DEQ

ENVIRONMENTAL RESPONSE DIVISION
DESIGN, O & M UNIT

FUNDAMENTAL PRINCIPLES OF BIOREMEDIATION
(An Aid to the Development of Bioremediation Proposals)

APRIL 1998

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Foreword

The purpose of this document is to provide fundamental information about factors that influence the rate and extent to which environmental contaminants are metabolized by microorganisms in the environment. This information is intended to help the reader "ask the right questions" when evaluating whether bioremediation is a viable strategy for remediation of a contaminated site. It is not intended to be a "road map" or "checklist" for the use of bioremediation technologies.

Decisions pertaining to the use, management, and monitoring of biological processes in a predictable and effective manner for site remediation are often complex and dependent on site-specific conditions such as the properties of the contaminated environmental matrix (soil, sediments, water, etc.), the contaminants present (physicochemical properties, biodegradability, kinds of mixtures, etc.) and microbial ecology (factors controlling microbial growth and activity, etc.). Because the effective use of bioremediation technologies requires a rather sophisticated understanding of site-specific conditions, it is often unwise to propose "formula" approaches for site remediation. Hopefully, this document will lead to the "right questions" being asked and the "right data" being gathered so that sound decisions can be made regarding the use of bioremediation for site remediation.

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I.   Introduction

Bioremediation is the use of microorganisms for the degradation of hazardous chemicals in soil, sediments, water, or other contaminated materials. Often the microorganisms metabolize the chemicals to produce carbon dioxide or methane, water and biomass. Alternatively, the contaminants may be enzymatically transformed to metabolites that are less toxic or innocuous. It should be noted that in some instances, the metabolites formed are more toxic than the parent compound. For example, perchloroethylene and trichloroethylene may degrade to vinyl chloride.

There are at least five critical factors that should be considered when evaluating the use of bioremediation for site clean up. These factors are:

1.   Magnitude, toxicity, and mobility of contaminants.

It is imperative that the site be properly investigated and characterized to determine the (a) horizontal and vertical extent of contamination; (b) the kinds and concentrations of contaminants at the site; (c) the likely mobility of contaminants in the future, which depends in part on the geological characteristics of the site.

2.   Proximity of human and environmental receptors.

Whether bioremediation is the appropriate cleanup remedy for a site is dependent on whether the rate and extent of contaminant degradation is sufficient to maintain low risks to human or environmental receptors.

3.   Degradability of contaminants.

The biodegradability of a compound is generally high if the compound occurs naturally in the environment (e.g., petroleum hydrocarbons). Often, compounds with a high molecular weight, particularly those with complex ring structures and halogen substituents, degrade more slowly than simpler straight chain hydrocarbons or low molecular weight compounds.   Whether synthetic compounds are metabolized by microorganisms is largely determined by whether the compound has structural features similar to naturally occurring compounds. The rate and extent to which the compound is metabolized in the environment is often determined by the availability of electron acceptors and other nutrients.

4.   Planned site use.

A critical factor in deciding whether bioremediation is the appropriate cleanup remedy for a site is whether the rate and extent of contaminant degradation is sufficient to reduce risks to acceptable levels.

5.   Ability to properly monitor.

There are inherent uncertainties in the use of bioremediation for contaminated soils and aquifers due to physical, chemical and biological heterogeneities of the contaminated matrix. It is important to recognize that biological processes are dynamic and, given current knowledge, often lack the predictability of more conventional remediation technologies. Thus, it is important to insure that unacceptable risks do not develop in the future. These risks may include migration of contaminants to previously uncontaminated media and the failure of bioremediation to achieve acceptable contaminant concentrations.

The remainder of this document will focus on the factors that influence the *rate and extent* of contaminant degradation by microorganisms. These can be broadly grouped into two classes of factors: (a) biological factors and (b) environmental factors. The biological factors are primarily concerned with the numbers of specific kinds of microorganisms present and the expression and activity of metabolic enzymes, in other words, the amount of "catalyst" present. The environmental factors include chemical and physical characteristics that influence the bioavailability of contaminants, the availability of other nutrients, the activity of biological processes (temperature and pH, for example), characteristics of the contaminants with respect to how they interact with the site's geochemical and geological characteristics.

II.   Potential Advantages and Disadvantages of Bioremediation Technologies

The use of intrinsic or engineered bioremediation processes offers several potential advantages that are attractive to site owners, regulatory agencies, and the public. These include:

- Lower cost than conventional technologies.
- Contaminants usually converted to innocuous products.
- Contaminants are destroyed, not simply transferred to different environmental media.
- Nonintrusive, potentially allowing for continued site use.
- Relative ease of implementation.

However, there are potential disadvantages to bioremediation as well, these include:

- May be difficult to control.
- Amendments introduced into the environment to enhance bioremediation may cause other contamination problems.
- May not reduce concentration of contaminants to required levels.
- Requires more time.
- May require more extensive monitoring.
- Lack of (hydraulic) control.
- Dynamic process, difficult to predict future effectiveness.

III. Presumptive Evidence for Bioremediation

No one piece of evidence can unambiguously prove that microbial processes are responsible for an observed decrease in contaminant concentration. Typically, a "weight of evidence" argument must be formulated based on several lines of evidence. The kinds of data consistent with bioremediation include:

- Accumulation of intermediates formed during the metabolism of contaminants.
- An elevated concentration of metabolic by-products formed through microbial activity.
- An increased ratio of compounds that are difficult to degrade relative to those that are readily degraded.
- Metabolism of contaminants at reasonable rates in laboratory microcosms that contain soils, sediments or water from the site.
- Depletion of oxygen or nutrients required for microbial growth.
- More rapid depletion of added oxygen in the contaminated areas as compared to unimpacted areas.
- Decreased contaminant levels following addition of oxygen or nutrients.
- An increase in the number of protozoans suggesting there has been an increase in the number of bacteria that they feed on.
- Changes in the isotope ratio of inorganic carbon consistent with the conversion of contaminant to carbon dioxide, carbonate, and related compounds.
- Documentation of the amount of contaminant removed by nonbiological processes using conservative tracers.
- Demonstrated conversion of contaminants to metabolites using isotopically labeled contaminants.

IV. Glossary of Terminology

The choice of bioremediation technologies used for site remediation is often dictated by whether it is possible or practical to physically manipulate the contaminated materials. If the contaminated materials can be removed and treated ex situ in a cost-effective manner, then this is often the approach selected because it offers the advantages of better process control, more rapid rates of degradation, and greater minimization of risk. However, if the amount of contaminated material is large or if the material is not easily accessed, then treatment of the material in situ is often the only practical and cost-effective alternative. This approach is also advantageous if there is no imminent risk to human health or the environment and if the time required to reach closure is not a critical factor.

Over the years, numerous terms have been used to describe various remediation technologies that rely on microbial processes for the destruction of contaminants. Often several different terms have been used to describe the same basic process and the terminology can be confusing. Here are explanations of a few of the most commonly used terms.

A.   Intrinsic Bioremediation

Often bioremediation can be accomplished without human intervention by microorganisms that are naturally found in the contaminated matrix. For this approach to be used, it is usually necessary for the rate of contaminant degradation to exceed the rate of contaminant migration. Knowledge of the following key site characteristics are required to evaluate the likely success of intrinsic remediation; the bioavailability of contaminants, levels of nutrients, the presence of minerals to buffer the pH of the matrix, adequate levels of electron acceptors (either oxygen, nitrate, ferric iron, or sulfate), and site specific contamination migration rates.

B.   Engineered Bioremediation

In some cases, it may be desirable to construct engineered systems to supply nutrients, electron acceptors or other materials that enhance the rate or extent of contaminant degradation. This is desirable when the rate of contaminant migration, the location of environmental receptors or other liability issues dictate that steps be taken to optimize the rate of contaminant degradation in order to mitigate contaminant migration. The key site characteristics for engineered bioremediation are the same as for intrinsic remediation. However, in addition, it is important to consider factors that affect the ability to introduce chemicals to the environment such as the permeability of the soils or subsurface and the uniformity of the subsurface.

C.   Combination of Technologies

Nonbiological treatment technologies or source removal may be used to reduce the total amount of contaminant present at the site before, or concurrent with, bioremediation. For example, excessively contaminated soils may be excavated at the source of contamination, volatile contaminants may be vacuum extracted, or undissolved pools of contaminants may be pumped from aquifers.

D.   In Situ Bioremediation

Bioremediation technologies that are used "in place" without removal of the contaminated matrix. Both intrinsic and engineered bioremediation technologies can be used in situ.

E.   Ex Situ Bioremediation

Bioremediation technologies that require removal of the contaminated matrix by excavation so it can be manipulated in some way through the use of slurry reactors, composting, biopiles, or other technologies.

V.   Microbial Ecology

A.   Microbial Diversity

1.   Numbers and Kinds of Microorganisms

In most environments there are large numbers of microorganisms present. In aquatic environments there can be 10^5 - 10^7 organisms per ml whereas in surface soils the number of organisms ranges from 10^7 - 10^9 per gram. However, it is important to recognize that often only a fraction of the total microbial community actually metabolizes the compound(s) of concern and a majority of organisms utilize other carbon sources that are present. As a result, the total number of microorganisms present per unit mass is a poor indicator of the rate of contaminant degradation. Thus, the rate of contaminant degradation is not always proportional to the total number of organisms present.

"In most environments there are large numbers of microorganisms present."

The rate of contaminant degradation is roughly proportional to the number of microorganisms present in a sample that actually metabolize the compound of concern in situ. However, for a variety of reasons, it can be difficult or impossible to accurately determine the numbers of active organisms in a sample or to make comparisons between regions of the same site or between different sites. For example, the recovery of organisms can be markedly affected by the procedures used and generally a fraction can be cultivated using any one procedure. Moreover, the numbers of organisms able to metabolize a contaminant that can be recovered from a sample may not be proportional to the rate of contaminant degradation in the sample since the organisms cultivated may not actively metabolize the contaminant in situ. For these and other reasons, it is difficult to reach definitive conclusions based simply on the numbers of organisms.

Numerous factors can influence the distribution and numbers of microorganisms found at a particular site. Moreover, organisms able to degrade some contaminants are widely distributed in nature and are likely to be found at most sites, whereas organisms able to degrade more recalcitrant compounds may not be widespread. Prevailing site conditions such as high concentrations of contaminants or toxicants (heavy metals, for example) and other factors such as extremes of pH, low moisture, and nutrient limitation, can restrict or preclude microbial growth. As a result there is merit to demonstrating that organisms able to degrade a given contaminant are present in reasonable numbers at a given site. In view of the limitations described above, it is often desirable but never sufficient to demonstrate that microorganisms able to degrade a specific contaminant are present at various locations at a site.

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In many microbial communities there is more than one microbial population able to metabolize a given contaminant. These populations will compete for and metabolize the available contaminant and work in concert to reduce the contaminant mass. Although the kinetic efficiency of contaminant metabolism is an important determinant of which of these populations

will become numerically dominant, there are other factors, such as predation, and nutritional requirements, that influence this as well.

This competition among functionally equivalent populations can be important because the properties of the microorganisms can differ significantly. For example, not all organisms able to metabolize a contaminant will do so at the same rate or to the same extent. In addition, the populations may metabolize the contaminants using different metabolic pathways that cause different metabolites to accumulate. This can be particularly problematic in processes that result in cometabolism (see section B.3) of the contaminants, since not all pathways are capable of cometabolism. For example, there are five known pathways for the metabolism of toluene by aerobic microorganisms, yet not all of these can efficiently cause cometabolism of trichloroethylene. Consequently, selection of certain populations can result in efficient metabolism of toluene, but little or no cometabolism of trichloroethylene.

2.   Bioaugmentation

In some instances, the rate of biological degradation can be increased through the addition of microorganisms that have been shown to degrade the contaminants of concern at high rates or are particularly well suited to remain active under prevailing site conditions. This process is referred to as bioaugmentation. This can be useful if the contaminants are particularly recalcitrant to degradation or if site conditions are extreme (high concentrations or toxicity of contaminants, for example).

To be effective, the introduced organism(s) must become distributed throughout the contaminated matrix and compete with the indigenous microorganisms for available nutrients. If they are not distributed throughout the matrix the positive effect will be localized. On the other hand if the introduced organisms compete poorly, they will not persist and the treatment effect will be short lived.

"if the introduced organisms compete poorly, they will not persist and the treatment effect will be short lived"

Problems encountered using this approach include biofouling of equipment, injection wells and seepage beds. Adjustments to the system, such as the use of new discharge areas, may be required to prevent this from occurring. This approach to bioremediation must be evaluated on a site specific basis.

B.   Resource Competition

1.   Competitive Advantage of Contaminant Use

The growth of microorganisms in many environments is limited by the availability of carbon. This is particularly true for terrestrial habitats. Consequently, organisms capable of metabolizing novel carbon sources will have a competitive advantage. Consequently, these organisms will be active and may increase in number relative to other microorganisms present.

2.   Carbon Source Mineralization

Many organic compounds can be completely metabolized to produce carbon dioxide or methane. When this occurs, the compound is said to be mineralized. A portion of the carbon derived from these processes is used for cell growth and reproduction. In addition, energy needed to sustain metabolic processes and cell growth is obtained. Often these compounds can serve as sole sources of carbon and energy for microbial metabolism (except when metabolism occurs through the concerted action of a microbial consortium - see section V.C).

3.   Cometabolism

Some compounds can only be partially metabolized by microbial populations and are transformed into metabolites that may either accumulate in the environment or be metabolized further by other microbial species present. These metabolic reactions do not provide benefit to the responsible organism because they do not gain either carbon or energy. These processes are typically fortuitous and occur because the responsible population produces one or more enzymes that are comparatively nonspecific and can react with compounds that are structural analogues of the "normal" substrate for the enzyme(s).

Cometabolism is important for the metabolism of many environmental contaminants particularly chlorinated solvents (trichloroethylene, for example), polychlorinated biphenyls, and many polyaromatic hydrocarbons.

For several reasons, processes that involve cometabolism either wholly or in part are more difficult to reliably use and manage. This is because transformation of the contaminant offers no competitive advantage to the responsible organism. Indeed the opposite is often the case; cometabolism may result in products that are toxic to the organism (epoxides, for example) or it may divert enzyme activity and energy toward processes that provide no benefit and are therefore futile reactions. Consequently, management approaches must be aimed toward stimulation and selection of these populations through indirect means. For example, certain, but not all, microbial populations able to metabolize methane can fortuitously cometabolize trichloroethylene. Consequently, stimulation of methane production in situ or injection of methane might be done in an effort to stimulate the cometabolism of trichloroethylene. Lack of knowledge about the competitive dynamics among microbial populations in natural ecosystems decreases the predictability of such approaches and dictates that empirical data be collected to demonstrate the effectiveness of such approaches.

C.   *Microbial Communities and Consortia*

Microbial communities are commonly comprised of many different microbial species and estimates of as many as 10,000 distinct species per gram of soil that is rich in organic matter have been reported. In many upland environments, the number of organisms present is roughly proportional to the amount of carbon present that can be metabolized, whereas in aquatic systems the number of organisms is dictated by the availability of other nutrients (phosphorous, for example). The factors that control the number of species present are more complex and less well understood. In part, species diversity is influenced by the variety of carbon sources (different kinds of compounds) present and the physical and chemical complexity of the environment.

In most situations and environments, the contaminants are metabolized by numerous species of microorganisms in an environment. These organisms may reflect functional redundancy in the community (multiple species all capable of metabolizing the contaminants independently of one another) or multiple species may act synergistically and be interdependent on each other either for specific metabolic processes or nutritional needs. Lack of knowledge about these complex interactions are a significant reason why it is difficult to predict the effect of efforts to enhance bioremediation processes or to predict rates and extent of contaminant degradation in the future as site conditions change.

> *"In most situations and environments, the contaminants are metabolized by numerous species of microorganisms in an environment."*

VI.   Factors Affecting Contaminant Biodegradability

A.   Natural versus Synthetic Compounds

Although a wide-array of organic contaminants can be metabolized by microorganisms, some are more readily degraded than others. Evolutionary processes have resulted in the development of enzymes and pathways for the metabolism of naturally occurring compounds (petroleum hydrocarbons, for example). These pathways are found in diverse species of microorganisms that are widespread and indigenous to most environments. Environments that are unusually harsh or those that have been severely stressed may have limited microbial diversity and not contain bacterial species able to metabolize all kinds of naturally occurring compounds. In all other cases, such organisms can be considered to be pandemic. (Also see Bioaugmentation, section V.A.3)

> *"Environments that are unusually harsh or those that have been severely stressed may have limited microbial diversity and not contain bacterial species able to metabolize all kinds of naturally occurring compounds."*

Synthetic compounds may or may not be readily degraded by microorganisms. Many of these compounds have been introduced in recent decades and pathways for their efficient metabolism may not have evolved. Moreover, some classes of compounds (chlorofluorocarbons, for example) are inherently recalcitrant to degradation and will be metabolized slowly or not at all. To a first approximation, the degradability of a synthetic compound can be gauged by the extent to which it is chemically analogous to naturally occurring compounds. Those compounds that resemble naturally occurring compounds are more likely to be readily metabolized whereas those that are chemically unique are less likely to be efficiently metabolized.

> *"the degradability of a synthetic compound can be gauged by the extent to which it is chemically analogous to naturally occurring compounds"*

B.   Effect of Halogenation

There are few naturally occurring chemicals that have halogen substituents. Consequently, synthetic chemicals with either carbon-chlorine or carbon-fluorine bonds are typically metabolized slowly and some are highly recalcitrant (i.e. resist biodegradation). This class of compounds include some of the most significant contaminants of soils and aquatic environments, such as polychlorinated biphenyls, chlorinated solvents and chlorinated aromatic compounds. These compounds are often metabolized by complex pathways that involve microbial consortia or cometabolism.

C.   Contaminant Mixtures

Many contaminated sites have mixtures of organic compounds and these are usually not metabolized at the same rate. Instead, the rates at which various compounds are metabolized will differ and be dependent on several variables (see section VII). It is not uncommon to observe a sequential depletion of compounds within the mixture. The sequence observed can vary either temporally or spatially. The latter is commonly observed in aquifers as the contaminant plume migrates through a gradient of electron acceptors in the groundwater.

High concentrations of contaminants can be toxic, in which case degradation of the contaminants may occur only at the fringe of the contaminated matric where the concentration of the contaminants is sufficiently low to avoid toxicity.

VII.   Site Evaluation and Factors Affecting Contaminant Degradation

A.   Biological Factors

1.   Rates of Contaminant Degradation

The rate of contaminant degradation is often dependent on the concentration of the contaminant and the amount of "catalyst" present. In this context, the amount of "catalyst" present represents the number of organisms able to metabolize the contaminant as well as the amount of enzymes(s) produced by each cell. Thus, any factor that affects concentration of contaminant, the number of microorganisms present or the expression of specific enzymes by the cells can increase or decrease the rate of contaminant degradation. It should be noted that the rate of degradation is generally not constant and independent of contaminant concentration but rather decreases as the concentration of contaminant decreases. This is a primary reason why it can be impractical to reduce contaminant concentrations to very low levels using biological processes.

*"any factor that affects concentration of contaminant, the
number of microorganisms present or the expression of specific enzymes by
the cells can increase or decrease the rate of contaminant degradation"*

2.   Extent of Contaminant Degradation

The extent to which contaminants are metabolized is largely a function of the specific enzymes involved and their "affinity" for the contaminant and the availability of the contaminant. The "affinity" of an enzyme is an inherent property of the enzyme that is determined by it's structure. This "affinity" varies among different enzymes, and even among enzymes with identical functions produced by different populations of microorganism (so long as the structure of the enzymes differ).

3.   General Indicators and Microbial Physiology

a.   C:N:P Ratios

Microbial cells are largely comprised of carbon (C), nitrogen (N) and phosphorus (P) at an average C:N:P ratio of 50:14:3. Sufficient amounts of these nutrients must be available in a usable form and in proper proportions for unrestricted microbial growth to occur. It is important to realize that measures of total carbon, nitrogen and phosphorus do not necessarily reflect the amounts that can actually be used by microorganisms for growth as they may be physically or chemically unavailable.

b.   Nutrient Availability

Organic compounds serve as sources of carbon and can be estimated based on concentrations of total organic carbon (TOC). All organic chemicals present in a sample, including compounds that are unavailable or not readily metabolized, are included in measures of TOC. Thus, TOC tends to overestimate the carbon available to microorganisms. In general, the total number of organisms present is proportional to the amount of carbon available (assuming other nutrients are not limiting). Thus, the microbial density of surface soils is greater than subsurface soils which in turn are generally greater than in aquifer sediments.

Provided that carbon is present in sufficient amounts, the total amount of nitrogen available to microorganisms in the form of organic nitrogen, ammonia (NH_4^+), nitrate (NO_3^-), and nitrite (NO_2^-) can significantly influence the rate of contaminant degradation. Total nitrogen is measured as Kjeldahl N, and total organic nitrogen (TON) is calculated from Kjeldahl N less the concentration of inorganic N (nitrate, nitrite and ammonia). A C:N ratio of less than 40 suggests that adequate nitrogen is present.

Microorganisms can utilize both soluble inorganic (ortho-phosphate) and organic forms of phosphorus. Phosphorus limitation can occur when the C:P ratio is more than 120:1 and a C:N:P ratio of 100:10:1 is considered optimal. High concentrations of calcium and magnesium may precipitate phosphates reducing the amount available for microbial metabolism.

In most soils other nutrients such as S, Ca, Mg, K, as well as trace amounts of metals, are typically found in adequate supply for metabolic needs.

c.   Terminal Electron Acceptors

The metabolism of organic contaminants by microorganisms often entails oxidation of the compound and this requires that the electrons derived from these processes be used to reduce other compounds. A wide range of organic and inorganic compounds can be used as terminal electron acceptors by microorganisms (see Table 1).

The energy derived by the organism from these respiratory processes varies. Aerobic respiration yields the most energy and methanogenesis the least (the processes are listed in Table 1 in the order of most to least energy conserved). As a first approximation, the rate of contaminant degradation is roughly proportional to the amount of energy obtained.

Table 1

Electron Acceptor	Chemical Symbol	Process
Oxygen	O_2	Aerobic Respiration
Nitrate and nitrite	NO_3^- and NO_2^-	Anaerobic Respiration (denitirification)
Ferric iron	Fe^{+3}	Anaerobic Respiration
Manganese	Mn^{+3} and Mn^{+2}	Anaerobic Respiration
Sulfate	SO_4^{-2}, $S_2O_3^{-2}$, SO_3^{-2}	Anaerobic Respiration (sulfate reduction)
Organic compounds	Many	Fermentation
Carbon dioxide	CO_2	Methanogenesis

In aerobic processes, oxygen is the terminal electron acceptor. The rate of contaminant degradation by aerobic processes is often limited by the availability of oxygen, which is supplied by diffusion from the atmosphere or dissolved in and transported by water. The diffusion of oxygen from the atmosphere into soil and water is slow and the solubility of oxygen in water is low (about 10mg/L at $25^{\circ}C$). Consequently, oxygen may be consumed faster than it is replaced and the system may become anaerobic. The availability of oxygen becomes rate limiting at dissolved oxygen concentrations below about 1mg/L.

As oxygen is depleted, other organic and inorganic compounds are used as terminal electron acceptors. This often results in a continuum of processes that extend away from the source of contamination with the regions nearest the source being anaerobic (no oxygen present) and extending to regions where oxygen is more readily available. Microbial processes coupled to the reduction of ionic compounds such as iron, manganese and sulfate compounds, can offer significant advantages since these compounds are more water-soluble than oxygen. Moreover, in some environments they are present in high concentration (nitrate in groundwater, for example) or they can readily be added in engineered bioremediation processes.

> *"As oxygen is depleted, other organic and inorganic compounds are used as terminal electron acceptors."*

d.   Soil Respirometry

Soil respirometry provides a measure of oxygen consumption or carbon dioxide production in soils and is an indication of net aerobic biological activity in situ. The determination is typically made by measuring the consumption of oxygen by contaminated soils over time and comparing the rate to that observed with soils from a nearby region that is not contaminated. Increased O_2 use, measured as lower O_2 levels, is taken as an indication of increased respiratory activity that is potentially due to metabolism of contaminant(s). However, there are numerous problems with the use of respirometry to demonstrate contaminant degradation. Perhaps the most significant is that this method measures oxygen consumption resulting from metabolism of all carbon sources, not just contaminant(s). The additional oxygen consumed as a result of contaminant metabolism may be a relatively small increment of the total if the contaminant represents a small fraction of the total organic carbon present in the sample. Moreover, the method is useful only for monitoring aerobic respiration and does not include anaerobic respiration or other processes that may be involved in contaminant degradation.

4. Temperature

Temperature directly influences the rate of biodegradation by controlling the rates of enzyme catalyzed reactions. The rate of biodegradation is decreased by roughly one-half for each $10^{o}C$ decrease in temperature. Rates of biodegradation are generally exceedingly low at $0^{o}C$. Conversely, higher soil temperatures result in higher microbial metabolic activity and higher rates of bidegradation up to a maximum of about $65^{o}C$. As a result, the rate of biodegradation may vary seasonally and microbial metabolic activity itself can increase soil temperature.

It should be noted that most research studies reported in the scientific literature have employed temperatures that range from 20-25°C and therefore the reported rates of contaminant degradation are higher than would be expected in subsurface environments of Michigan where average soil temperatures at depth are around $10^{o}C$. A better estimate of rates of natural biodegradation based on literature values is possible when soil temperature is known.

5. Moisture

Moisture (water) influences the rate of contaminant metabolism because it influences the kind and amount of soluble materials that are available as well as the osmotic pressure and pH of terrestrial and aquatic systems. As mentioned above, the amount of water in the pore spaces of soil also affects the exchange of oxygen. Under saturated conditions, oxygen can be consumed faster than it is replenished in the soil vapor space and the soil can become anaerobic. This can retard the rate of biodegradation and cause major changes in microbial metabolic activity to occur. Conversely, soil moisture content should be between 25-85% of the water holding capacity, and a range of 50-80% is optimal for biodegradation. The water holding capacity of soil is sometimes expressed as the "field capacity" which is the percentage of water remaining in a soil after it has been saturated and gravitational drainage has ceased (also see Soil Matric Potential, section VII.B.3).

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6.   pH

Soil pH is a measure of the acidity or alkalinity of water. Biodegradation can occur under a wide-range of pH; however, a pH of 6.5 to 8.5 is generally optimal for biodegradation in most aquatic and terrestrial systems and values ranging from 5 to 9 are considered acceptable. Soil pH may affect the availability of nutrients. For example, the solubility of phosphorus, an important nutrient in biological systems, is maximal at a pH value of 6.5 and decreases at pH that are either higher or lower than this value.

B.   Environmental Factors

1.   Geologic and Hydrogeologic Factors

a.   Adsorption and Absorption

The binding of an organic compound to the surface of a solid is called *adsorption*. The extent of adsorption that occurs is determined by the relative affinity of the compound for a solid matrix, the surface area of the matrix and the volatility or solubility of the compound in water. In general, hydrophobic chemicals (those that are not very soluble in water) will tend to adsorb to insoluble organic matter and other hydrophobic materials. Similarly, hydrophilic (water soluble) chemicals may bind to minerals.

Adsorbed contaminants are in equilibrium with the dissolved or volatilized contaminants. So as the concentration in the aqueous or gaseous phases is reduced, the amount of contaminants adsorbed onto a surface will decrease. Constituents that adsorb tightly to solid matrices will be less subject to transport in aqueous and gaseous phases and thus their mobility in a system is retarded. In contrast, constituents that are not tightly adsorbed can be transported through soils, aquatic systems, and the atmosphere.

The potential for adsorption is closely associated with soil type and soil organic matter content. The fraction of organic carbon in surficial soils typically ranges from 1 to 3.5 percent. The organic matter content in subsurface soils is typically an order of magnitude lower because most organic residues are incorporated or deposited on the surface soils and do not readily migrate to subsurface soils. In some cases, surfactants or cosolvents can be added to the aqueous phase of a system to increase the relative solubility or mobility of the contaminant and decrease adsorption.

Absorption is an analogous process wherein a contaminant penetrates into the bulk mass of the soil matrix. Both adsorption and absorption reduce the availability of the contaminant to most microorganisms and the rate at which the chemical is metabolized is proportionately reduced.

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> *of the contaminant to most microorganisms and the rate at which*
> *the chemical is metabolized is proportionately reduced."*

b.   Contaminant Migration in Groundwater

Contaminant migration within an aquifer is controlled by many chemical and physical properties of the contaminants, and of the hydrogeologic setting in which they are found. Hydraulic conductivity is one of the primary aquifer characteristics that must be understood to effectively predict contaminant migration and evaluate the possibilities for adding materials to enhance bioremediation. It may vary horizontally and vertically and is measured in units of length per time, such as feet per day or centimeters per second, reflecting the rate at which water will flow through rock or unconsolidated materials. Particle size and rock type affect hydraulic conductivity with smaller particle size or increasing density of rock resulting in lower rates of hydraulic conductivity. Typically, hydraulic conductivities for soils in the range of 10^{-5} - 10^{-3} cm/sec will be conducive to bioremediation, although values below 10^{-5} cm/sec may be acceptable if other site specific conditions are favorable.

Variations in porosity of the unsaturated and saturated zones of the aquifer matrix may influence the movement of other fluids in addition to water. The ability of the matrix to transmit gases, such as oxygen, methane and carbon dioxide, is reduced in fine grained sediments and also when soils become more saturated with water. This can affect the rate and type of biodegradation taking place. Conversely, fine grained clays and silts tend to retard the movement of contaminants making widespread contamination less likely.

Other factors to consider are dispersion and diffusion of contaminants. Dispersion is the mechanical mixing and spreading of the contaminants that occurs within the aquifer and includes diffusion, the movement of contaminants along a concentration gradient due to their kinetic energy. These are primarily physical processes that reduce contaminant concentrations within the plume and are of particular importance for sites where natural attenuation is the proposed remedy.

        2.   **Bioavailability**

The fraction of contaminant actually available to microorganisms is said to be *bioavailable*. The concentration of bioavailable contaminant is often less than the total concentration measured (following solvent extraction, for example). It is important to note that the rate and extent of contaminant degradation is proportional to the concentration of bioavailable contaminant and not the total concentration.

        3.   **Soil Matric Potential**

The soil matric potential is a measure of the energy required to overcome capillary and adsorptive forces and thus reflects the "work" that microbial cells must do to extract water from the soil. The matric potential of soil is strongly dependent on soil type and is correlated with soil moisture content. Soil moisture contents of 50-80% roughly correspond to matric potentials in the range of -0.01 to -0.015 Mpa (-0.1 to -0.15 bar) in sands and -0.03 to -0.05 Mpa (-0.3 to -0.5 bar) in medium to fine textured soils. In general, microbial activity is low in soils with a matric potential of about -0.1 Mpa (-1 bar) and ceases at about -8 Mpa (-80 bars). Matric potentials

that range from -0.5 Mpa (-5 bars) to -1.5 Mpa (-15 bars) are generally considered adequate. If the matric potential is too low then contaminant degradation rates will also be low.

4. Redox Potential

The oxidation-reduction potential (redox potential) of a soil provides a measurement of the electron density of the system. Biological energy is obtained from the oxidation of compounds in which electrons are transferred to various more oxidized compounds referred to as electron acceptors. A low electron density (Eh greater than 50 mV) indicates oxidizing, aerobic conditions, whereas high electron density (Eh less than 50 mV) indicates reducing, anaerobic conditions. High positive Eh values (+100 mV to +400 mV) indicate well aerated conditions that are optimal for biodegradation. It should be noted that Eh may vary considerably over a very small distance within soils and it is generally not practicable to directly measure this parameter for a specific site. Redox potential is often inferred from other parameters that demonstrate the type of bioremediation taking place.

VIII.   Guidelines for Site Remediation Proposals for Bioremediation Technologies

A proposal for the use of biological processes for site remediation should contain the following basic elements:

A.   *Site Characterization*

Sufficient remedial investigation work must be performed prior to proposing a bioremediation remedy to adequately characterize the magnitude and extent of contamination. This work should at a minimum encompass the following factors:

* Fully determine the horizontal and vertical extent of contamination.
* List the parameters and locations to be sampled and the rationale for their choice.
* Describe the methods to be used for sample acquisition and analysis to be performed.

B.   *Proposed Strategy for Bioremediation*

The strategy itself needs to consider a variety of approaches along with the rationale for the specific remedy chosen, which should address the following:

* Analyze alternative approaches for site remediation.
* Justify the alternative chosen and explain why it is likely to be effective.
* Evaluate factors that could potentially limit the rate or extent of contaminant biodegradation.
* Estimate or measure contaminant degradation rates.
* evaluate potential for transport of contaminants (i.e. site hydrogeological conditions)

- Is intended future site use consistent with degree of risk reduction achieved by remediation?
- Is intended future use consistent with local zoning requirements?
- Will treatment create any nuisances with regard to aesthetics or adjacent property use (e.g. noise, odor, dust, etc.)?
- Is the contaminant plume contained on-site, or has migration to offsite properties already occurred or likely to occur?
- List actions required if the contaminant plume approaches and/or crosses the site boundary.

C.   *Proposed Monitoring Plan*

An integral part of any proposal must be a monitoring plan that addresses parameters necessary to demonstrate the efficacy of the remedy as well as issues related to site control and regulatory compliance.

- Describe plans to monitor the process(es) that focus on determining the potential of further contaminant migration
- Identify monitoring parameter rationale (items such as choice of analytes as they relate to biological activity and decreasing concentrations of the contaminants).
- Minimum of quarterly monitoring after monthly monitoring for the first quarter.
- Target analytes and methods for analysis identified.
- Describe sampling procedures.
- Data must demonstrate decreasing contamination over time.
- Provisions for detecting migration as a cause of decreasing concentrations rather than biodegradation (include upgradient background monitoring well and at least one sentinel well beyond leading edge of plume for soil gas or groundwater monitoring).
- Are any devices, such as blocking wells, capture wells, or slurry walls, needed to prevent off-site contaminant migration during remediation?
- A means to measure the type of degradation occurring and the presence of necessary chemical and physical conditions (i.e. nutrients, electron acceptors, by-product concentrations).
- Provisions for routine and emergency site maintenance.
- Permitting requirements addressed (NPDES, stormwater runoff, air discharge, construction codes, groundwater discharge permit or exemption, etc.).
- Site security described.
- Identify sampling parameters, methods, locations and media for demonstrating closure.
- Closure sampling plan should comply with Verification of Soils Remediation guidance document.

IX Information on Other Bioremediation References

A wide variety of technical documents covering bioremediation are available through the Internet that may be useful in providing additional specific information necessary in reviewing bioremediation proposals. Those identified in this document are provided by the U.S. Environmental Protection Agency within their Internet addresses (URLs) listed in the table of contents of this document. They are provided in Adobe™ portable document format (PDF). The program used to read these files (Adobe Acrobat Reader™) is available for free downloading at various internet sites including EPA's web site or from Adobe Systems Inc. at http://www.adobe.com/.

The intent of providing guides to these references is to assist State project managers and other interested parties in evaluating various types of bioremediation proposals and in finding the portions of the reference materials that may be most relevant to that evaluation. Since there are many different types of bioremediation, each with a unique set of processes or questions particular to that treatment type, these guides are not meant to be exhaustive or all-encompassing. Answering "yes" or "no" to any of the questions, or having parameters fall outside the stated ranges, does not imply "approval" or "denial" of the proposed plan on that factor. Each item of consideration must be made in the context of the entire proposal and the site specific conditions which it addresses.

There are two broad categories of bioremediation technologies, *in-situ* (treating the contaminants where they are found), and *ex-situ* (moving the contaminated soil and/or groundwater to a different location for treatment). The guides are meant to highlight areas of the references that answer some questions likely to be asked about a proposed remediation. They aren't meant to replace a careful reading of a specific reference, to limit the use of other references, or to replace a careful review of the proposal itself. Not all bioremediation approaches have been addressed in this document.

A. In-Situ Treatments:

1. BIOVENTING

EPA/540/R-95/534a   Manual - Bioventing Principles and Practice
Volume I: Bioventing Principles
Volumes II: Bioventing Design

Reference	Page Nos.	Points for Consideration
EPA/540/R-95/534a	V1, p11	Is the soil gas permeability sufficient to allow air flow of at least 0.25 to 0.5 pore volumes per day?
	V1, p14	Are significant quantities of contaminant in the capillary fringe or below the water table?
	V1, p14; V2, p17	Has the oxygen radius of influence been calculated for the venting wells?
	V1, p16	Will excess soil moisture inhibit air flow?
	V1, p18	Is the soil pH between 5 and 9?
	V1, p19	Will exceedingly cold ($<10^o$C) or exceedingly hot ($>40^o$C) temperatures inhibit bioremediation?
	V1, p20	Are existing site levels of nutrients, especially nitrogen and phosphorus sufficient to support bioremediation?
	V1, p22	Will contaminant concentrations or types of contaminants be toxic to microorganisms?
	V1, p22-23	Is the vapor pressure of the contaminants between 1 and 760 mm Hg?
	V1, p22; V2, p2	Are the contaminants biodegradable under aerobic conditions?
	V2, p1	Preliminary tests needed to determine if bioventing will be effective.
	V2, p2	Has free product been removed?
	V2, p3	Has a soil gas survey been performed to evaluate O_2, CO_2 and TPH levels?
	V2, p33	Are there sufficient numbers of properly placed and constructed monitoring points?
	V2, p33, 43	How does the in situ respiration rate compare to that in uncontaminated soils?

2. ENHANCED IN-SITU BIOREMEDIATION OF HYDROCARBONS IN GROUNDWATER

EPA/540/S-92/003   In-Situ Bioremediation of Contaminated Groundwater

Reference	Page Nos.	Points for Consideration
EPA/540/S-92/003	p1	What are the optimal site characteristics for successful implementation of enhanced in-situ bioremediation?
	p3	What is a reasonable time frame to expect cleanup of petroleum hydrocarbons?
	p3	What factors limit the success of innoculants?
	p3, 7	Have source removal and free product removal been performed?
	p4	Has the aquifer been adequately characterized?
	p4	What factors control the rate of biological activity?
	p5	What methods are available to characterize subsurface microorganisms?
	p6	Have the chemical and physical properties of the contaminants been evaluated in relation to their bioavailability?
	p6	Is the hydraulic conductivity of the aquifer sufficient to allow transport of electron acceptors or nutrients?
	p7	Has a treatability study been performed using solids and groundwater from the site?
	p7	Have hydraulic controls been designed to optimize treatment and contain the plume?
	p9	Will biomass plugging of the aquifer or injection bed/well be a problem?
	p9	What parameters are proposed to assess the effectiveness of the treatment system?
	p9	What are the advantages and limitations of enhanced in-situ bioremediation?

3. NATURAL ATTENUATION OF SOILS

EPA/625/K-96/001   Seminars - Bioremediation of Hazardous Waste Sites: Practical Approaches to Implementation

Reference	Page Nos.	Points for Consideration
EPA/625/K-96/001	p16-1	Is contamination limited to the unsaturated zone and groundwater not threatened?
	p16-2, 16-7	Is the Redox potential reducing (Eh<50mV) or oxidizing (Eh>100mV) (i.e. does the Redox environment match conditions required for the expected degradation pathway)?
	p16-2, 16-7	Is the soil pH between 5 and 9?
	p16-2, 16-8	Is soil moisture adequate to support biodegradation (50-80% of field capacity)?
	p16-4	Have CO_2 production and O_2 consumption been measured to determine respiration rates?
	p16-4, 16-13	Are the contaminants likely to biodegrade in a reasonable time frame?
	p16-5	Are institutional controls adequate to ensure protection of human health and the environment?
	p16-9, 16-10, 16-11	For contaminants other than petroleum products, have tests been performed to demonstrate the presence and ability of microorganisms that will biodegrade the contaminants under site conditions?
	p16-1, 16-12	Have all relevant human and ecological receptors been evaluated?
	p21-1	Will more toxic intermediate products (e.g. vinyl chloride) be produced?
	p16-3, 16-8	Is the temperature range acceptable?
	p16-3, 16-9	Are adequate nutrients available?
	p16-11, 16-13, Section 21	Have monitoring frequency and parameters been addressed?

4. NATURAL ATTENUATION OF GROUND WATER

EPA/600/R-94/162   Symposium on Natural Attenuation of Ground Water
EPA/625/K-96/001   Seminars - Bioremediation of Hazardous Waste Sites: Practical Approaches to Implementation

Reference	Page Nos.	Points for Consideration
EPA/600/R-94/162	p22, 123, 124	What steps are required to implement natural attenuation as a remedial option for contaminated groundwater?
	p21	What are the necessary objectives of site characterization for natural attenuation?
	p23	What site-specific parameters are needed to characterize the site?
	p41	Are existing C:N:P ratios within the 300 - 100:10:1 range?
	p41	Have Redox potentials been evaluated throughout the plume?
	p80, 116	Has natural attenuation of groundwater been documented at similar contaminated sites in Michigan?
	p86	What mathematical models are available for simulating plume behavior?
	p104	Which chlorinated solvents have the greatest likelihood of being naturally attenuated within a reasonable time frame (20yrs)?
	p106	What are the anaerobic transformation pathways for chlorinated aliphatic hydrocarbons?
EPA/625/K-96/001	p15-3	What geochemical trends document the occurrence of natural attenuation?

5. NATURAL ATTENUATION OF CHLORINATED ALIPHATIC HYDROCARBONS (CAHs)

EPA/540/R-97/504   Proceedings of the Symposium on Natural Attenuation of Chlorinated Organics in Ground Water

Reference	Page Nos.	Points for Consideration
EPA/540/R-97/504	p5	What is a reasonable time frame to achieve remediation of CAHs by natural attenuation (NA)?
	p5	Is source control necessary when using NA?
	p6, 23, 39	What kinds of site specific data support the use of NA?
	p7, 8	How are CAHs transformed and what are their breakdown products?
	p12	What biological mechanisms result in the degradation of CAHs?
	p19	What Redox conditions favor the biodegradation of CAHs?
	p23	When are microcosm studies needed to evaluate the efficacy of NA?
	p24, 25	What geochemical indicators are used to characterize subsurface conditions that affect NA?
	p38, 39	What are the advantages and limitations of NA?
	p42	How does the chemical composition of the plume influence plume behavior and the ability to predict contaminant degradation pathways?
	p44	What decision making process should be followed to evaluate the use of NA?
	p45-47	What soil and groundwater sampling protocols should be used with NA?
	p48, 50	What data are required to make a preliminary assessment of NA for a specific site?
	p53-57	What types of site characterization data are needed to support NA?
	p58, 59	What types of monitoring data are needed to track the effectiveness of NA?

B. Ex-Situ Treatments:

1. BIOPILES

EPA/625/K-96/001   Seminars - Bioremediation of Hazardous Waste Sites: Practical Approaches to Implementation

Reference	Page Nos.	Points for Consideration
EPA/625/K-96/001	p10-1	Will the treatment system have controls to maintain aerobic/anaerobic conditions as needed?
	p10-1	What contaminants have been successfully biodegraded using biopiles?
	p10-1	How do costs compare with other remedial alternatives?
	p10-1	Will moisture content of the pile be maintained within 40-85% of field capacity?
	p10-1	Will the C:N ratio be maintained within the 10:1 to 100:1 range?
	p10-1	How will the pH level affect biodegradation?
	p10-1	Are soil amendments needed to increase the water holding capacity of the soil?
	p10-1	What bulking agents will be used to promote soil porosity and aeration?
	p10-2	Will alternating aerobic/anaerobic conditions be used to promote dehalogenation?
	p10-3	How is a biopile constructed?
	p10-3	Will leachate be controlled?

2. BIOREACTORS - FIXED FILM

EPA/625/K-96/001   Seminars - Bioremediation of Hazardous Waste Sites: Practical Approaches to Implementation

Reference	Page Nos.	Points for Consideration
EPA/625/K-96/001	p13-1	What are some typical applications of fixed film bioreactors?
	p13-1, 5	What kinds of media can be used in a bioreactor?
	p13-1	What are the advantages and disadvantages of using granular activated carbon (GAC) as the medium?
	p13-1, 5, 6	What are the different types of fixed film bioreactors?
	p13-1	What are the limitations of using inert media?
	p13-2	What are the best uses for anaerobic expanded- or fluidized-bed GAC filters?
	p13-2, 3	What types of contaminants are recalcitrant to biodegradation in a fixed film reactor?
	p13-2	How is biomass plugging of the media controlled?
	p13-3, 7	How efficient are fixed film bioreactors?
	p13-8	How do costs compare with other treatment options?

3. BIOREACTORS - SUSPENDED GROWTH

EPA/625/K-96/001   Seminars - Bioremediation of Hazardous Waste Sites: Practical Approaches to Implementation

Reference	Page Nos.	Points for Consideration
EPA/625/K-96/001	p14-1, 4	What are the different types of suspended growth reactors?
	p14-1	What is the minimum amount of organic carbon required to support a stable microbial culture?
	p14-4	What are typical loading ranges for suspended growth processes?
	p14-1, 4	What are the components of a typical onsite treatment system?
	p14-2	Will tertiary treatment of the effluent be required?
	p14-2	Is discharge of effluent to a municipal waste water treatment plant an option?
	p14-2	How do suspended growth reactors compare with fixed film reactors?
	p14-3, 5	How efficient are suspended growth reactors?

4. WHITE ROT FUNGI

EPA/625/K-96/001   Seminars - Bioremediation of Hazardous Waste Sites: Practical Approaches to Implementation

Reference	Page Nos.	Points for Consideration
EPA/625/K-96/001	p11-1	How do white rot fungi biodegrade contaminants?
	p11-2	Will temperature be optimized for the appropriate fungal strain used?
	p11-2	What are the results of field scale evaluations of the use of white rot fungi?
	p11-2	What compounds are recalcitrant to biodegradation by white rot fungi?
	p11-5	What is the rationale for using white rot fungi for biotreament?
	p11-5	What contaminants have been considered for biodegradation by white rot fungi?
	p11-6	What is the range of soil types at sites where white rot fungi have been used?
	p11-7	What are the time frames required to reduce contaminant levels using white rot fungi?
	p11-7	What is the process used to treat soils using white rot fungi?

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