gov)


Bioremediation Technologies
Liquid Phase
(Biologically Assisted Soil Washing)
Gas Phase
(Biologically Assisted Soil Venting)
Bioreactor
(Liquid)
Nutrients
Air
Electron Donors
TEA
Microbes
Nutrients
Bioventing
Infiltration
gallery
Contaminated Zone
Water (Saturated with O_2)
Microbes
Nutrients (N_2O PO_4 CH_4 NH_3)
Water and
Contaminants
Contaminated Zone
Water
Table
Bioreactor
(Gas)
Water
Nutrients
Excavated Soil
Prepared Bed
Biopile
Phytoremediation
Air and
Contaminants
Contaminated Zone
Air (Saturated with Water)
Microbes (Aerosol)
Nutrients (N_2O PO_4 CH_4 NH_3)

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.

Factors that Affect Biodegradation

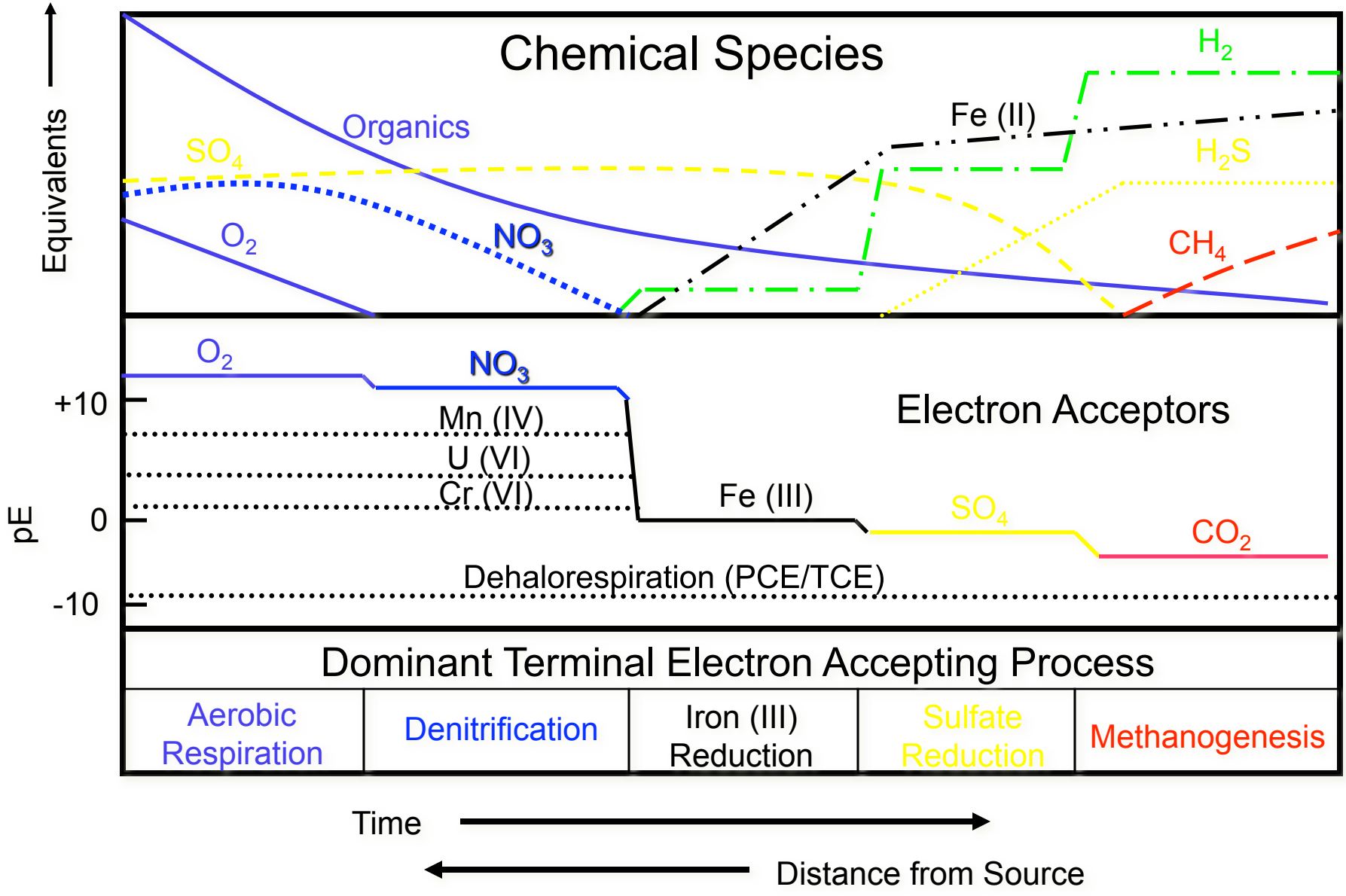
The Contaminant

- molecular size, shape, charge and functional groups, concentration
- solubility in water; lipid/water partition coefficient
- solid/liquid/gas; volatilization
- toxicity
- possibility of spontaneous nonenzymatic reactions

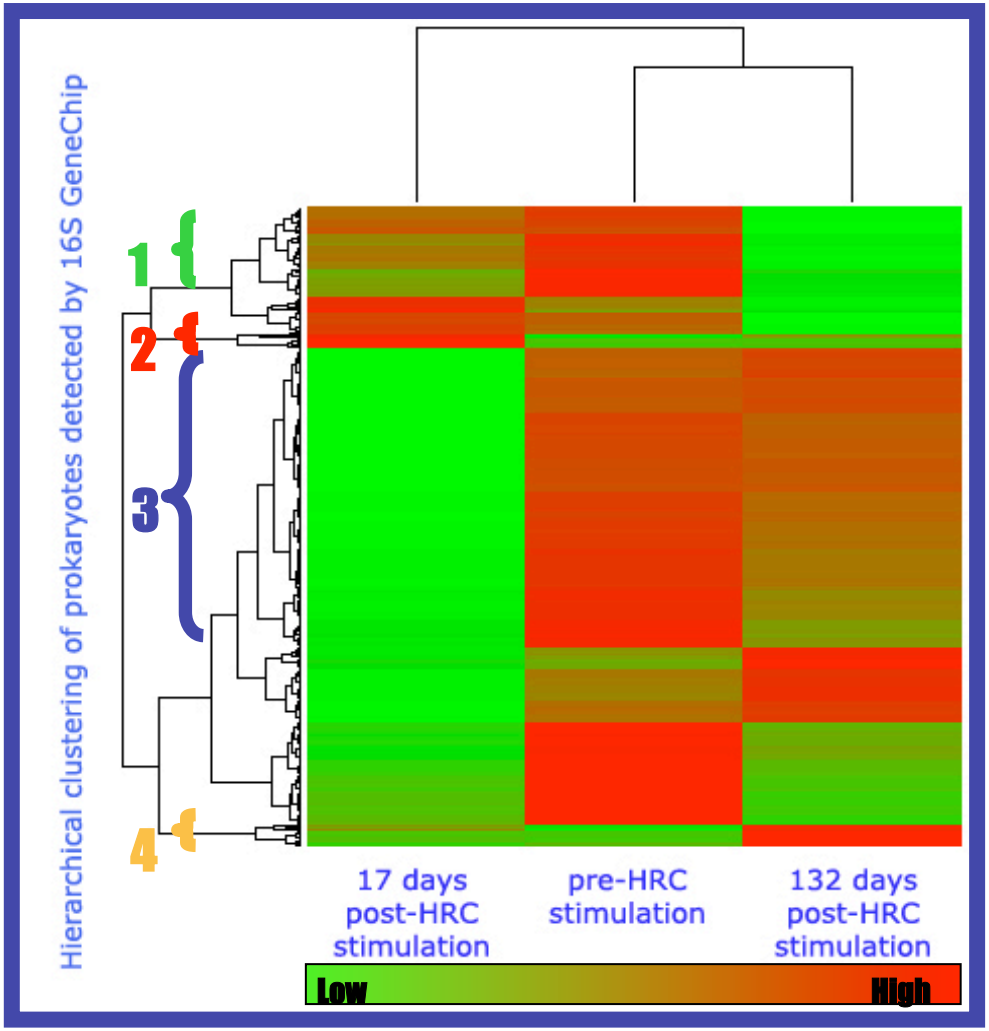
The Environment

- mechanical accessibility
- pH, pO_2, temperature, redox potential
- presence of interfaces
- ionic composition and concentration
- water and wind speed, light quality and intensity
- presence of co-metabolites, essential nutrients, reactive radicals, etc.
- presence of appropriate organisms or plasmids

Critical Biogeochemistry



High Density Microarray Analysis



Hierarchical clustering and heatmap plot of 16S GeneChip analysis of microbial community sub-families detected during chromate bioremediation. PCA groups are indicated by brackets.

Bacteria and Archaea Detected

Grouped according to response to HRC during chromate remediation

Group1 organisms decline
Pseudomonas, Burkholderia (Denitrifiers)
Acidithiobacillus, Thiothrix (Sulfur oxidizers)
Leptothrix (Iron oxidizer)

Group2 organisms increase then decline
Acidovorax, Thauera (denitrifiers)
Flavobacteria (aerobes, use glycerol)

Group3 organisms decline then return
Mainly oligotrophic bacteria

Group 4 organisms increase in late stages
Legionella, Chlamydophila, Flectobacillus.

Systems Biology Approach

<u>Ecosystem</u>
Identify key factors (i.e., stresses) that drive community structure and composition and impact the survival and efficacy of hydrocarbon degraders

<u>Community and Population</u>
Understand impacts on structure/function relationships

<u>Cell</u>
Analyze DNA, RNA, and protein at the cellular level to understand cellular effects in terms of bioremediation