United States
Environmental Protection
Agency

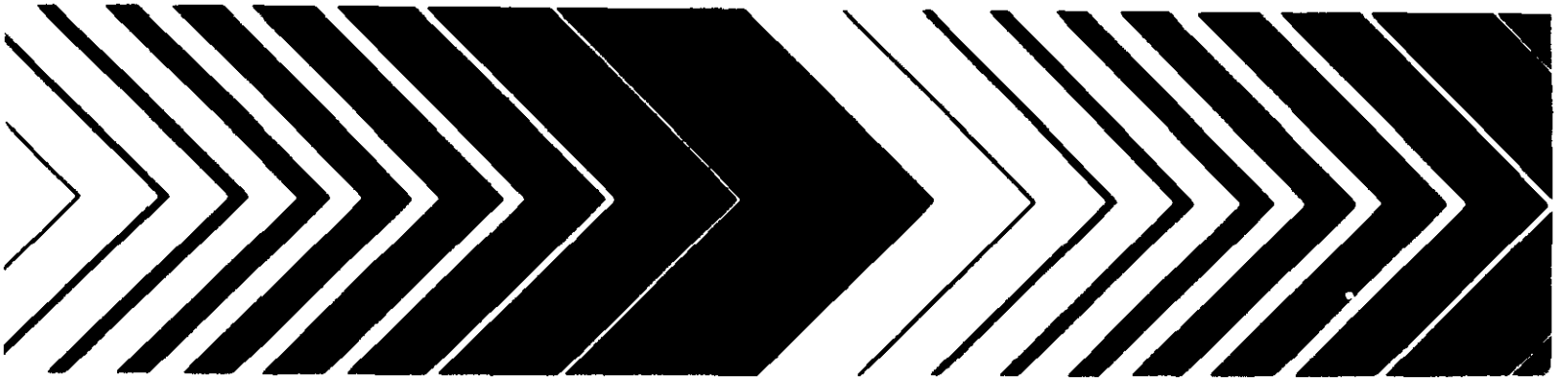
Robert S. Kerr
Environmental Research Laboratory
Ada, OK 74820

EPA/600/9-89/073
August 1989

Research and Development

EPA

Bioremediation of Contaminated Surface Soils



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Bioremediation of Contaminated Surface Soils

J.L. Sims, R.C. Sims, and J.E. Matthews

August 1989

This report was developed by the
Robert S. Kerr Environmental Research Laboratory
U.S. EPA, ORD
Ada, Oklahoma 74820

Contents

1. Introduction

Biological remediation of soils contaminated with organic chemicals is an alternative treatment technology that can often meet the goal of achieving a permanent clean-up remedy at hazardous waste sites, as encouraged by the U.S. Environmental Protection Agency (U.S. EPA) for implementation of The Superfund Amendments and Reauthorization Act (SARA) of 1986. Bioremediation is consistent with the philosophical thrust of SARA, for it involves the use of naturally occurring microorganisms to degrade and/or detoxify hazardous constituents in the soil at a contaminated site to protect public health and the environment. Bioremediation of contaminated soils, including applications and limitations, has been addressed at several recent scientific meetings and conferences [1, 2, 3, 4]. With regard specifically to wood preserving contaminated sites McGinnis et al. [5] have stated that reliable, safe, economical bioremediation techniques using soil systems are attractive and warrant thorough study and evaluation. The use of bioremediation techniques in conjunction with chemical and physical treatment processes, i.e., the use of a "treatment train," is an effective means for comprehensive site-specific remediation [6].

Wilson [7] identified biological processes, including microbial degradation, as a mechanism for attenuating contaminants during transit through the vadose zone to the groundwater. (The vadose zone is the region extending from the ground surface of the earth to the upper surface of the principal water-bearing formation [8]). On-site soil remedial measures using biological processes can reduce or eliminate groundwater contamination, thus reducing the need for extensive groundwater monitoring and treatment requirements [7, 9, 10]. Lehr [11] also emphasized that monitoring for attenuation of contaminants occurring in the vadose zone provides information for understanding their movement in and through the vadose zone and in the groundwater.

On-site bioremediation of contaminated soils generally is accomplished by using one of three types of systems:

(1) In situ;
(2) Prepared bed; or
(3) Bioreactor (e.g., slurry reactors) systems.

This discussion focuses on in situ and prepared bed systems, which utilize the soil as the treatment medium, as contrasted to bioreactor systems, in which contaminated soil is treated in an aqueous medium.

An in situ system consists of treating contaminated soil in place. Contaminated soil is not moved from the site. In general, naturally occurring microorganisms are allowed to treat the contaminants. Treatment often may be enhanced by a variety of physical/chemical methods, such as fertilization, tilling, soil pH adjustment, moisture control, etc. In some instances, addition of supplemental populations of adapted organisms may serve to enhance treatment.

In a prepared bed system, the contaminated soil may be either (1) physically moved from its original site to a newly prepared area, which has been designed to enhance bioremediation and/or to prevent transport of contaminants from the site; or (2) removed from the site to a storage area while the original location is prepared for use, then returned to the bed, where the treatment is accomplished. Preparation of the bed may consist of such activities as placement of a clay or plastic liner to retard transport of contaminants from the site, or addition of uncontaminated soil to provide additional treatment medium. Treatment may also be enhanced with physical/chemical methods, as with in situ systems.

2. Overview of Soil Biodegradation and Other Soil Processes

Bioremediation of a soil contaminated with organic chemicals is accomplished by degradation of specific organic constituents, i.e., the "parent" compounds. The term degradation may refer to complete mineralization of the constituents to carbon dioxide, water, inorganic compounds, and cell protein. The ultimate products of aerobic metabolism are carbon dioxide and water. However, biodegradation of a compound is frequently a stepwise process involving many enzymes and many species of organisms. Therefore, in the natural environment, a constituent may not be completely degraded, but only transformed to intermediate product(s) that may be less, equally, or more hazardous than the parent compound, as well as more or less mobile in the environment. Under anaerobic conditions (i.e., in the absence of oxygen), metabolic activities result in the formation of incompletely oxidized simple organic substances such as organic acids as well as other products such as methane or hydrogen gas.

The goal of on-site bioremediation is degradation that results in detoxification of a parent compound to a product or product(s) that are no longer hazardous to human health and/or the environment. Information on degradation and detoxification of a parent compound may be obtained using chemical and bioassay analyses [12, 13, 14]. Chemical analysis and identification of intermediate products may yield information about biochemical degradation pathways

and products, but are often time consuming and expensive. Bioassays may be used to demonstrate detoxification of parent compounds and are usually less expensive and time consuming. Before bioremediation is implemented at a contaminated site, degradation pathways for specific constituents present and/or detoxification demonstrations require investigation to ensure that environmental and health protection can be achieved.

Degradation of most organic compounds in soil systems may be described by monitoring their disappearance in a soil through time. Disappearance, or rate of degradation, is often expressed as a function of the concentration of one or more of the constituents being degraded. This is termed the order of the reaction and is the value of the exponential used to describe the reaction [15]. Either zero or first order power rate models are often used in environmental studies.

Zero order reactions are ones in which the rate of transformation of an organic constituent is unaffected by changes in the constituent concentration because the reaction rate is determined by some other factor than the constituent concentration. If a constituent C is transformed to X, the rate of change of C is:

$$dC/dt = -k \qquad (1)$$

On integration, the equation becomes:

$$C_t = C_o - kt \qquad (2)$$

where C_t = concentration of constituent remaining at time t; C_o = initial concentration of constituent; and k = zero order rate constant. A useful term to describe the reaction kinetics is the half-life, $t_{1/2}$ which is the time required to transform 50% of the initial constituent:

$$C_t = C_o/2, \text{ then } t_{1/2} = C_o/2k \qquad (3)$$

The first order rate model (Equation 4) is widely used because of its effectiveness in describing observed results as well as its inherent simplicity. Its use also allows comparison of results obtained from different studies. In a first order rate reaction, the rate of transformation of a constituent is proportional to the constituent concentration:

$$dC/dt = -kC \qquad (4)$$

where C = contaminant concentration (mass/mass); t = time; and k = first order rate constant (1/time). After integration of Equation 4 and rearrangement of the integrated equation, Equation 5 may be used to graphically determine the rate constant, k:

$$\ln(C_t/C_o) = -kt \qquad (5)$$

where C_t = concentration of constituent remaining at time t; and C_o = initial concentration of constituent. A plot of $\ln(C_t/C_o)$ versus t is linear with a slope of -k. The rate constant k is independent of the concentration of constituent, since the slope is constant over time. To calculate the time required to transform one-half of the initial constituent ($C_t = C/2$), the following equation is used:

$$\ln((C_o/2)/C_o) = -kt_{1/2} \qquad (6)$$

which is equal to:

$$t_{1/2} = 0.693/k \qquad (7)$$

where $t_{1/2}$ = half-life of the constituent.

First order kinetics generally apply when the concentration of the compound being degraded is low relative to the biological activity in the soil. However, very low concentrations may be insufficient to initiate enzyme induction or support maintenance requirements necessary for microbial growth, even if the compound can be used as an energy source [16].

A second model used to describe degradation in soils is the hyperbolic rate model, which is similar to Michaelis-Menten enzyme kinetics. This model is expressed as:

$$dC/dt = -k_1 C/k_2 + C \qquad (8)$$

where k_1 and k_2 are constants. The constant k_1 represents the maximum rate of degradation that is approached as the concentration increases. This model simulates a catalytic process in which degradation may be catalyzed by microorganisms.

Often an organic compound that cannot be used as a sole carbon and energy source for microorganisms is degraded. Biodegradation of the compound does not lead to energy production or cell growth. This biodegradation process is referred to as cometabolism [17] or co-oxidation if the transformation involves an oxidation reaction [18]. Cometabolism occurs when an enzyme produced by an organism to degrade one substance that supports growth also degrades another nongrowth substrate that is neither essential for, nor sufficient to, support microbial growth. The nongrowth substrate is only incompletely oxidized, or otherwise transformed, by the microorganism involved, although other microorganisms may utilize by-products of the cometabolic process. Cometabolism may be a prerequisite for the mineralization of many recalcitrant substances found in the environment, such as polycyclic aromatic hydrocarbons [19].

Measurement of physical abiotic loss mechanisms and partitioning of organic constituents in a soil should be used in conjunction with conventional degradation studies to ensure that information generated from modeling degradation represents only biological degradation of parent compounds, and not other possible disappearance mechanisms of the constituents in the soil system.

The soil is a complex system, consisting of four phases (Figure 1): (1) soil gases; (2) soil water; (3) inorganic solids; and (4) organic solids. Gases and water, which are found in the pore spaces of a soil, together comprise about 50% (by volume) of a typical soil. An organic constituent,

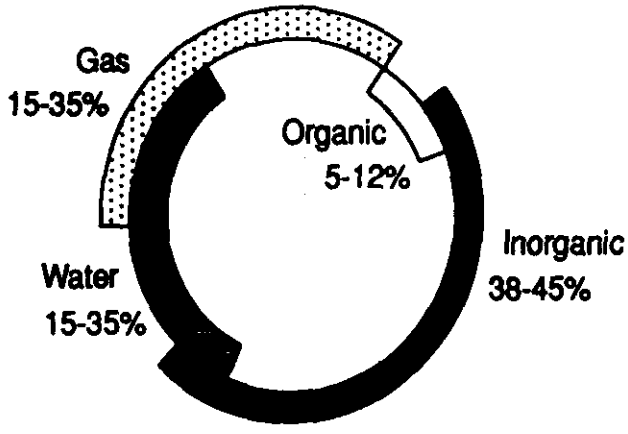


Figure 1. Typical Volumetric Composition of Soil.

depending upon its solubility and its tendency to volatilize, may be found in varying proportions in these two phases. Pore sizes and continuity and relative proportions of water and air in the pores are examples of factors that affect the mobility of contaminants (both upward out of the soil and downward to the saturated zone) in a specific soil.

Depending upon site-specific soil characteristics and constituent-specific chemical and physical properties, constituents in these two phases may be relatively mobile or immobile.

Soil solids are comprised of organic and inorganic components. The inorganic components are comprised of sparingly soluble chemicals known as minerals, which are primarily sand, silt, and clay particles in most soils. The solids may contain highly reactive charged surfaces that play an important role in immobilizing organic constituents in a specific soil. Certain types of clays are especially high in negative charges, thus exhibiting what is termed as a high cation exchange capacity. Clays may also contain positively charged surfaces and act as anion exchange media for negatively charged constituents.

Soil organic matter also has many highly reactive charged surfaces and may aid in retaining organic constituents in a soil system. The term humus refers to the relatively stable portion of soil organic matter that remains in soil after the chemicals comprising plant and animal residues have decomposed. Hydrophobic organic constituents may partition from soil water into soil organic matter and thus become less mobile in the soil system. Immobilization of constituents may result in additional time for biodegradation to occur. However, immobilization also could result in less bioavailability to microorganisms. Research is required to discover whether such immobilization constitutes adequate treatment if the constituent is so tightly and irreversibly bound that it poses no harm to human health and the environment.

Soil solid/organic chemical interactions may be quite complex. The structure of an organic constituent, as it affects such properties as molecular volume, water solubility, octanol-water partition coefficients, and vapor pressure, determines the magnitude of sorption onto the surfaces of a specific soil. The specific aspects of chemical structure that affect sorption onto soil surfaces, as summarized by Dragun [20], include:

(1) molecular size- in general, the larger the molecule, the greater its tendency to exist in the adsorbed state. This is attributed to multiple Van der Waal's forces arising from many points of contact between the soil surface and the adsorbed molecule;

(2) hydrophobicity or lipophilicity-hydrophobicity refers to the preferential migration to and accumulation of an organic chemical in hydrophobic solvents or on hydrophobic surfaces such as soil organic matter, in preference to aqueous solvents or hydrophilic surfaces. In general, molecular groups comprised of carbon, hydrogen, bromine, chlorine, and iodine are hydrophobic groups, while molecular groups containing nitrogen, sulfur, oxygen, and phosphorus are primarily hydrophilic groups. The net hydrophobicity of a molecule is determined by the combined effects of hydrophobic and hydrophilic groups that comprise the molecule;

(3) molecular charge- some organic chemicals contain functional groups with permanent positive negative or positive charges. These compounds will interact with charged soil solids and adsorb onto soil surfaces. Soils typically possess a significantly greater number of negative surfaces than positive ones, thus negatively charged organic anions may be repelled by soil surfaces. Some organic chemicals contain functional groups that may or may not possess a positive or negative charge, depending upon the acidity of the soil/water system. The pK_a of a chemical is a mathematical description of the effect of acidity on the charge of the chemical. The relative ratio of charged to uncharged molecules at a pH level in a soil/water system may be estimated and used in identification of the effect of a molecular charge on the extent of adsorption. For chemicals that possess both types of functional groups, i.e., ones that can acquire a positive charge and ones that can acquire a negative charge, the isoelectric point (IP) may be used to predict the effect of pH on the adsorption of these chemicals. The IP is the pH at which the organic chemical has zero charge. Above the IP, the organic chemical has a net negative charge; below the IP, the organic chemical has a net positive charge. The IP represents a general summation of the effects of the pK_as of each functional group in the molecule;

(4) organic molecular functional groups that undergo hydrogen bonding- hydrogen bonding occurs when a hydrogen atom serves as a bridge between two

electronegative atoms. The hydrogen atom is linked to one electronegative atom by a covalent bond and to the other by an electrostatic bond;

(5) three-dimensional arrangement and interaction of molecular functional groups- adsorption potential of a chemical is affected by intramolecular reactions of adjacent molecular groups or fragments or interference with a particular adsorption mechanism caused by the presence of one or more functional groups or molecular fragments; and

(6) molecular functional groups that undergo coordination bonding- coordination is the formation of a weak bond between an organic molecule that is capable of donating electrons and adsorbed cations that are capable of accepting electrons. The net result is a partial overlap of orbitals and a partial exchange of electron density. Coordination can occur between organic chemicals and cations in the water phase of a soil system as well as with soil particle surfaces and with adsorbed cations.

Many chemical properties of a specific organic chemical are the result of sums and interactions of functional group contributions to each specific property.

Other abiotic loss mechanisms in addition to surface *sorption/desorption reactions that may account for loss of* parent compounds include:

(1) hydrolysis- a chemical reaction in which an organic chemical reacts with water or a hydroxide ion;

(2) substitution and elimination- reactions where other chemicals in the soil react with an organic chemical;

(3) oxidation- the reaction resulting in the removal of electrons from a chemical. This removal generally occurs by two different pathways: (a) heterolytic or polar reactions, (an electrophilic agent attacks an organic molecule and removes an electron pair leading to the formation of an oxidized product); or (b) homolytic or free-radical reaction (an agent removes only one electron to form a radical that undergoes further reaction); and

(4) reduction- which is a reaction that results in a net gain of electrons [20].

Successful bioremediation depends upon a thorough characterization and evaluation of the pathways of movement and potential mechanisms of removal of organic constituents at a specific site, as illustrated in Figure 2. To assess the potential for use of bioremediation, the rate of transport of the constituents may be compared to the rate of degradation to determine if the rate of transport is significant in relation to the rate of degradation.

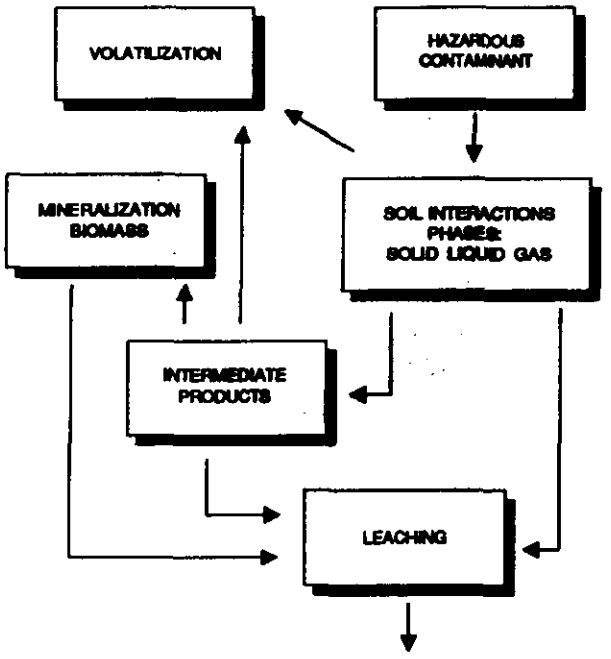


Figure 2. Fate of Hazardous Contaminants in Soil

A means of predicting rate of transport of a constituent through a soil system is to describe its mobility (or relative immobility) by predicting its retardation. Retardation is a factor that describes the relative velocity of the constituent compared to the rate of movement of water through the soil, i.e.,:

$$R = V_w/V_c \qquad (9)$$

where R = retardation factor; V_w = average water velocity; and V_c = average constituent velocity. A retardation factor greater than one indicates that a constituent is moving more slowly than water through a soil. A factor developed from a transport model combined with a description of sorption processes, as defined by a linear Freundlich isotherm [21, 22], can be calculated from the following equation:

$$R = 1 + (\rho K_d/\theta) \qquad (10)$$

where ρ = soil bulk density; K_d = soil water partition coefficient, which describes the partitioning between the soil solid phase and soil water; and θ = volumetric moisture content. This information can be used to manage a contaminated soil system (i.e., through control of soil moisture, changes in bulk density, or addition of amendments to the soil that affect the soil water partition coefficient) so that constituents can be "captured" or contained within the system, thus allowing time for implementation and performance of bioremediation treatment techniques.

3. Waste and Soil Characterization

Interfacing "soil-based behavioral characteristics" of specific organics with specific site and soil properties allows a determination of potential for bioremediation of a site and potential for contamination of other media, i.e., the ground water under the contaminated area, the atmosphere over the site or at the site boundaries, surface waters, etc. Specific characteristics important for describing and assessing the environmental behavior and fate for organic constituents in soil are listed in Table 1. For each chemical, or chemical class, the information required can be summarized as: (1) characteristics related to potential leaching, e.g., water solubility, octanol/water partition coefficient, solid sorption coefficient; (2) characteristics related to potential volatilization, e.g., vapor pressure, relative volatilization index; (3) characteristics related to potential biodegradation, e.g., half-life, degradation rate, biodegradability index; and (4) characteristics related to chemical reactivity, e.g., hydrolysis half-life, soil redox potential [21].

An adequate site characterization, including surface soil characteristics, subsurface hydrogeology, and microbiological characteristics, is the basis for the rational design of a bioremediation system. Site constraints may limit rate and/or extent of treatment of the contaminated vadose zone; therefore, a thorough site characterization is necessary to determine both the three-dimensional extent of contamination as well as engineering constraints and opportunities.

Important soil hydraulic, physical, and chemical properties that affect the behavior of organic constituents in the vadose zone are presented in Table 2. In this zone, water primarily coexists with air, though saturated regions may occur. Perched water tables may develop at interfaces of layers with differing textures. Prolonged infiltration may also result in saturated conditions. The vadose zone usually consists of topsoils, typically three to six feet deep, which are weathered geological materials, arranged in more or less well developed profiles. Water movement in the vadose zone is usually unsaturated, with soil water at less than atmospheric pressure. Weathered topsoil materials gradually merge with underlying earth materials, which may include residual or transported clays or sands. The topsoil differs from the material lying below it in that it is more weathered, contains organic matter, and is the zone of plant root growth. In some regions, the entire vadose zone may be hundreds of feet thick and the travel time of constituents to ground water hundreds or thousands of years. Other regions may be underlain by shallow potable aquifers that are especially susceptible to contamination due to short transport times and reduced potential of soil materials and processes for pollutant attenuation.

Table 1. Soil-Based Waste Characterization [21]

Chemical Class	Soil Sorption Parameters	Soil Degradation Parameters	Chemical Properties
Acid	Freundlich sorption constants (K,N)	Half-life ($t_{1/2}$)	Molecular weight
Base	Sorption based on organic carbon content (K_{oc})	Rate constant (first order)	Melting point
Polar neutral	Octanol water partition coefficient (K_{ow})	Relative biodegradability	Specific gravity
Nonpolar neutral			Structure
Inorganic			Water solubility

Volatilization Parameters	Chemical Reactivity	Soil Contamination Parameters
Air:water partition coefficient (K_w)	Oxidation	Concentration in soil
Vapor pressure	Reduction	Depth of contamination
Henry's law constant ($1/K_w$)	Hydrolysis	Date of contamination
Sorption based on organic carbon content (K_{oc})	Precipitation	
Water solubility	Polymerization	

Table 2. Site and Soil Characteristics Identified as Important in in Situ Treatment [21]

Site location/topography and slope
Soil type, and extent
Soil profile properties
boundary characteristics
depth
texture*
amount and type of coarse fragments
structure*
color
degree of mottling
bulk density*
clay content
type of clay
cation exchange capacity*
organic matter content*
pH*
Eh*
aeration status*
Hydraulic properties and conditions
soil water characteristic curve
field capacity/permanent wilting point
water holding capacity*
permeability* (under saturated and a range of unsaturated conditions)
infiltration rates*
depth to impermeable layer or bedrock
depth to groundwater,* including seasonal variations
flooding frequency
runoff potential*
Geological and hydrogeological factors
subsurface geological features
groundwater flow patterns and characteristics
Meteorological and climatological data
wind velocity and direction
temperature
precipitation
water budget

*Factors that may be managed to enhance soil treatment

Microbiological characterization of a contaminated site should be conducted to ensure that the site has a viable community of microorganisms to accomplish biodegradation of the organic constituents present at the site. Approaches for estimating the kinds, numbers, and metabolic activities of soil organisms include:

(1) determination of the form, arrangement, and biomass of microorganisms in the soil;

(2) isolation and characterization of subgroups and species; and

(3) detection and measurement of metabolic processes [15].

Examples of techniques to accomplish these activities include direct microscopy of soil (e.g., fluorescent staining, buried-slide technique), biomass measurement by chemical techniques (e.g., measurement of ATP), measurement of enzyme activity, and cultural counts of microorganisms (e.g., plate counts, dilution counts, isolation of specific organisms). Biotransformation studies that measure the disappearance of contaminants or mineralization studies that indicate complete destruction of contaminants to carbon dioxide and water may be used to confirm the potential for biodegradation of specific organic chemicals. Specific techniques include batch culture and electrolytic respirometer studies. Controls to detect abiotic transformation of the contaminants and tests to detect toxic effects of contaminants on microbial activity should be included in the studies.

Information from waste and soil/site characterization studies of a specific site and from laboratory evaluations of biodegradation and immobilization potential of specific constituents at the site may be integrated by the use of predictive mathematical models. The resulting mathematical description may be used to: (1) evaluate the effectiveness of use of on-site bioremediation for treatment of the contaminated soil; (2) develop appropriate containment structures to prevent unacceptable waste transport from the treatment zone; and (3) design performance monitoring strategies.

4. Microbial Factors Affecting Biodegradation

The upper layers of soil contain large numbers and diversity of microorganisms. Biodegradation of organic constituents is accomplished by enzymes produced by the microorganisms. Since many enzymes are not released by microbial cells, substances to be degraded must contact or be transported into the cells. Enzymes are generally specific in the substances they affect, so many types may be required to complete biodegradation of organic constituents. The production of enzymes is genetically controlled, thus mutations and adaptations of the native soil microbial populations can improve the ability of the populations to degrade organic substances [23].

Microbial ecologists have identified ranges of critical environmental conditions that affect the activity of soil microorganisms (Table 3). Many of these conditions are controllable and can be changed to enhance biodegradation of organic constituents.

Water is necessary for microbial life, and the soil water matric potential against which microorganisms must extract water from the soil regulates their activity. (The soil matric

Table 3. Critical Environmental Factors for Microbial Activity [15, 21, 23]

Environmental Factor	Optimum Levels
Available soil water	25 - 85% of water holding capacity; -0.01 MPa
Oxygen	Aerobic metabolism: Greater than 0.2 mg/l dissolved oxygen, minimum air-filled pore space of 10%; Anaerobic metabolism: O_2 concentrations less than 1%
Redox potential	Aerobes and facultative anaerobes: greater than 50 millivolts; Anaerobes: less than 50 millivolts
pH	5.5 - 8.5
Nutrients	Sufficient nitrogen, phosphorus, and other nutrients so not limiting to microbial growth (Suggested C:N:P ratio of 120:10:1)
Temperature	15 - 45° C (Mesophiles)

potential is the energy required to extract water from the soil pores to overcome capillary and adsorptive forces). Soil water also serves as the transport medium through which many nutrients and organic constituents diffuse to the microbial cell, and through which metabolic waste products are removed. Soil water also affects soil aeration status, nature and amount of soluble materials, soil water osmotic pressure, and the pH of the soil solution [15].

Microbial respiration, plant root respiration, and respiration of other organisms remove oxygen from the soil atmosphere and enrich it with carbon dioxide. Gases diffuse into the soil from the air above it, and gases in the soil atmosphere diffuse into the air. However, oxygen concentration in a soil may be much less than in air while carbon dioxide concentrations may be many times that of air. Even so, a large fraction of the microbial population within the soil depends on oxygen as the terminal electron acceptor in metabolism. When soil pores become filled with water, the diffusion of gases through the soil is restricted. Oxygen may be consumed faster than it can be replaced by diffusion from the atmosphere, and the soil may become anaerobic. Clay content of soil and the presence of organic matter also may affect oxygen content in soil. Clayey soils tend to retain a higher moisture content, which restricts oxygen diffusion, while organic matter may increase microbial activity and deplete available oxygen. Loss of oxygen as a metabolic electron acceptor induces a change in the activity and composition of the soil microbial population. Facultative anaerobic organisms, which can use oxygen when it is present or can switch to alternative electron acceptors such as nitrate or sulfate in the absence of oxygen, and obligate anaerobic organisms become the dominant populations.

Another soil parameter that describes the effect of the soil environment on metabolic processes is the redox potential of the soil [15]. Biological energy is obtained from the oxidation of reduced materials. Electrons are removed from organic or inorganic substrates to capture the energy that is available during the oxidative process. Electrons from reduced compounds are moved along respiratory or electron transport chains composed of a series of compounds. In an aerobic process, O_2 acts as the terminal electron acceptor. In some cases where O_2 is not available, nitrate (NO_3^-), iron (Fe^{3+}), manganese (Mn^{2+}), and sulfate (SO_4^{2-}) can act as electron acceptors if the organisms have the appropriate enzyme systems. A measurement of the oxidation-reduction potential (redox potential) of a soil provides a measurement of the electron density of the system. As a system becomes reduced, O_2 is depleted, and other substances are used as terminal electron acceptors. There is a corresponding increase in electron density, resulting in a progressively increased negative potential. Redox potential is measured as E_h, expressed in millivolts, or as P_E, which is equal to $-\log [e^-]$ where $[e^-]$ is the concentration of negatively charged electrons.

Oxygen levels in a soil system can be maintained by:

(1) prevention of saturation with water;

(2) presence of sandy and loamy soil materials (excessive clay contents are undesirable);

(3) moderate tilling;

(4) avoidance of compaction of soil; and

(5) limited addition of additional carbonaceous materials [23].

Soil pH also affects the activity of soil microorganisms. Fungi are generally more tolerant of acidic soil conditions (below pH 5) than are bacteria. The solubility of phosphorus, an important nutrient in biological systems, is maximized at a pH value of 6.5. A specific contaminated soil system may require management of soil pH to achieve levels that maximize microbial activity. Control of pH to enhance microbial activity may also aid in the immobilization of hazardous metals in a soil system (a pH level greater than 6 is recommended to minimize metal transport).

Microbial metabolism and growth is dependent upon adequate supplies of essential macro- and micronutrients. Required nutrients must be present and available to

microorganisms in: (1) a usable form; (2) appropriate concentrations; and (3) proper ratios [20]. If the wastes present at the site are high in carbonaceous materials and low in nitrogen (N) and phosphorus (P), the soils may become depleted of available N and P required for biodegradation of the organic constituents. Fertilization may be required at some contaminated sites as a management technique to enhance microbial degradation. Biodegradation of organic constituents declines with lowering of soil temperature due to reduced microbial growth and metabolic activity. Biodegradation has been shown to essentially stop at a temperature of 0° C. Soils exhibit a variation in the temperature of the surface layers, both diurnally and seasonally. Diurnal changes of temperature decrease with depth of the soil profile. Due to the high specific heat of water, wet soils are less subject to large diurnal changes than dry soils [15]. Factors that affect soil temperature include soil aspect (direction of slope), steepness of slope, degree of shading, soil color, and surface cover.

The environmental factors presented in Table 3, as well as soil and waste characteristics, interact to affect microbial activity at a specific contaminated site. Computer modeling techniques are useful to attempt to describe the interactions and their effects on treatment of organic constituents in a specific situation.

5. Treatability Studies for Determination of Bioremediation Potential

Treatability studies for sites contaminated with organic wastes are used to provide specific information concerning the potential rate and extent of bioremediation of surficial soil and deeper vadose zone soils by providing information on fate and behavior of organic constituents at a specific contaminated site. Treatability studies can be conducted in laboratory microcosms, at pilot scale facilities, or in the field. To determine whether a specific site is suitable for bioremediation, information from treatability studies is combined with information concerning site and waste characteristics in order to determine potential applications and limitations of the technology. Ultimate limitations to the use of bioremediation at a specific site are usually related to: (1) time required for cleanup, (2) level of cleanup attainable, and (3) cost of cleanup using bioremediation.

Information from treatability studies also is used to prepare an approach to the engineering design and implementation of a bioremediation system at a specific site. An engineering design to accomplish bioremediation at the site is generally based upon information from simulations (e.g., mathematical modeling) or estimates of pathways of migration of chemicals. These simulations or estimates are generated from treatability data and site/soil characterization data in order to: (1) determine containment requirements to prevent contamination of off-site receiver systems; (2) develop techniques to maximize mass transfer of chemicals affecting microorganism activity (addition of mineral nutrients, oxygen, additional energy sources, pH control products, etc.; removal of toxic products) in order to enhance bioremediation; and (3) design a cost-effective and efficient monitoring program to evaluate effectiveness of treatment.

Table 4. Materials Balances and Mineralization Approaches to Biodegradation Assessment

Biodegradation Approach	Process Examined
Materials balances	Recovery of parent compound in the air, soil water, soil solids (extractable)
	Recovery of transformation products in the air, soil water, and soil solids (extractable)
Mineralization	Production of carbon dioxide, and/ or methane from the parent compound
	Release of substituent groups, e.g., chloride or bromide ions

During the performance of a treatability study, biodegradation, detoxification, and partitioning (immobilization) processes are evaluated as they affect the fate and behavior of organic constituents in the soil.

To assess the potential for biological degradation at a specific contaminated site, the use of treatability studies incorporating materials balance and mineralization approaches to determine the environmental fate and behavior of the constituents in the specific soil is recommended (Table 4). Rate of degradation is calculated by measuring the loss of parent compound and the production of carbon dioxide with time of treatment. Degradation rate is often reported as half-life, which represents the time required for 50 percent of the compound to disappear based upon a first-order kinetic model.

Calculation of the rate of decrease of parent compound, however, by itself does not provide complete information concerning mechanisms and pathways by which organic constituents are interacting with the soil environment [24]. Further information is necessary to understand whether a constituent is simply transferred from one phase (e.g., solid phase) to another (e.g., air phase) through a process of interphase transfer, or is chemically altered so that the

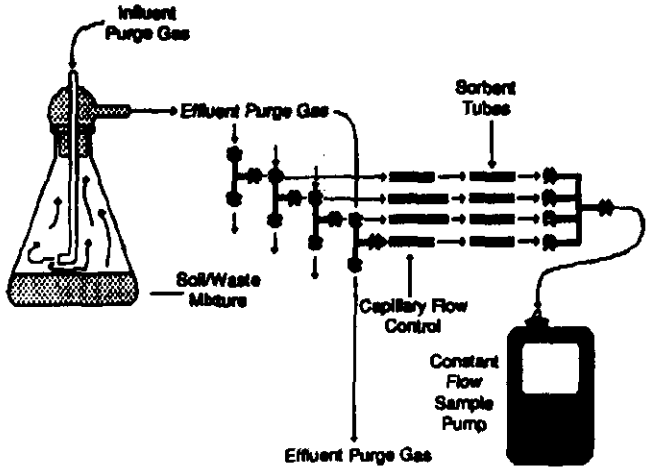


Figure 3. Laboratory Flask Apparatus Used for Mass Balance Measurements.

properties of the parent compound are destroyed. Evaluation of the fate of a constituent in a soil therefore also requires identification and measurement of the distribution of the constituent among the physical phases that comprise the system as well as differentiation of the mechanisms by which constituent may be chemically altered in a soil system.

A laboratory flask apparatus that can be used as a microcosm to measure interphase transfer and biodegradation potential in a laboratory treatability study is illustrated in Figure 3. The contaminated soil material is placed in a flask, which is then closed and incubated under controlled conditions for a period of time. During the incubation period, air is drawn through the flask and then through a sorbent material. Volatilized materials are collected by the sorbent and are measured to provide an estimate of volatilization loss of the constituents of interest. At the end of the incubation period, a portion of the contaminated soil is treated with an extracting solution to determine the extent of loss of the constituents in the soil matrix. This loss can be attributed to biodegradation and possible immobilization in the soil materials. Selection of an appropriate extracting solution is necessary to maximize constituent recovery from the soil. Another portion of the soil is leached with water to determine leaching potential of remaining constituents. Abiotic processes involved in removal of the parent compound are also evaluated by comparing microbially active soil/waste mixtures with mixtures that have been treated with a microbial poison, e.g., mercuric chloride or propylene oxide. The use of a procedure incorporating features illustrated by the use of this microcosm is crucial in order to obtain a materials balance of waste constituents in the soil system. Examples of such protocols may be found in [14, 24, 25, 26]. A certain amount of material is added to the soil, and tracking the fate of the material as it moves through the multiple phases of the soil system provides a materials balance.

Transformation refers to the partial alteration of hazardous constituents into intermediate products. Intermediate products may be less toxic or more toxic than the parent compound, and therefore the rate and extent of detoxification of the contaminated material should be evaluated. Samples generated from the different phases of the soil system in the microcosm studies can be analyzed for intermediate degradation products and used in bioassay studies to provide information concerning transformation and detoxification processes.

Bioassays to quantify toxicity measure the effect of a chemical on a test species under specified test conditions [14]. The toxicity of a chemical is proportional to the severity of the chemical on the monitored response of the test organism(s). Toxicity assays utilize test species that include rats, fish, invertebrates, microorganisms, and seeds. The assays may utilize single or multiple species of test organisms. The use of a single bioassay procedure does not provide a comprehensive evaluation of the toxicity of a chemical in the soil/organic chemical-impacted system. Often a battery of bioassays is utilized that may include measurements of effects on general microbial activity (e.g., respiration, dehydrogenase activity) as well as assays relating to activity of subgroups of the microbial community (e.g., nitrification, nitrogen fixation, cellulose decomposition). Bioassays utilizing organisms from different ecological trophic levels may also be used to determine toxicological effects. However, use of a single assay as a screening test to identify relative toxicity reduction in the environment is a common procedure employed in treatability studies. Assays using microorganisms are often used due to their speed, simplicity, ease in handling, cost effectiveness, and use of a statistically significant number of test organisms that is required to detect the effects of potentially toxic materials in the environment [27, 28].

Two microbial bioassays that have been used to evaluate toxicity of wastes in soil systems are the Ames *Salmonella typhimurium* mammalian microsome assay and the Microtox™ test system. The Ames assay is a measure of the mutagenic potential of hazardous compounds [29, 30] and has been widely used to evaluate environmental samples [31, 32, 33, 34, 35]. A high correlation has been shown between carcinogenicity and mutagenicity, where about 90% of known carcinogens tested mutagenic in the Ames assay [36]. Special strains of *Salmonella typhimurium* that require histidine to grow are used to test for mutagenicity. When plated on a histidine-free medium, the only bacteria able to form colonies are those that have reverted to the "wild" state and are able to produce their own histidine. Without the addition of test chemicals, this back mutation occurs at a rate specific to each strain type (spontaneous reversion rate). The addition of chemicals that are mutagenic increases the reversion rate. Several dose levels of a chemical, mixture of chemicals, or an environmental sample are added to obtain a dose response. Some mutagens act directly on the bacterial cells while others require activation by mammalian

microsomes. These microsomes are generally obtained from liver extracts of Aroclor 1254-induced rats (i.e., rats injected with the polychlorinated biphenyl (PCB), Aroclor 1254). The extract, referred to as the S-9 fraction, contains enzymes that metabolically convert certain chemicals to active mutagens, simulating the activity that occurs in living mammalian systems. Several strains of *Salmonella typhimurium* have been developed in order to detect different types of mutagens. The recommended strains for general mutagenicity testing include TA97, TA98, TA100, TA102. TA97 and TA98 detect frameshift mutagens. TA100 detects mutagens causing base-pair substitutions, while TA102 detects a variety of mutagens not detected by the other strains.

The Microtox™ assay is an aqueous general toxicity assay that measures the reduction in light output produced by a suspension of marine luminescent bacteria in response to an environmental sample [37]. Bioluminescence of the test organism depends on a complex chain of biochemical reactions. Chemical inhibition of any of the biochemical reactions causes a reduction in bacterial luminescence. Therefore, the Microtox™ test considers the physiological effect of a toxicant and not just mortality. Matthews and Bulich [38] have described a method of using the Microtox™ assay to predict the land treatability of hazardous organic wastes. Matthews and Hastings [39] described a method using the Microtox™ assay to determine an appropriate range of waste application loading for soil-based treatment systems. Symons and Sims [40] utilized the assay to assess the detoxification of a complex petroleum waste in a soil environment. The assay was also included as a recommended bioassay in the U.S. EPA Permit Guidance Manual on Hazardous Waste Land Treatment Demonstrations [25].

Immobilization refers to extent of retardation of the downward transport (leaching potential) and upward transport (volatilization potential) of waste constituents. Interphase transfer potential for waste constituents among soil, oil (waste), water, air, and solid (organic and inorganic) phases is affected by the relative affinity of the waste constituents for each phase, and may be quantified through calculation of partition coefficients [25]. Partition coefficients are calculated as the ratio of the concentration of a chemical in the soil, oil, or air phase to the concentration of a chemical in the water phase, and are expressed as Ko (oil/water), Kh (air/water), and Kd (solid/water). Calculation of retardation factors (Equations 9 and 10) also may be used to predict immobilization of constituents in a soil system [22, 41].

Either laboratory microcosm, pilot scale reactors, or field plots may be used to generate treatability data. The set of experimental conditions, e.g., temperature, moisture, waste concentration, etc., under which the studies were conducted should be presented along with experimental results.

Treatability study results provide information relating to rates and extent of treatment of hazardous organic constituents when mass transfer rates of potential limiting substances are not limiting the treatment. Treatability studies usually represent optimum conditions with respect to mixing, contact of soil solid materials with waste constituents and with microorganisms, and homogeneous conditions throughout the microcosm. Therefore, treatability studies provide information concerning potential levels of treatment achievable at a specific site. Under field conditions, the rate and extent of bioremediation is generally limited by accessibility and rate of mass transfer of chemical substances (oxygen, nutrients, etc.) to the contaminated soil as well as by mass transfer of the contaminants to the microbial population and removal of microbial degradation products.

6. Integration of Information from Site Characterization and Treatability Studies

Information from the performance of site characterization and treatability studies may be integrated with the use of comprehensive mathematical modeling. In general, models are used to analyze the behavior of an environmental system under both current (or past) conditions and anticipated (or future) conditions [42]. A mathematical model provides a tool for integrating degradation and partitioning processes with site/soil- and waste-specific characterization for simulating the behavior of organic constituents in a contaminated soil and for predicting the pathways of migration through the contaminated area, and therefore pathways of exposure to humans and to the environment. Models may also be used to approximate and estimate the rates and extent of treatment that may be expected at the field scale under varying conditions. DiGiulio and Suffet [43] have presented guidance on the selection of appropriate vadose zone models for site-specific applications, focusing on recognition of limitations of process descriptions of models and difficulties in obtaining input parameters required by these process descriptions.

The Regulatory and Investigative Treatment Zone Model (RITZ Model, developed at the U.S. EPA Robert S. Kerr Environmental Research Laboratory by Short [44]) is an example of a model that has been used to describe the potential fate and behavior of organic constituents in a contaminated soil system [45]. The Ritz Model is based on an approach by Jury [46]. An expanded version of RITZ, the Vadose Zone Interactive Processes (VIP) model, incorporates predictive capabilities for the dynamic behavior of organic constituents in unsaturated soil systems under conditions of variable precipitation, temperature, and waste loadings [25, 47, 48, 49, 50]. Both models simulate vadose zone processes, including volatilization, degradation, sorption/desorption, advection, and dispersion [51].

Once interphase transfer potential and pathways of escape have been identified by treatability studies and simulation modeling, containment requirements for the constituents of interest at the site can be determined. If the major pathway of transport is volatilization, containment with respect to volatilization control is required. An inflatable plastic dome erected over a contaminated site is a containment method that has been used to control escape of volatile constituents. Volatiles are drawn from the dome through a conduit and treated in an above ground treatment system. If leaching has been identified as important, control of soil water movement should be implemented. For example, if contaminated materials are expected to leach downward from the site, the contaminated materials can be temporarily removed from the site, and a plastic or clay liner placed under the site. When downward, as well as upward, migration is significant, both volatilization and leaching containment systems can be installed. Some hydrophobic chemicals do not tend to volatilize or to leach but are persistent within the soil solid phase; therefore, containment efforts may not be required.

A critical and cost-effective use of modeling is in the analysis of proposed or alternative future conditions, i.e., the model is used as a management or decision-making tool to help answer "what if" type questions [42]. Attempting to answer such questions through data collection programs would be expensive and practically impossible in many situations. For example, information can be generated to evaluate the effects of using different approaches for enhancing microbial activity and for accelerating biodegradation and detoxification of the contaminated area by altering environmental conditions that affect microbial activity.

Results of modeling also can aid in the identification of constituents that will require treatment in the air (volatile) phase, in the leachate phase, and in the solid (soil) phase. Monitoring efforts therefore can be concentrated on monitoring the appropriate environmental phase to evaluate treatment effectiveness. If a comprehensive and thorough evaluation of a specific contaminated system has been conducted, not all chemicals need to be monitored in each phase.

7. Potential Applications and Limitations of Bioremediation Technology

Existing information for constituents of interest at a specific site/soil contaminated system should be collected as a first step in the investigation of the application of bioremediation as a potential treatment technology. Many organic constituents from a wide range of chemical classes have been shown to be amenable to biodegradation in laboratory studies, using both single strains of microbial species or consortia of microbial populations. Biodegradation has also been demonstrated in both aqueous cultures or soil microcosm studies. A summary of biodegradation and disappearance rates for almost 300 chemicals has been prepared by Dragun [20]. Examples of specific chemical classes shown to be biodegradable include: amines and alcohols [14]; polycyclic aromatic hydrocarbons (PAHs) [5, 12, 13, 52, 53]; chlorinated and non-chlorinated phenols [14]; chlorinated aromatic hydrocarbons [54]; polychlorinated biphenyls (PCBs) [55], halogenated aliphatic compounds [50, 57]; pesticides [13, 47, 58, 59, 60, 61]; and various hazardous substances [13, 62]. Industrial wastes from petroleum refining, wood preserving, leather tanning, coal gasification/liquefaction, food processing, pulp and paper manufacturing, organic chemical production, animal production, munitions production, textile manufacturing, pesticide manufacturing, and pharmaceutical manufacturing, as well as municipal wastewaters, sludges, and septage from septic tanks, have all been successfully treated in land treatment systems [14, 20].

RSKERL, as part of its responsibilities to manage research programs to determine the fate, transport, and transformation rates of pollutants in the soil, the unsaturated and the saturated zones of the subsurface environment, initiated a research program to develop comprehensive screening data on the treatability in soil of specific listed hazardous organic chemicals and specific listed hazardous wastes. Research results have been presented by Sims et al. [13], Loehr [14], and McGinnis et al. [5]. A Soil Transport and Fate (STF) Data Base was also developed for RSKERL [63]. The Data Base contains quantitative and qualitative information on degradation, transformation, partitioning among the soil phases, and toxicity of hazardous organic constituents in soil systems. It may be used as a tool for contaminated site assessment and remediation activities. The Data Base provides input data concerning degradation rates, partition coefficients, and chemical property data for mathematical models simulating the behavior and fate of chemical constituents in contaminated surface and subsurface soils. The information is also useful for providing assistance in determining treatment potential at contaminated sites using in situ techniques. Chemicals may be evaluated with respect to the importance of natural processes in controlling persistence and transport potential, and therefore, the susceptibility to degradation or retardation within a subsurface environment.

A report was prepared for the U.S. EPA evaluating the effectiveness of soil treatment practices at Superfund sites, EPA Office of Research and Development tests, Department of Defense and Department of Energy studies, state remediation efforts, private party studies, and vendor demonstrations [64, 65]. Bioremediation was shown to successfully treat many non-halogenated compounds, but was less successful with halogenated compounds. Removal efficiencies for non-halogenated aromatics, heterocyclics and other polar compounds were greater than 95%. Halogenated aliphatic compounds were also

successfully treated, with removal efficiencies averaging 98%; however, volatilization may have contributed to observed losses. More complex halogenated and nitrated compounds exhibited lower removal efficiencies, ranging from 50 to 85%.

Even though a specific organic constituent has been shown to biodegrade under laboratory conditions, whether or not it will degrade in a specific soil/site system is dependent on many factors [54]. Potential degradability requires investigation in site-specific treatability studies. Available oxygen may be limiting in some cases, while other compounds may require the presence of anaerobic conditions. Other environmental conditions that may place restrictions on biological activity include pH, temperature, and moisture. Upon exposure to the soil environment, the constituent may be biologically or chemically altered so as to be rendered persistent and/or toxic in the environment.

The system may lack other nutrients required for microbial activity. Other chemicals present may serve as preferred substrates, or act to repress required enzyme activities. High concentrations of metal salts may be inhibitory or toxic to many microorganisms.

Most chemicals require the presence of a consortium of microbial species for mineralization, some of which may not be present at the specific site. Also, most organisms require a period of acclimation to the constituent before metabolism occurs. During this period, the level of constituent must be high enough to promote acclimation without being toxic or inhibitory. Prior exposure to the constituent or similar constituents may help to shorten the acclimation period.

8. Example of Bioremediation Potential for Polycyclic Aromatic Hydrocarbons (PAHs) in a Soil System

To demonstrate the potential effectiveness of bioremediation, results are presented for the semi-volatile chemical class of compounds known as the polycyclic aromatic hydrocarbons (PAHs). These compounds are of environmental significance because of their recalcitrance to biological degradation, their chronic toxic effects on humans, and their widespread occurrence at contaminated waste sites. Specifically, PAH compounds are associated with oily wastes, such as wastes from petroleum refining operations and wastes from the wood preserving industry. The higher molecular weight PAH compounds are of special concern, because they exhibit mutagenic, carcinogenic, and teratogenic potential.

Table 5. Degradation of PAHs Present in a Complex Oily Waste, Applied at 2% Oil and Grease in Clay Loam Soil [66]

Compound	C_0* µg/g	$t_{1/2}$* days	R^2	95% Confidence Interval ($t_{1/2}$) (days)	
				Lower	Upper
Fluoranthene	351	15	0.966	13	18
Pyrene	283	32	0.884	26	41
Benzo(a) anthracene	86	139	0.397	87	347
Benzo(g,h,i)perylene	8	1661	0.006	139	ND
Indeno-pyrene	5	69	0.559	43	139

* C_0 = Initial Concentration

* $t_{1/2}$ = Half-life (first order kinetics)

Table 6. Effect of Manure and pH Amendments on PAH Degradation in a Complex Waste Incorporated into Soil [67]

PAH Compound	Half-Life in Waste/Soil Mixture (days) Without Amendments	With Amendments
Acenaphthylene	78	14
Acenaphthene	96	45
Fluorene	64	39
Phenanthrene	69	23
Anthracene	28	17
Fluoranthene	104	29
Pyrene	73	27
Benz(a)anthracene	123	52
Chrysene	70	42
Benzo(b)fluoranthene	85	65
Benzo(k)fluoranthene	143	74
Benzo(a)pyrene	91	69
benzo(ghi)perylene	74	42
Dibenz(a,h)anthracene	179	70
Indeno(1,2,3-cd)pyrene	57	42

Table 7. Effect of Soil Moisture on PAH Degradation [67]

Moisture	Half-life in Waste/Soil Mixture (Days) Anthracene	Phenanthrene	Fluoranthene
20-40% field capacity	43	61	559
60-60% field capacity	37	54	231

The degradation of PAH compounds in soils has been demonstrated in laboratory treatability studies [66]. The results presented in Table 5 for PAH compounds present in a complex oily waste show that the half-lives for four of the five compounds ranged from only 15 to 139 days. However, the half-life for benzo(g,h,i)perylene, a higher molecular weight PAH compound, was still quite long (1661 days). McGinnis et al. [5] in a laboratory soil treatability study of PAH compounds present in creosote waste sludges also found that degradation of PAH was dependent on molecular weight and number of aromatic rings. PAHs with two rings generally exhibited half-lives less than ten days, while three- ring compounds in most cases exhibited longer half-lives, which were usually less than one hundred days. Most of the four- or five-ring PAHs exhibited half lives of one hundred days or more. The results of these two studies suggest that means of enhancing biological degradation of more recalcitrant PAH compounds should be investigated.

When additional carbon and energy sources were provided and soil pH was adjusted from 6.1 to 7.5, the half-lives of PAH waste constituents present in a complex fossil fuel waste added to a soil were decreased, as shown in Table 6 [67]. In this laboratory study using first-order kinetic modeling of degradation, the use of manure as an amendment and control of soil pH significantly decreased the $t_{1/2}$ of the PAH constituents studied. For example, the half-life of phenanthrene decreased from 69 to 23 days, benz(a)anthracene from 123 to 52 days, and benz(a)pyrene from 91 to 69 days.

The control of soil moisture also resulted in enhanced biodegradation of PAHs, as shown in Table 7 [67]. Soil moisture in this study was described in terms of percent of field capacity. Field capacity is defined as the percentage of soil moisture remaining in a soil after having been saturated and after free drainage has practically ceased. Therefore, soils with moisture levels of 60 to 80% of field capacity are wetter than soils with levels of 20 to 40% of field capacity. At higher levels of soil moisture, the half-life of the PAH constituents studied decreased. For example, for fluoranthene, the half-life decreased from 559 days to 231 days. At a specific site where containment has been achieved, the addition and control of soil moisture may be a tool to accomplish faster degradation of the constituents.

An increase in soil temperature also can decrease the time required to accomplish degradation, especially the loss of lower molecular weight PAHs [68]. In a laboratory study, for example, the half-life of fluorene decreased from 60 days to 47 days to 32 days at 10°, 20°, and 30° C, respectively (Table 8). At a field site, soil temperature may be difficult to control. However, if a cover is used at the site to control the release of volatile materials, an increase in soil temperature may also occur. Seasonal climatic changes will affect the rate of degradation of organic constituents, as well as geographical location of a specific contaminated site.

If a soil has been exposed previously to similar or the same type of contamination, the soil microbial population may have become acclimated to the waste, and waste degradation may occur at a faster rate. In a laboratory study investigating the acclimation of a soil to a fossil fuel waste, a greater reduction in concentration of all the waste PAH compounds studied was achieved in 22 days in an acclimated soil, compared to the reduction seen in 40 days in an unacclimated soil (Table 9) [67]. These results show that at a site that has been contaminated for a period of time, the indigenous microbial population may become acclimated to the presence of wastes, and techniques to stimulate microbial activity may produce significant degradation. Mixing of a small amount of a contaminated soil that has developed an acclimated population with the

Table 8. Percentages of PAH Remaining at the End of the 240 Day Study Period and Estimated Apparent Loss Half Lives [68]

Compound	Percent of PAH Remaining			Estimated Half Life (day)*		
	10°C	20°C	30°C	10°C	20°C	30°C
Acenaphthene	5	0	0	<60	<10	<10
Fluorene	8	3	2	60(+11/-10)	47(+6/-5)	32(+5/-3)
Phenanthrene	36	19	2	200(+40/-40)	<60	<60
Anthracene	83	51	58	460(+310/-140)	260(+160/-70)	200(+90/-30)
Fluoranthene	94	71	15	+	440(+560/-160)	140(+40/-20)
Pyrene	93	89	43	+	1900(+6200/-800)	210(+160/-60)
Benz(a)anthracene	82	71	50	680(+300/-160)	430(+110/-70)	240(+40/-40)
Chrysene	85	88	86	980(+520/-270)	1000(+900/-250)	730(+370/-180)
Benzo(b)fluoranthene	77	75	62	580(+520/-180)	610(+590/-200)	360(+150/-80)
Benzo(k)fluoranthene	93	95	89	910(+690/-270)	1400(+3300/-560)	910(+4400/-410)
Benzo(a)pyrene	73	54	53	530(+1700/-230)	290(+570/-120)	220(+160/-60)
Dibenz(a,h)anthracene	88	87	83	820(+1100/-300)	750(+850/-260)	940(+12000/-450)
Benzo(g,h,i)perylene	81	76	75	650(+650/-230)	600(+570/-190)	590(+1800/-250)
Indeno(1,2,3-c,d)pyrene	80	77	70	600(+310/-150)	730(+1100/-270)	630(+2500/-280)

* $t_{1/2}$ (95 percent confidence interval)

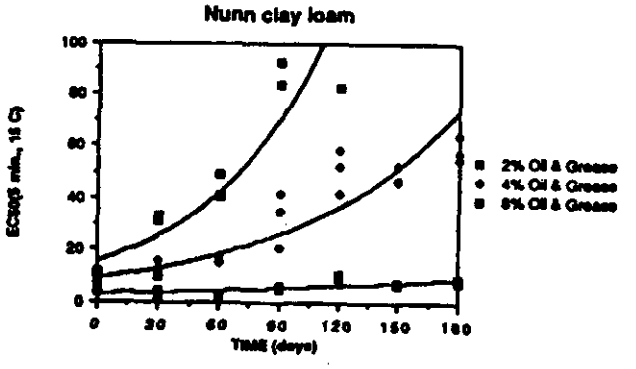
\+ Least squares slope (for calculation of $t_{1/2}$) = zero with 95% confidence.

Table 9. Acclimation of Soil to Complex Fossil Fuel Waste [67]

PAH Compound	Unacclimated Soil		Acclimated Soil	
	Initial Soil Concentration (mg/kg-dry wt)	Reduction in 40 days (%)	Soil Concentration after First Reapplication of Waste (after 168 days incubation at initial level) (mg/kg-dry wt)	Reduction in 22 days (%)
Naphthalene	38	90	38	100
Phenanthrene	30	70	30	83
Anthracene	38	58	38	99
Fluoranthene	154	51	159	82
Pyrene	177	47	180	86
Benz(a)anthracene	30	42	40	70
Chrysene	27	25	33	61
Benz(a)pyrene)	10	40	12	50

contaminated soil to be treated may also result in faster cleanup of a site. Amendment of the soil with exogenous microorganisms developed in laboratory batch cultures would not be required.

One method to assess detoxification of waste constituents in a soil system involves use of the Microtox™ assay [38]. The assay is used to measure acute toxicity of aqueous solutions or water soluble fraction extracts. The Microtox™ system is a standardized instrumental-based system that utilizes a suspension of marine luminescent bacteria (*Photobacterium phosphoreum*) as bioassay organisms. The bioassay organisms are handled like chemical reagents. Suspensions of about one million bacteria are "challenged" with additions of serial dilutions of an aqueous sample or extract. Light output from each bacterial suspension is measured before and after each addition of sample. Results are presented as EC50 values, which are defined as sample concentrations resulting in a 50% decrease of light produced by the luminescent bacteria. High EC50 values indicate lower toxicity than low values. Detoxification of a contaminated soil system is indicated by increased Microtox™ EC50 values approaching 100%. A value of 100% is considered as non-toxic.



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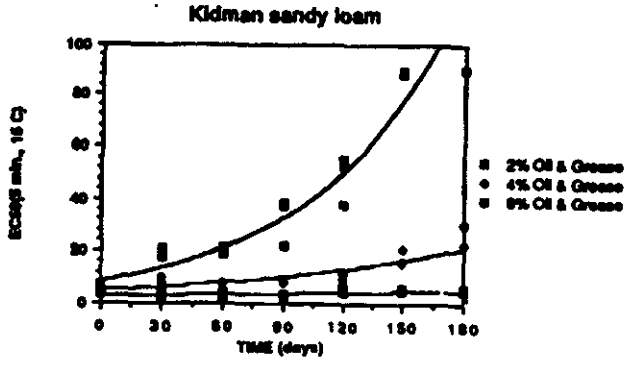

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Figure 4. EC50 as a Function of Time for Two Soils and Three Waste Loading Rates

In a clay loam soil, a petroleum refinery waste was added to soil at application rates of 2%, 4%, and 8% by weight of oil and grease [40]. The results of the study are shown in Figure 4. Time of incubation is plotted on the x-axis, and EC50 values, as determined by the Microtox™ assay,on the y-axis. At the 2% loading rate, the waste material was detoxified to an EC50 value of 100 in a period of about 100 days (Figure 4a). At the highest level of contamination (8% loading rate), the materials remained toxic, even after 180 days. In addition to providing evidence of detoxification of waste constituents, this study also showed the potential for enhancement of biodegradation by mixing uncontaminated soil with contaminated soil to produce a treatment medium with waste contents at levels not toxic to microbial populations.

In a sandy loam soil amended with the same contaminated material, a longer period (about 170 days) was required to detoxify the 2% contamination level to an EC50 value of 100% (Figure 4b). Results of these studies show that mixing of contaminated soils with uncontaminated soils can result in detoxification. However, since the rate of detoxification may be a function of soil type, these results also illustrate the site specificity of bioremediation efforts and underscore the need to perform site-specific characterization of the contaminated area.

Another technique to assess detoxification of organic constituents in a soil system is the use of the Ames *Salmonella typhimurium* assay [29, 30, 52]. The assay utilizes the bacterium *S. typhimurium* to indicate the presence of mutagenic constituents, which may include

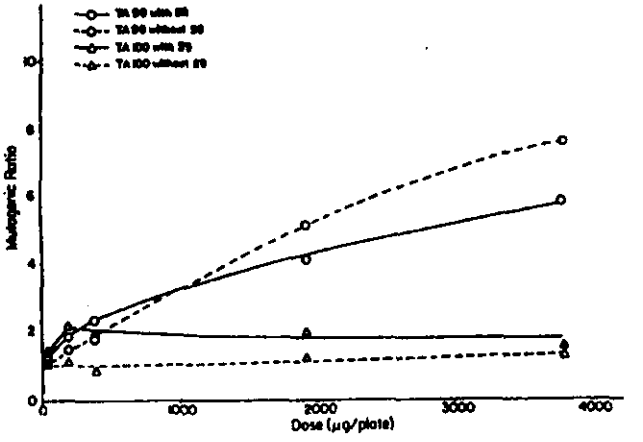

Figure 5. Ames Assay Results for Waste; Soil Mixture Immediately After Waste Incorporation into Soil [67]

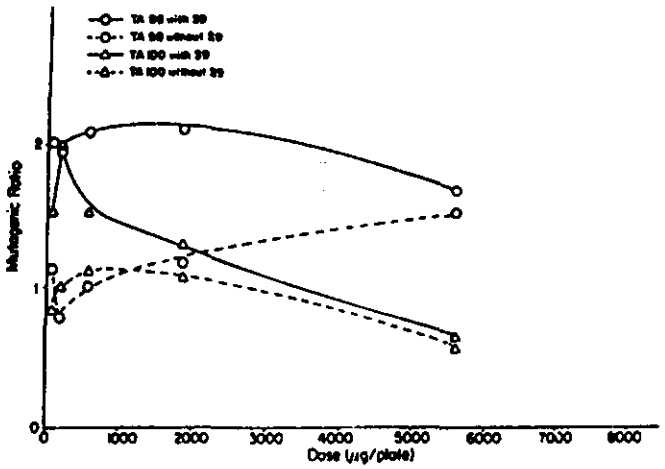

Figure 6. Ames Assay Results for Waste; Soil Mixture After Forty-two Days Incubation [67]

transformation products of parent compounds. Different strains of *S. typhimurium* are selected to indicate the mechanism of mutagenicity, i.e., point mutations or frame-shift mutations. Mutagenicity is measured as a ratio of the number of colonies that grow in the presence of a test sample (e.g., chemical, mixture of chemicals, or extract of an environmental sample) to the number of colonies in the absence of the test sample. Since growth occurs in proportion to mutagenic potential, growth will be greater in the presence of a mutagen, and will increase as the dose of mutagen is increased. The increase in growth in response to dose is depicted graphically in dose-response curves. The minimum mutagenic ratio (ratio of number of colonies that form in the presence of a test sample to the number of colonies on a control growth plate) is 2.0. Therefore, a sample exhibiting a mutagenic ratio greater than 2.0 is considered to possess mutagenic properties.

A study to evaluate detoxification of mutagenic potential of a complex fossil fuel waste containing PAH compounds treated in a soil system was conducted utilizing the Ames assay [67]. Mutagenic ratios for *S. typhimurium* strain TA98 (a test strain used to detect frameshift mutagens such as PAHs) with metabolic activation (to simulate mammalian metabolism by the addition of a mammalian liver extract (referred to as the S9 fraction)). and without metabolic activation (without the addition of the S9 fraction) were determined immediately after waste incorporation and after 42 days of incubation of the waste in the soil. Results as shown in dose response curves showed that the mutagenic ratios decreased from about 4.5 and 7.0 at the highest dose levels tested immediately after waste incorporation (Figure 5) to borderline mutagenic levels (i.e., mutagenic ratios of about 2) after 42 days of treatment (Figure 6). For a different *S. typhimurium* strain, (TA100, a test strain used to detect mutagens causing base-pair substitutions), no dose-response effects or mutagenic activity were measured during the study.

Results of a pilot scale field study have also demonstrated that bioremediation of PAH contaminated soils is a technology that can result in significant cleanup of contaminated soils [69]. A coal gasification waste was thoroughly mixed into a soil at a one-half acre site. Sampling of soil cores was performed at 10 feet intervals across 100 feet rows. Data presented in Table 10 are composite values from the sampling efforts. In all cases, concentrations of the PAH compounds in the soil were greatly reduced after 91 days. Data quality was poorer at the 91-day sampling period, as measured by the coefficient of variation (CV), which is the mean value measured (AVG) divided by the standard deviation (SD). The poorer data quality was attributed to increased analytical difficulties when levels of constituents near detection limits are measured.

The fate and environmental impact of transformation products is an area of bioremediation that needs more consideration. In a laboratory study, the transformation of a ^{14}C radio-labelled PAH compound, 7,12- dimethylbenz(a)-anthracene, in a sandy loam soil was investigated for a 28-day incubation period [70]. At time 0, 62% of the applied parent compound was recovered from the soil, which represents the extraction efficiency of the test (Table 11). After 28 days of incubation, only 20% of the parent compound was recovered. Since recoveries in control reactors poisoned with mercuric chloride were not significantly different over the 28-day incubation period, biological treatment was the proposed mechanism of compound removal from the soil system. Table 11 also shows that the decrease in parent ^{14}C was accompanied by an increase in the metabolite ^{14}C fraction. The appearance of transformation products increased from 4% of the total ^{14}C applied at time 0 to 53% after 28 days. None of the radiolabelled carbon appeared as CO_2 in this study, but 12 to 17% of the radiolabelled material was associated with the solid phase of the soil during the incubation period. The mass balance for the study ranged from 78 to 90% recovery of the applied radiolabelled carbon. Therefore, the appearance, toxicity, fate, and behavior of a metabolite fraction may need to be evaluated on a site-specific basis.

The environmental significance and fate and behavior of many transformation products of PAH constituents, as well as transformation products from many other organic constituents, are not yet known. Therefore, incorporating detoxification assessment into a bioremediation plan is recommended to evaluate these concerns.

Table 10. Field Results for Soil Treatment of PAHs in Coal Gasification Wastes [69]

Compound	C_0*(µg/g)			C After 91 days (µg/g)		
	AVG	SD	CV(%)	AVG	SD	CV(%)
Naphthalene	186	68	37	3	1.8	61
Acenaphthene	729	276	38	1	1.8	157
Phenanthrene	78	28	36	2.6	0.6	23
Benz(a) anthracene	86	42	49	2	0.8	38
Dibenz(a,h) anthracene	52	36	69	ND	--	--

*C_0 = Initial Soil Concentration

Table 11. Transformations of (^{14}C) DMBA by McLaurin Sandy Loam Soil* [70]

	^{14}C appearing in each fraction, percent				
	Soil extract				
Time, days	DMBA, parent compound	Metabolites	Soil Residue	CO_2	Total
0	62(69)	4(6)	12(13)	0(0)	78(88)
14	26	43	16	0	85
28	20(60)	53(11)	17(16)	0(0)	90(87)

* Poisoned (control) data in parentheses.

Table 12. Wood Preserving Sites Where Bioremediation has been Proposed for Soil or Lagoon Sediments [71]

Site Name	State (U. S. EPA Region)	Proposed Remediation
L.A. Clark and Son	VA (III)	Bioremediation
Brown Wood Preserving	FL (IV)	Bioremediation
Burlington Northern (Brainard)	MN (V)	Landfarm
North Cavalcade Street	TX (VI)	Bioremediation
United Creosoting Company	TX (VI)	In Situ remediation
Baxter/Union Pacific	WY (VIII)	Bioremediation
Burlington Northern Somers)	MT (VIII)	Landfarm
Libby (Champion International)	MT (VIII)	In Situ Bioremediation and Landfarm
Koppers, Co.	CA (IX)	Bioremediation
J.H. Baxter	CA (IX)	Bioremediation

9. Implementation of Bioremediation at Sites Contaminated with Organic Wastes

A recent survey conducted for the U.S. EPA concerning the use of bioremediation at sites with soils contaminated with wood-preserving wastes identified ten sites that currently plan to use bioremediation techniques to cleanup contaminated soils and sediments (Table 12) [71]. Sims observed a wide range of variability in target clean-up levels. A wide variability also was observed in criteria for selecting target levels (maximum contaminant levels (MCLs) based on drinking water standards vs. negotiated levels vs. risk assessment-based levels) and in selection of soil phases that must meet target levels (solid phase, leachate phase, and/or air phase). Target levels were determined on a site-specific basis.

An example of a bioremediation plan for a facility identified in the survey was presented by Lynch and Genes [72]. On-site treatment of creosote-contaminated soils from a shallow, unlined surface impoundment was demonstrated at a disposal facility for a wood-preserving operation in Minnesota. The contaminated soils contained creosote constituents consisting primarily of PAHs at concentrations ranging from 1,000 to 10,000 ppm. Prior to implementation of the full scale treatment operation, bench-scale and pilotscale studies simulating proposed full-scale conditions were conducted to define operation and design parameters. Over a four-month period, 62% to 80% removal of total PAHs were achieved in all test plots and laboratory reactors. Two-ring PAH compounds were reduced by 80-90%, 3-ring PAHs by 82-93%, and 4+-ring PAHs by 21-60%.

The full-scale system involved preparation of a treatment area within the confines of the existing impoundment. A lined waste pile for temporary storage of the sludge and contaminated soil from the impoundment was constructed. All standing water from the impoundment was removed, and the sludges were excavated and segregated for subsequent free oil recovery. Three to five feet of "visibly" contaminated soil was excavated and stored in the lined waste pile. The bottom of the impoundment was stabilized as a base for the treatment area. The treatment area was constructed by installation of a polyethylene liner, a leachate collection system, four feet of clean backfill, and addition of manure to achieve a carbon:nitrogen ratio of 50:1. A sump for collection of stormwater and leachate and a center pivot irrigation system were also installed. The lined treatment area was required because the natural soils at the site were highly permeable. A cap was also needed for residual contaminants left in place below the liner. Contaminated soil was periodically applied to the treatment facility and roto-tilled into the treatment soil. Soil moisture was maintained near field capacity with the irrigation system. During the first year of operation, greater than 95% reductions in concentration were obtained for 2- and 3-ring PAHs. Greater than 70% of 4- and 5-ring PAH compounds were degraded during the first year. Comparison of half-lives of PAHs in the full-scale facility were in the low end of the range of half-lives reported for the test plot units. Only two PAH compounds were detected in drain tile water samples, at concentrations near analytical detection limits.

Bioremediation of a Texas oil field site with storage pit backfill soils contaminated with styrene, still bottom tars,

and chlorinated hydrocarbon solvents was demonstrated on a pilot scale [73]. The remediation efforts also included chemical and physical treatment strategies. The pilot scale, solid-phase biological treatment facility consisted of a plastic film greenhouse enclosure, a lined soil treatment bed with an underdrain, an overhead spray system for distributing water, nutrients, and inocula, an organic vapor control system consisting of activated carbon absorbers, and a fermentation vessel for preparing microbial inoculum or treating contaminated leachate from the backfill soils. Soils were excavated from the contaminated area and transferred to the treatment facility. Average concentrations of volatile organic compounds (VOCs) were reduced by more than 99% during the 94 day period of operation of the facility; most of the removal was attributed to air stripping. Biodegradation of semivolatile compounds reduced average concentrations by 89% during the treatment period.

A solid-phase treatment system to remediate petroleum contaminated soil at a hazardous waste site in California was described by Ross et al. [6]. The treatment process involved stimulating the existing microbial population in the soil to degrade petroleum hydrocarbon contaminants. A biotreatability evaluation prior to full-scale operation demonstrated that the existing microorganisms in the soil could degrade the petroleum hydrocarbons, but that the nutrient levels in the soil were not sufficient to maintain growth and support complete degradation of the hydrocarbon contaminants. With adequate nutrients, hydrocarbons decreased from 3500 ppm to less than 100 ppm in 4 weeks in bench scale studies. The degradation process exhibited biphasic kinetics, likely due to the fact the petroleum hydrocarbons were a mixture of a lighter diesel fuel and more recalcitrant waste motor oils. The full scale facility, which began operation in 1988, consists of a four acre treatment site that has had 30 inches of contaminated soil applied to the surface. Bioremediation of the top 15 inches was proceeding by the addition of nutrients, daily tilling and maintenance of adequate soil moisture levels. When the first 15 inches of contaminated soil have been remediated to the target cleanup level of 100 ppm, it will be removed and the second 15 inches will be treated. During the first four weeks of operation, the average concentration of petroleum hydrocarbons was reduced from 2,800 to 280 mg/kg. The rate of hydrocarbon biodegradation measured in the field was consistent with the rate measured in the laboratory.

A solid phase treatment system to clean up pesticides in soil contaminated as a result of a fire at a chemical storage facility was also described by Ross et al. [6]. Water used to extinguish the fire carried large amounts of insecticides and herbicides into the soil beneath the warehouse facility. Laboratory biotreatability studies showed that moderately contaminated soils (90 mg/kg of 2,4-D) could be treated in a soil treatment system to meet regulatory criteria (total MCPA and 2,4-D = 10 mg/kg), while highly contaminated soils (2,4-D concentrations greater than 200 mg/kg) required treatment in a soil/water slurry bioreactor. A five acre soil treatment area was constructed with an engineered clay liner 12 inches thick and a drainage system to control water movement. Ten thousand cubic yards of soil contaminated with a complex mixture of herbicides and insecticides, including 2,4-D, alachlor, trifluralin, carbofuran, and MCPA, were spread on the treatment bed to an average depth of 15 inches. During operation, soil conditions were optimized for biological activity by daily tilling and by maintenance of soil moisture content between 8% and 15% by weight. During three months of operation, the combined 2,4-D and MCPA concentrations decreased from 86 ppm to 5 ppm.

Brubaker and Exner [74] reported on two case histories that involved microbial degradation of chemical contaminants to remediate chemical spills. Both sites also involved other remediation tools in addition to microbial remediation, emphasizing the need to examine complementary and synergistic remediation techniques. At the first site, residual contamination from a formaldehyde spill was treated using chemical oxidation with hydrogen peroxide, followed by microbial "polishing" to complete the remediation. A commercial inoculum of microorganisms acclimated for formaldehyde degradation and a nutrient solution were mixed in an aeration tank and then sprayed on the site. Water was collected in a sump and recycled through the aeration tank. Treatment effectiveness was measured by reduction of concentration of formaldehyde in the aeration tank. After 25 days, concentrations had dropped from over 700 mg/l to less than 1 mg/l. At the second site, a gasoline leak from an underground storage tank was remediated with enhanced bioreclamation techniques, which consisted of addition of nutrients and hydrogen peroxide as an oxygen source. A series of injection and recovery wells were used to recycle water through the site. Soil samples showed a decrease in volatile fuel hydrocarbons from an average of 245 ppm at the initiation of the bioreclamation process to 0.8 ppm after 200 days.

Bioremediation of a site contaminated with PCBs, which have generally been considered resistant to biodegradation in the environment, has been demonstrated at a drag-racing track in New York [55]. Laboratory treatability studies using contaminated soils from the sites inoculated with pure resting cell cultures of PCB-degrading organisms that had been isolated from environmental samples showed substantial PCB biodegradation, up to 51% of the PCBs present in three days. Follow-up laboratory studies were conducted using only 3-4% of the number of cells used in the earlier studies, lower moisture content, lower temperatures, and no shaking or aeration of the reaction mixtures. PCB degradation was not observed until 30 days after the initiation of the study. In an undisturbed soil sample inoculated three times weekly with the PCB-degrading microorganisms, 50% of the PCBs in the top 1 cm of soil was degraded in 15 weeks. Only 10% degradation was seen at depths below 1 cm. When a duplicate of the undisturbed soil experiment was mixed at three months with continued inoculation, the redistributed soil again exhibited the highest degradation rate at the surface. In experiments where soils were inoculated three times weekly and mixed after each application, 35% of the PCBs were degraded after 23 weeks at all depths. This degree of degradation represents a greater amount of PCB

destruction since the PCBs were degraded throughout the whole sample and not just at the surface. Thus mixing was identified as an important site management variable. Preliminary results at a field scale test site at the drag-racing track indicated significant PCB degradation after eight to ten weeks.

10. Conclusions

Consideration of bioremediation for remediation of a site contaminated with organic constituents requires a detailed site, soil, and waste characterization that must be conducted in order to evaluate the potential application of the technology at the site and to demonstrate the feasibility of the approach. A sound and thorough engineering remediation plan developed at the onset of the project will allow cost-effective and efficient use of resources for implementation of site clean-up. The use of treatability studies and simulation modeling are also necessary components of the bioremediation plan so that necessary data to evaluate potential use and to identify pathways of migration are collected in a cost-effective manner. Bioremediation of sites contaminated with organic chemicals is a promising technology, especially if it is incorporated in a remediation plan that uses an integrated approach to the cleanup of the complete site, i.e., a plan that involves the concept of a "treatment train" of physical, chemical, and/or biological processes to address remediation of all sources of contaminants at the site.

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☆U.S. GOVERNMENT PRINTING OFFICE: 1990 - 748-159/20428

EPA/600/9-89/073