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Guide for Conducting Treatability Studies Under CERCLA

Biodegradation Remedy Selection

Interim Guidance

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GUIDE FOR CONDUCTING TREATABILITY STUDIES UNDER CERCLA: BIODEGRADATION REMEDY SELECTION

INTERIM GUIDANCE

U.S. Environmental Protection Agency
Risk Reduction Engineering Laboratory
Office of Research and Development
Cincinnati, Ohio 45268

and

Office of Emergency and Remedial Response
Office of Solid Waste and Emergency Response
Washington, D.C. 20460

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FOREWORD

Today's rapidly developing and changing technologies and industrial products and practices frequently carry with them the increased generation of materials that, if improperly dealt with, can threaten both public health and the environment. The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the nation's land, air, and water resources. Under a mandate of national environmental laws, the agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. These laws direct EPA to perform research to define our environmental problems, measure the impacts, and search for solutions.

The EPA Risk Reduction Engineering Laboratory is responsible for planning, implementing, and managing research, development, and demonstration programs to provide an authoritative, defensible engineering basis in support of the policies, programs, and regulations of the EPA with respect to drinking water, wastewater, pesticides, toxic substances, solid and hazardous wastes, and Superfund-related activities. This publication is one of the products of that research and provides a vital communications link between the researcher and the user community.

The primary purpose of this guide is to provide standard guidance for designing and implementing a biodegradation treatability study in support of remedy selection testing. Additionally, it describes a three-tiered approach that consists of 1) remedy screening testing, 2) remedy selection testing, and 3) remedial design/remedial action testing. It also presents a guide for conducting treatability studies in a systematic and stepwise fashion for determination of the effectiveness of biodegradation in remediating a site regulated under the Comprehensive Environmental Response, Compensation, and Liability Act. The intended audience for this guide includes Remedial Project Managers, On-Scene Coordinators, Potentially Responsible Parties, consultants, contractors, and technology vendors.

E. Timothy Oppelt, Director
Risk Reduction Engineering Laboratory

ABSTRACT

Systematically conducted, well-documented treatability studies are an important component of the remedial investigation/feasibility study process and the remedial design/remedial action process under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). These studies provide valuable site-specific data necessary to aid in the selection and implementation of the remedy. This manual focuses on biodegradation treatability studies conducted in support of remedy selection testing prior to developing the Record of Decision (ROD).

This manual presents a standard guide for designing and implementing a biodegradation remedy selection treatability study. The manual describes and discusses the applicability and limitations of biodegradation technologies, and defines the prescreening and field measurement data needed to determine if treatability testing is required. It also presents an overview of the process of conducting treatability tests and the applicability of tiered treatability testing for evaluating biodegradation technologies. The specific goals for each tier of testing are defined and performance levels are presented, which should be met at the remedy selection testing level in support of the ROD. The elements of a treatability study work plan are also defined and detailed discussions on the design and execution of the remedy selection treatability studies are provided.

The manual is not intended to serve as a substitute for communication with experts or regulators or as the sole basis for the selection of biodegradation as a particular remediation technology. This manual is designed to be used in conjunction with the Guide for Conducting Treatability Studies Under CERCLA (Final)[52] and the Guide for Conducting Treatability Studies Under CERCLA: Aerobic Biodegradation Remedy Screening (Interim Guidance).[53] The intended audience for this guide includes Remedial Project Managers, On-Scene Coordinators, Potentially Responsible Parties, consultants, contractors, and technology vendors.

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ABBREVIATIONS, ACRONYMS, AND SYMBOLS

ANOVA	analysis of variance
ARAR	applicable or relevant and appropriate requirement
ASTM	American Society for Testing and Materials
ATP	adenosine triphosphate
ATTIC	Alternative Treatment Technology Information Center
BBS	bulletin board system
BDAT	Best Demonstrated Available Technology
BOD	biological oxygen demand
BTEX	benzene, toluene, ethylbenzene, and xylene
CAMU	Corrective Action Management Unit
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
CLU-IN	Cleanup Information Database
CMP	chemical manufacture production
COC	contaminant of concern
COD	chemcial oxygen demand
COLIS	Computerized On-Line Information System
CPAH	carcinogenic polynuclear aromatic hydrocarbon
DNAPL	dense non-aqueous phase liquid
DO	dissolved oxygen
EPA	U.S. Environmental Protection Agency
ERT	Emergency Response Team
ETSC	Engineering Technical Support Center
FSP	Field Sampling Plan
FY	fiscal year
GC	gas chromatography
GC/MS	gas chromatography/mass spectroscopy
HDPE	high density polyethylene
HPLC	high-performance liquid chromatography
HWSFD	Hazardous Waste Superfund Collection Database
IR	infrared spectrometry
LAN	local area network
LDR	Land Disposal Restrictions
LNAPL	light non-aqueous phase liquid
MPN	most probable number
NAPL	non-aqueous phase liquid
NPDES	National Pollutant Discharge Elimination System
NPL	National Priorities List
NTIS	National Technical Information Service
O&G	oil and grease
OERR	Office of Emergency and Remedial Response
ORD	Office of Research and Development
OSC	On-Scene Coordinator
OSWER	Office of Solid Waste and Emergency Response

ABBREVIATIONS (continued)

PAH	polynuclear aromatic hydrocarbon
PCB	polychlorinated biphenyl
PCP	pentachlorophenol
PMN	premanufacturer notification
POTW	publicy-owned treatment works
ppb	parts per billion
PPE	personal protection equipment
ppm	parts per million
ppmv	parts per million volume
PRP	Potentially Responsible Party
QA	quality assurance
QAPP	Quality Assurance Project Plan
QC	quality control
RCRA	Resource Conservation and Recovery Act
RD/RA	remedial design/remedial action
RI/FS	remedial investigation/feasibility study
RITZ	Regulatory and Investigative Treatment Zone
ROD	Record of Decision
RPD	relative percent difference
RPM	Remedial Project Manager
RREL	Risk Reduction Engineering Laboratory
RSKERL	Robert S. Kerr Environmental Research Laboratory
SAIC	Science Applications International Corporation
SAP	Sampling and Analysis Plan
SITE	Superfund Innovative Technology Evaluation
SMOS	Subsurface Modeling Support
SRT	Subsurface Remediation Technology
STF	Soil Transport and Fate
SVOC	semivolatile organic compound
SW-846	Test Methods for Evaluating Solid Waste, Third Ed., SW-846
T_0	time zero
TCE	trichloroethylene
TIO	Technology Innovation Office
TOC	total organic carbon
TSC	Technical Support Center
TSCA	Toxic Substance Control Act
TSDF	treatment, storage, and disposal facility
TSP	Technical Support Project
UST	underground storage tank
VIP	Vadose Zone Interactive Processes
VISITT	Vendor Information System for Innovative Treatment Technologies
VOC	volatile organic compound

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SECTION 1
INTRODUCTION

1.1 BACKGROUND

Section 121(b) of the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) mandates the U.S. Environmental Protection Agency (EPA) to select remedies to restore hazardous waste sites that "utilize permanent solutions and alternative treatment technologies or resource recovery technologies to the maximum extent practicable" and to prefer remedial actions in which treatment that "permanently and significantly reduces the volume, toxicity, or mobility of hazardous substances, pollutants, and contaminants is a principal element." Treatability studies provide data to support treatment technology selection and remedy implementation. If treatability studies are used, they should be performed as soon as it is evident that insufficient information is available to select and implement a technology. Conducting treatability studies early in the remedial investigation/feasibility study (RI/FS) process reduces uncertainties associated with selecting the remedy based on limited information and provides a sound basis for the Record of Decision (ROD). EPA regional planning should factor in the time and resources required for these studies.

Treatability studies conducted during the RI/FS phase indicate whether the technology can meet the cleanup goals for the site, whereas treatability studies conducted during the remedial design/remedial action (RD/RA) phase establish design and operating parameters for optimization of technology performance. Although the purpose and scope of these studies differ, they complement one another since information obtained in support of the remedy selection process may also be used to support RD/RA.[75]

This document refers to three levels, or tiers, of treatability studies: remedy screening, remedy selection, and RD/RA testing. Three tiers of treatability studies are also defined in the Guide for Conducting Treatability Studies Under CERCLA, Final, hereinafter referred to as the "generic guide."[52] The generic guide refers to the three treatability study tiers, based largely on the scale of test equipment, as laboratory screening, bench-scale testing, and pilot-scale testing. Laboratory screening is typically used to screen potential remedial technologies and is equivalent to remedy screening. Bench-scale testing is typically used for remedy selection testing; however, it may fall short of providing enough information for remedy selection. Bench-scale studies can, in some cases, provide enough information for full-scale design. Pilot-scale studies are normally used for RD/RA, but may be required for remedy selection testing in some cases. Because of the overlap of these tiers, and because of differences in the applicability of each tier to different technologies, the functional descriptions of the treatability study tiers (i.e., remedy screening, remedy selection, and RD/RA testing) are used in this document.

Some or all of the treatability study levels may be needed on a case-by-case basis. The time and cost necessary to perform the studies are balanced against the improved confidence in the selection of treatment alternatives. These decisions are based on the quantity and quality of data available and on other factors (e.g., State and community acceptance of the remedy, additional site data, and experience with the technology). The need for each level of treatability testing is a management decision. Section 3 discusses in greater detail how treatability studies are used in remedy evaluation. Section 6 provides guidance on interpreting treatability study results and generating cost estimates.

1.2 PURPOSE AND SCOPE

This guide helps ensure a reliable and consistent approach to conducting remedy selection studies. Although there has been increased interest in using microbes to treat media contaminated with inorganics and metals, this document is limited to providing guidance on performing remedy selection studies that evaluate treatment alternatives for media contaminated with organic contaminants. The remedy screening level of treatability testing is discussed in the Guide for Conducting Treatability Studies Under CERCLA: Aerobic Biodegradation Remedy Screening (Interim Guidance), hereinafter referred to as the "biodegradation screening guide."[53] Remedy screening studies provide quick and relatively inexpensive indications of whether biodegradation is a potentially viable remedial technology. Remedy selection treatability testing provides data to help determine if a technology can be used singly or in combination with another technology to reduce contaminant concentrations to levels that comply with site cleanup goals. Remedy selection studies also provide preliminary estimates of the cost and performance data necessary to design either an RD/RA study or a full-scale remediation system.

In general, RD/RA studies will be required to optimize full-scale system design. Presumably, before RD/RA studies are conducted, remedy selection testing has al-

ready been used to determine that biodegradation is an economically and technically viable treatment alternative. RD/RA testing will be site-specific and will utilize equipment employed during full-scale treatment. Consequently, an in-depth discussion of RD/RA testing is beyond the scope of this guidance document.

1.3 INTENDED AUDIENCE

This document is intended for use by Remedial Project Managers (RPMs), On-Scene Coordinators (OSCs), Potentially Responsible Parties (PRPs), consultants, contractors, and technology vendors. Each has a different role in conducting treatability studies under CERCLA. Specific responsibilities for each can be found in the generic guide.[52]

1.4 USE OF THIS GUIDE

This guide is organized into seven sections and reflects the basic information required to perform treatability studies during the RI/FS process. Section 1 is an introduction that defines the role of the guide, describes the purpose and scope of the guide, and outlines its intended audience. Section 2 describes different biodegradation processes currently available and discusses how to conduct preliminary screening to determine if biological treatment is a potentially viable solution. Section 2 also identifies factors that may limit the feasibility of biodegradation. Section 3 provides an overview of the different levels of treatability testing and discusses how to determine the need for treatability studies. Section 4 provides an overview of remedy selection treatability studies, describes the contents of a typical Work Plan, and discusses the major issues to consider when conducting a treatability study. Section 5 discusses the Sampling and Analysis Plan (SAP), including the Field Sampling Plan (FSP) and the Quality Assurance Project Plan (QAPP). Section 6 explains how to interpret the data produced from treatability studies and how to determine if further RD/RA testing is justified. Section 7 lists the references. Although each section has been written to address a specific topic, they have been designed to provide enough background information to allow the reader to understand the topic being addressed without needing to refer to another section of the document for clarification of secondary issues. Although some repetition exists within this document, every effort was taken to minimize redundancy.

This guide is one of a series of guidance documents being developed by EPA. It is a companion document to the generic guide[52] and the biodegradation screening guide.[53] In an effort to avoid redundancy, supporting information in these and other readily available guidance documents is not repeated in this document.

Treatability studies for biodegradation are in their infancy. Procedures for conducting mathematical modeling and for performing field- and larger-scale tests have not been standardized. There are numerous site-specific conditions that can impact biodegradation; many of these cannot be accounted for or controlled during testing and/or remediation, and controversy exists concerning the usefulness of these tools. The lack of consensus stems, in part, from uncertainties associated with the use of in situ technologies. In order to thoroughly address the various design considerations associated with biological treatability studies, this document provides guidance on the available alternatives, including a discussion of their relative advantages and disadvantages. Hopefully, this information will provide a sound basis for approaching the treatability study process. This document is not intended to serve as a substitute for communication with regulators or experts in the field of biodegradation. This document should never be the sole basis for the selection of biodegradation as a remedial alternative or the exclusion of biodegradation from consideration.

As treatability study experience is gained, EPA anticipates further comments and possible revisions to this document. For this reason, EPA encourages constructive comments from outside sources. Direct written comments to:

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SECTION 2
TECHNOLOGY DESCRIPTION AND PRELIMINARY SCREENING

This section presents a brief description of various full-scale biological treatment technologies and a discussion of the information necessary for prescreening the technology before committing to a treatability test program. Subsection 2.1 describes several types of full-scale remediation systems. Subsection 2.1 is divided into three additional subsections: the first two subsections address in situ and ex situ technologies separately; the third section discusses anaerobic applications. The distinction between in situ and ex situ is made in other sections throughout the document and reflects the significant differences that exist between in situ and ex situ treatment. Subsection 2.2 discusses available literature, databases, and technical assistance, and reviews field data necessary to prescreen these technologies. Technology limitations are also reviewed in this subsection.

2.1 TECHNOLOGY DESCRIPTION

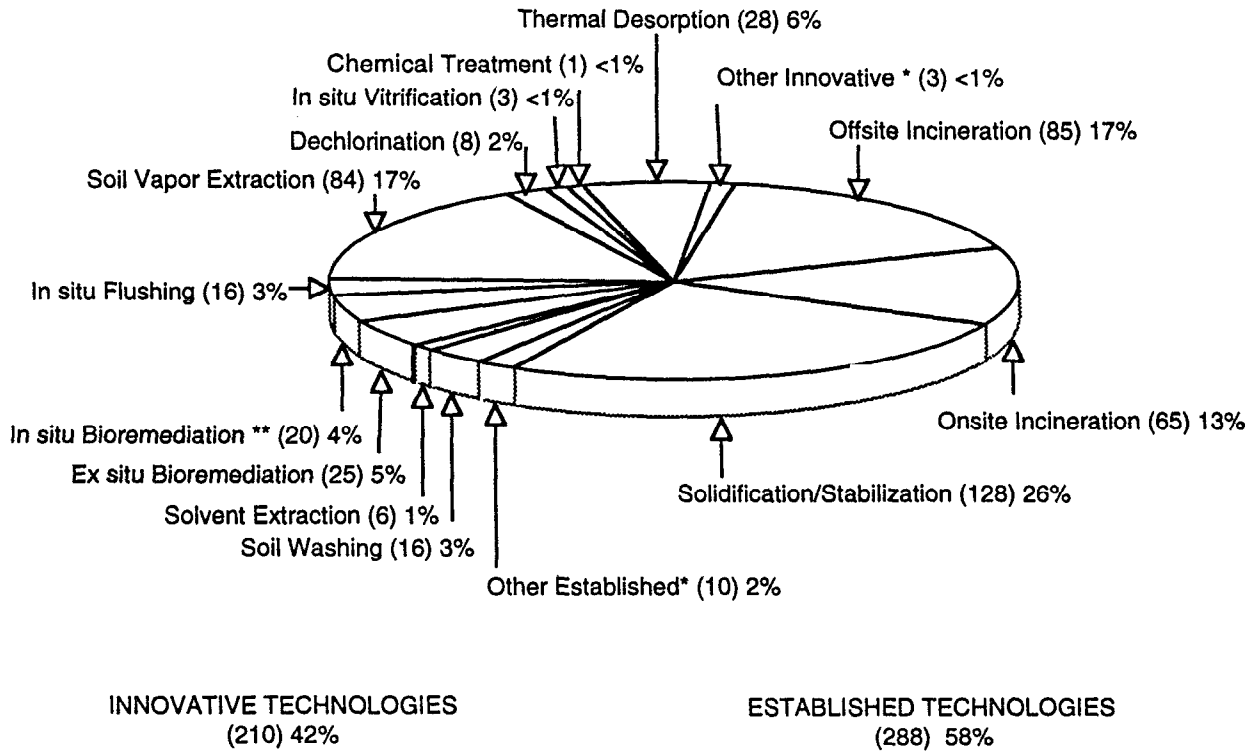
Bioremediation generally refers to the breakdown of organic compounds (contaminants) by microorganisms. Solid-phase, slurry-phase, soil heap bioremediation, bioventing, in situ bioremediation, and composting technologies can be used to remediate contaminated soils biologically.[30][74] In situ technologies encourage contaminant biodegradation by promoting biological activity (e.g., nutrient and oxygen availability) without relocating the impacted media. Disadvantages associated with in situ treatment include a limited ability to control the site-specific variables affecting biodegradation and the potential for offsite contaminant migration. In contrast, ex situ techniques physically isolate the contaminants from the environment prior to or during treatment, thereby limiting the potential for contaminant migration during treatment, while increasing the ability to control conditions that regulate biological degradation. These advantages must be balanced against the high costs associated with materials handling, space requirements, and an increased potential for fugitive emissions during media excavation and transport. As the number of variables requiring control increases, the more complicated (i.e., problematic) implementation becomes. For example, it would be less problematic to implement a remedial design, which modified only one parameter during treatment (i.e., oxygen concentrations), than an application that required the modification of multiple factors (e.g., pH, oxygen concentrations, nutrients, microbes, or buffering agents).

Biodegradation can be used as the sole treatment technology at a site or in conjunction with other technologies in a treatment train. The technology shows promise for degrading or transforming a large number of organic compounds commonly found at contaminated sites to environmentally-acceptable or less mobile compounds. Recently, biological mechanisms have been used to reduce the toxicity of metals as well as to increase metals recoveries. Bioremediation has also been used to treat water contaminated with nitrate, phosphate, and other inorganic compounds. These applications, however, are not discussed extensively in this guide.

As of October 1992, approximately 149 CERCLA, Resource Conservation and Recovery Act (RCRA), underground storage tank (UST), and other governmentally regulated sites have been identified by EPA Regions and States as considering (e.g., performing treatability studies), planning, operating full-scale, or having used biological treatment systems. Approximately 62 percent of the sites are CERCLA, 14 percent are RCRA, and 10 percent are UST sites. The remaining 14 percent represent Toxic Substance Control Act (TSCA) and other Federal and State efforts.[41] Of the 149 sites discussed above, approximately 27 percent are presently operational at the full-scale level, and 14 percent have been completed.

At the end of EPA fiscal year 1991 (FY91), there were 45 Superfund sites where bioremediation had been selected. These sites represent 9 percent of the total number of Superfund sites (Figure 2-1).[59] Historically, bioremediation has been primarily applied at sites containing petroleum hydrocarbons, creosote, pesticides, herbicides, and solvents. Bioremediation is presently being investigated at a number of sites contaminated with explosives and polychlorinated biphenyls (PCBs). Although full-scale applications have yet to occur, bioremediation has been selected in a number of RODs as a potential technology for treating media contaminated with explosives and PCBs. Full-scale applications are scheduled to begin at these and other non-CERCLA sites in the near future.

Brief discussions of in situ and ex situ technologies follow. The majority of the text in these subsections was adapted from material presented in the biodegradation screening guide.[53] Except for the addition of two new subsections describing bioventing and biofilters, and a table (Table 2.1) that synopsizes the advantages, disadvantages, and the appropriate applications of the different technologies, the majority of the text in Subsections 2.1.1 (In Situ Biological Technologies) and 2.1.2 (Ex Situ



Total Number of Technologies - 498***

NOTES:

( ) Number of times this technology was selected or used.
* "Other" established technologies are soil aeration, in situ flaming, and chemical neutralization. "Other" innovative technologies are air sparging and contained recovery of oily wastes.
** Includes nine in situ groundwater treatment remedies.
*** Data are derived from 1982-1991 Records of Decision (RODs) and anticipated design and construction activities as of February 1992. More than one technology per site may be used.

Figure 2-1. Superfund remedial actions: summary of alternative treatment technologies through FY91.

Biological Technologies) underwent minor technical and editorial changes. Significant modifications, however, were made to Subsections 2.2.1 through 2.2.4. In Subsections 2.2.1 (Literature/Database Review) and 2.2.2 (Technical Assistance), additional literature and database sources, as well as organizations to contact for technical assistance, were recommended. Subsections 2.2.3 (Prescreening Characteristics) and 2.2.4 (Technology Limitations) were completely rewritten, with significant changes made to the technical scope of these discussions.

A series of engineering bulletins is being prepared by EPA's Risk Reduction Engineering Laboratory (RREL) in Cincinnati, Ohio.[45][46] Readers interested in more detailed discussions of certain biodegradation technologies are encouraged to utilize these documents. These bulletins provide additional information on certain biodegradation technologies including the applicability of the technology, the most current performance data, the status of the technology, and sources for further information.

2.1.1 In Situ Biological Technologies

In situ biological technologies treat contaminants in place, eliminating the need for soil excavation and limiting volatile releases into the atmosphere. As a result, many of the risks and costs associated with materials handling are reduced or eliminated. Under some circumstances, these technologies can be used to clean up soil contamination responsible for impaired groundwater quality; they have been most frequently employed to treat soils with moderate to high permeabilities. In situ biological technologies may enhance traditional pump and treat technologies by reducing the time needed to achieve aquifer cleanup standards.

In Situ Bioremediation

During in situ bioremediation, contaminant biodegradation within the subsurface soil and water is enhanced

Table 2-1. Comparison of Biological Remediation Technologies*.

Technology	Advantages	Disadvantages	Typical application
In situ	• inexpensive • low exposure risks • excavation not required	• low degradation rates • control of operating parameters is difficult • hydrological characteristics can affect treatment	• saturated soils • aerobic or anaerobic • permeable soils
Bioventing	• excavation not required • faster degradation than other in situ technologies	• hydrological characteristics can affect treatment • contaminant volatilization can occur during treatment	• permeable soils • unsaturated soils
Solid-phase	• simple procedure • inexpensive • currently accepted method	• some exposure risks**	• surface contamination • aerobic
Soil heaping	• inexpensive	• some exposure risks**	• surface contamination • aerobic
Composting	• inexpensive • self-heating	• needs bulking agents • some exposure risks** • residual contamination	• surface contamination • aerobic
Slurry bioreactors	• good operational control • good microbe/compound contact • enhanced desorption of compound from soil • high degradation rates	• high capital outlay • limited by reactor size • some exposure risks**	• surface contamination • recalcitrant compounds • soils that bind compound tightly • aerobic or anaerobic
Biofilters	• can be operated cyclically without loss in performance • can treat a heterogeneous mixes of contaminants • high degradation rates	• prone to clogging • odors may result • filter media must be installed by hand • air loading rates are low	• gaseous contamination • light aliphatic compounds • chlorinated aliphatic and aromatic compounds

* Adapted from reference number 28.
** Fugitive emissions may occur during excavation.

without using excavation. The technology usually involves enhancing natural biodegradation processes by adding nutrients, oxygen (if the process is aerobic), and in some cases, microorganisms to stimulate the biodegradation of contaminants. Moisture control may also be required to enhance biodegradation in unsaturated soils. If oxygen is the rate-limiting parameter, oxygen sources such as air, oxygen, or hydrogen peroxide (H_2O_2) may be used. If the percolation of aqueous amendments is being considered, rough calculations should be made to estimate the amount of oxygenated water that will be required to mineralize the contaminants at the site (see Subsection 4.2.7 for an expanded discussion of this analysis). This concept is equivalent to estimating biological or chemical oxygen demand (BOD or COD) and can be used to verify that sufficient oxygen (i.e., electron acceptor) will be present. Laboratory and field studies have indicated that the addition of methane or other primary substrates may aid in the co-metabolic biodegradation of low molecular weight chlorinated organics. Recent evidence suggests that anaerobic processes that use nitrate as a terminal electron acceptor may be effective for the in situ treatment of benzene, toluene, xylenes,[14][13] and some polynuclear aromatic hydrocarbons (PAHs).[17]

In situ bioremediation is often used in conjunction with a groundwater-pumping and soil-flushing system to circulate nutrients and oxygen through a contaminated aquifer and associated soils. The process usually involves introducing aerated, nutrient-enriched water into the contaminated zone through a series of injection wells or infiltration trenches and recovering the water downgradient. Highly water-soluble contaminants are usually flushed out of a permeable soil before significant biodegradation can occur; less soluble contaminants usually remain in the soil and may be biodegraded. The recovered water can then be treated, if necessary, and reintroduced to the soil on site or discharged to the surface (Figure 2-2). Whether amendments can stimulate in situ biodegradation depends in part on contaminant accessibility. Water table fluctuations within the treatment zone can impact in situ bioremediation by affecting critical factors such as: nutrient and oxygen concentrations, air permeability, contaminant distribution, moisture content, and microbial composition. A low permeability soil (low hydraulic conductivity) can hinder the movement of water, nutrients, and, to a lesser extent oxygen, through the contamination zone. The soil's hydraulic conductivity must be low enough to allow the microbes sufficient time to incorporate needed nutrients as amendment-laden water percolates through the soil. Hydraulic conductivity also affects delivery of aqueous-phase electron

acceptors (e.g., hydrogen peroxide and nitrate). Variable hydraulic conductivities in different soil strata within a contaminated area can complicate the design of flow control. The ability to reinject or discharge water to the surface is dependent upon local regulations. Recovered groundwater may require pretreatment followed by discharge to a publicly-owned treatment works (POTW).

Except for bioventing, in situ technologies have primarily been used to treat saturated soils. Generally unsaturated soil treatment has been limited to fairly shallow regions over groundwater that is already contaminated. The bioremediation of contaminants present in unsaturated soils has been limited, in part due to difficulties associated with ensuring that sufficient time is available for microbes to utilize amendments present within the percolating water. Attempting to overcome these difficulties by increasing the flow of amendment-laden water into the soil can lead to a decrease in the soil's air permeability. This decrease in permeability is associated with increased soil saturation and inhibited gaseous oxygen (air) delivery. Futhermore, since the solubility of oxygen in water is limited (i.e., less than 8 mg/L at 20°C), in most situations oxygenated water will be unable to meet oxygen requirements. Thus, in order to operate effectively, percolation techniques used to introduce amendment-laden water to the soil should be combined with air injection or vacuum extraction techniques used to oxygenate the unsaturated soil. Alternate electron acceptors may be utilized as an option. It should be noted, in situations where underlying groundwater is not contaminated, the risk of contaminating the groundwater by infiltration from the overlying treatment zone often limits the application of bioremediation to the unsaturated zone.

Bioventing

In situ bioventing uses relatively low-flow soil aeration techniques to enhance the bioremediation of soil contaminated with organic contaminants. Aeration systems similar to those employed during soil vapor extraction are used to supply oxygen to the soil. Typically a vacuum extraction, air injection, or combination vacuum extraction and air injection system is employed.[77] An air pump, one or more air injection or vacuum extraction probes, and emissions monitors at the ground surface (Figure 2-3) are commonly used. Although no peer-reviewed data have been released on the use of vapor-phase nutrients (e.g., ammonia and phosphorus) at least one vendor has developed a system designed to provide these nutrients to the subsurface.[77] However, in most field applications to date, nutrient additions have been found to provide no additional benefits.[78]

In general, low air pressures and air flow rates are used to maximize biodegradation while minimizing contaminant volatilization. Some systems, however, utilize higher air flow rates, thereby combining bioventing with soil vapor extraction.[36][39][41][74] Although the technology is predominantly used to treat reasonably permeable unsaturated soils, research is being performed regarding its applicability to less permeable soils, saturated soils, and ground-

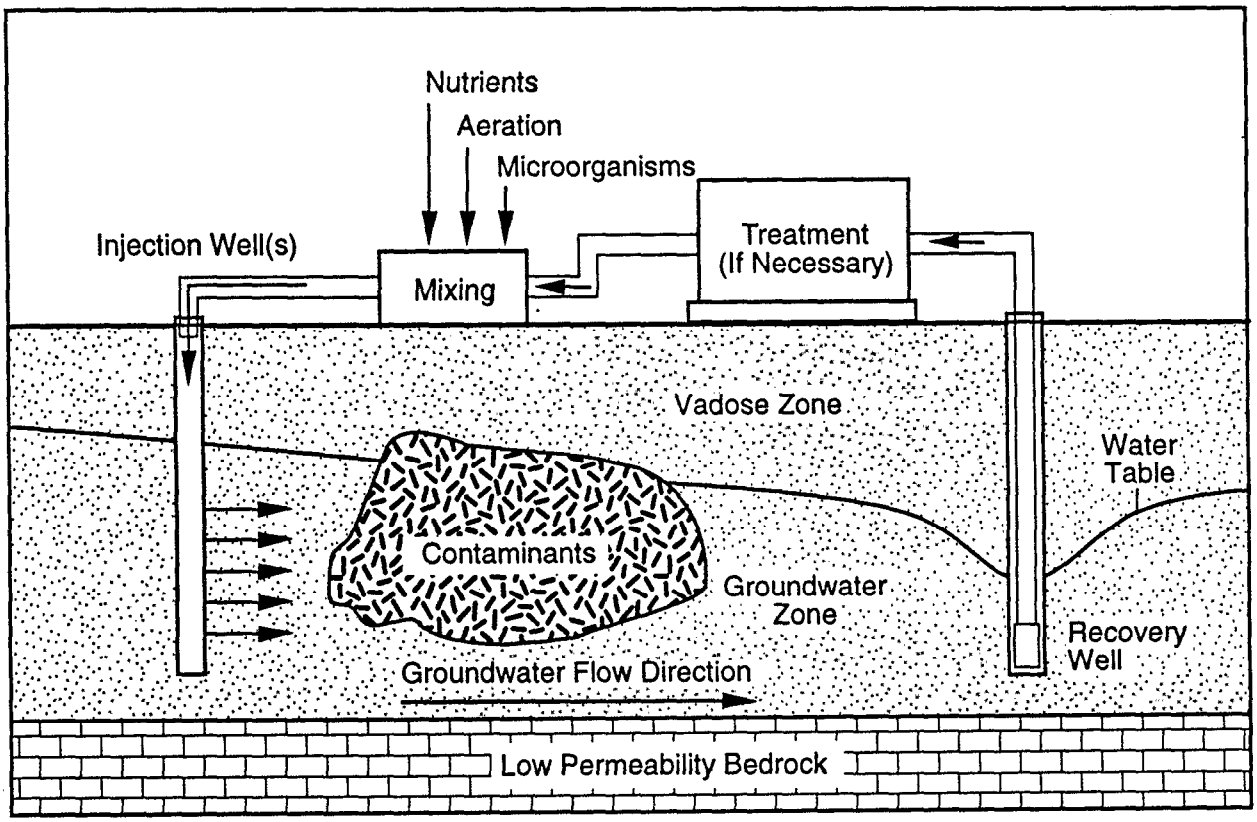


Figure 2-2. In situ bioremediation of saturated soils and groundwater.

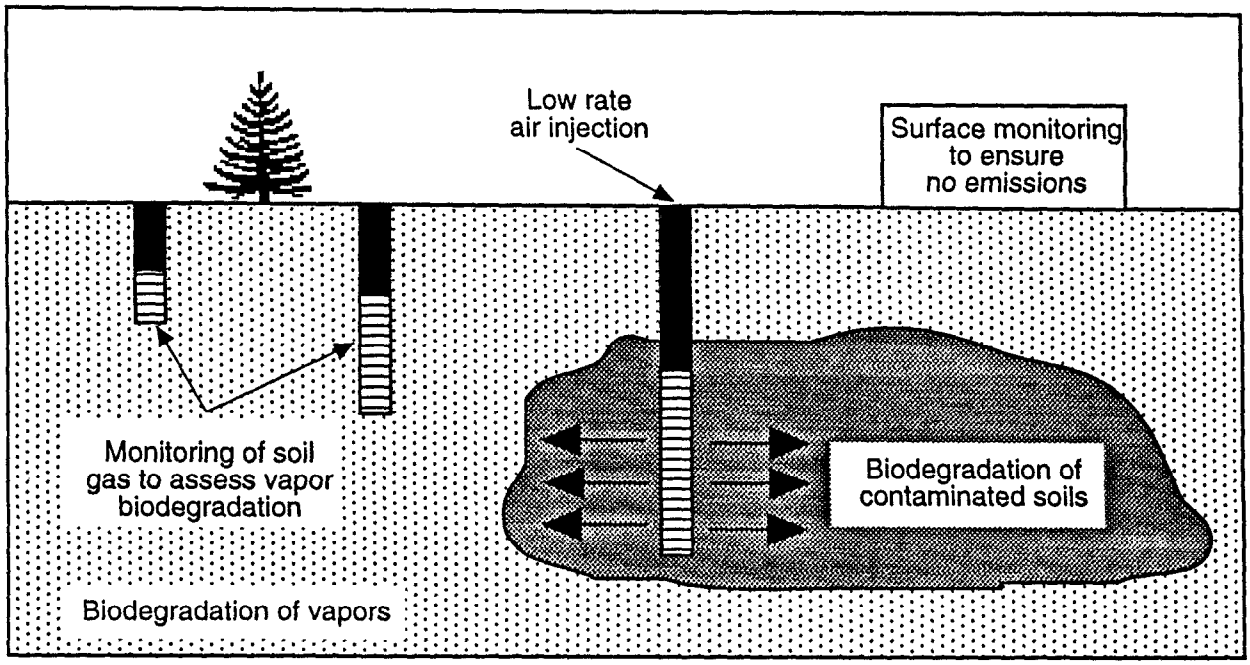


Figure 2-3. Bioventing.

water (using air sparging techniques).[22] A Test Plan and Technical Protocol for a Field Treatability Test for Bioventing has been developed by the U.S. Air Force.[35] This document has been reviewed and is supported by EPA.

2.1.2 Ex Situ Biological Technologies

Solid-Phase Bioremediation

Solid-phase bioremediation (sometimes referred to as land treatment or landfarming) is a process that treats soil in above-ground treatment systems using conventional soil management practices to enhance the microbial degradation of contaminants. Solid-phase bioremediation, in many instances, can be performed without triggering land disposal restrictions (LDRs). Subsection 3.2 further discusses the applicability of LDRs to bioremediation projects.

Solid-phase bioremediation at CERCLA sites usually involves placing excavated soil in an above-grade soil treatment area (Figure 2-4). If required, nutrients and microorganisms are added to the soil, which is tilled at regular intervals to improve aeration and contact between the microorganisms and the contaminants. During the operation of a solid-phase bioremediation system, pH, temperature, nutrient concentrations, and moisture content are maintained within ranges conducive to microbial activity (optimal ranges for these parameters are discussed in Subsection 4.2). If necessary, highly contaminated soil can be mixed with less contaminated soil from the same site to reduce the contaminant concentrations to levels that do not inhibit microbial activity. Depending on the nature of the contaminant, the type of soil, and a number of other site-specific factors, mixing may not reduce toxicity at a micro-environment level. Additionally, regulatory approval may be required before a less contaminated soil may be mixed with a more highly contaminated soil. Solid-phase treatment systems can be modified to contain and to treat soil leachate by adding underdrain and liquid treatment systems. Volatile organic compounds (VOCs) can be contained by adding an optional cover. Conventional VOC treatment can be added as part of a treatment train.

A variety of processes in addition to bioremediation influence the fate of contaminants during solid-phase treatment. These include physical and chemical processes such as leaching, adsorption, desorption, photodecomposition, oxidation, volatilization, and hydrolysis. The physical and chemical properties of the contaminants interact with site-specific variables (i.e., soil properties) to influence the fate of the contaminants. The contaminants may be degraded or transformed to environmentally-acceptable or less mobile compounds.[19] While most of these reactions occur in the top 6 to 12 inches of the treatment zone, some contaminant decomposition and immobilization occurs within underlying layers.

Soil Heap Bioremediation

Soil heap bioremediation, which is very similar in nature to solid-phase bioremediation, involves piling contaminated soil in heaps up to several meters high (Figure 2-5). If required, aeration is usually provided by pulling a vacuum through the heap. Simple irrigation techniques are generally used to maintain moisture content, pH, and nutrient concentrations within ranges conducive to the biodegradation of contaminants. The system can be designed to control the release of VOCs by passing the exhaust from the vacuum through activated carbon or biofilters. Moisture control and flow rates can be varied to favor biodegradation rather than volatilization. Simi-

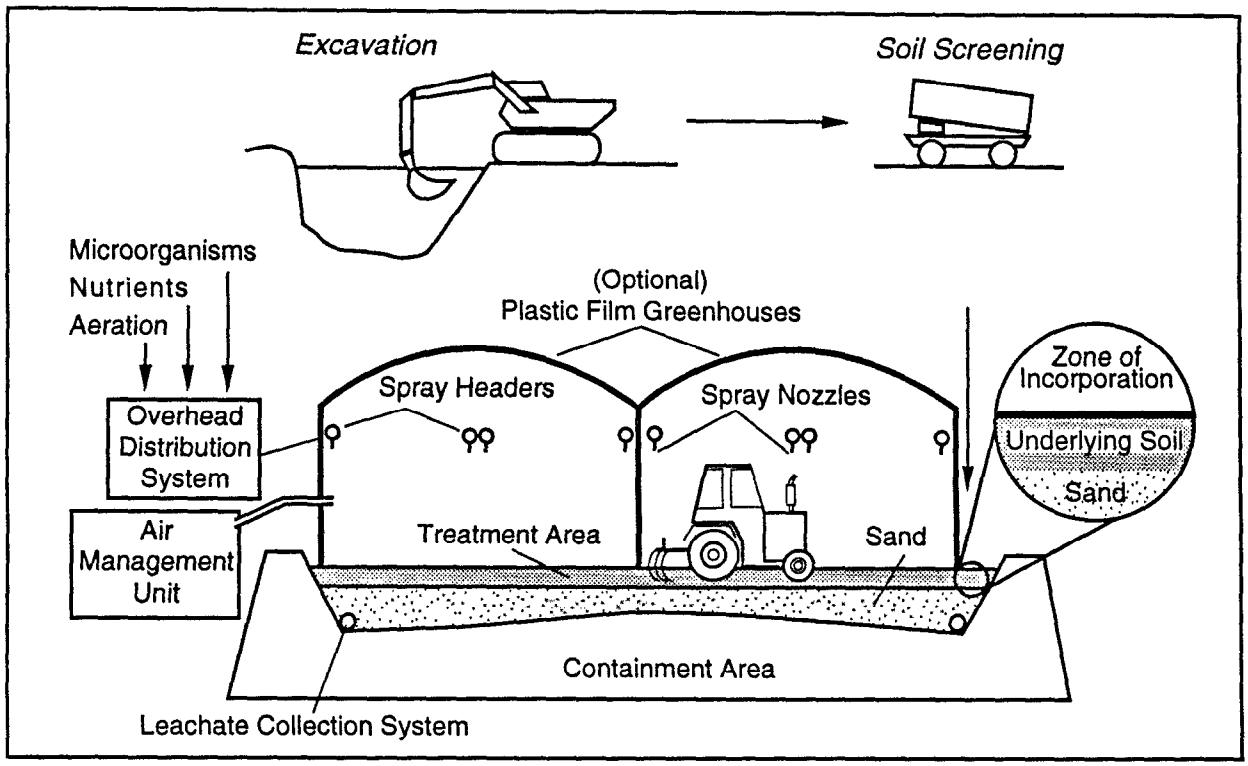


Figure 2-4. Solid-phase bioremediation.

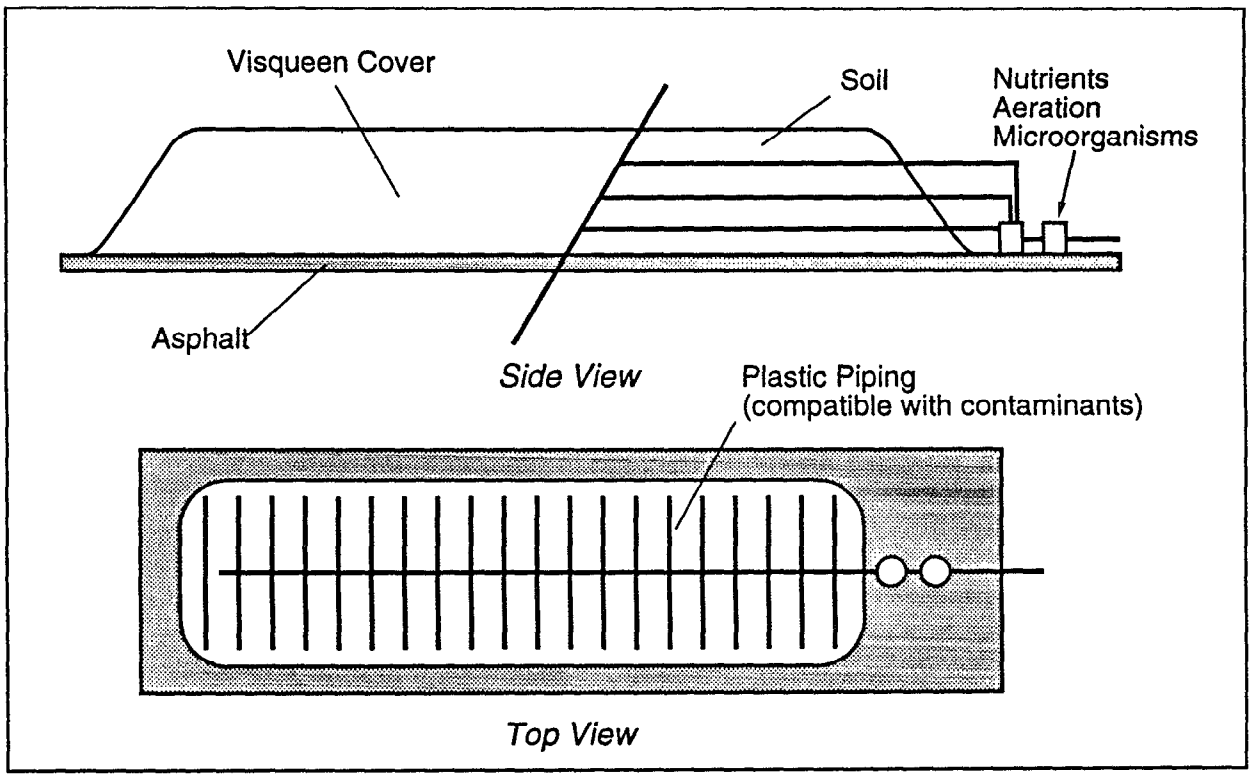


Figure 2-5. Soil heap bioremediation.

lar modifications are employed during bioventing (Subsection 2.1.1).

Composting

Like soil heap bioremediation, composting is similar to solid-phase bioremediation. In contrast, composting technologies typically employ a bulking agent and encourage the thermophilic degradation of the contaminants of interest.

Composting involves the storage of biodegradable waste with a bulking agent (e.g., chopped hay or wood chips). The structurally-firm bulking agent is usually biodegradable. Typically, two parts bulking agent are mixed with one part contaminated soil to improve the soil permeability. Adequate aeration; optimum temperature, moisture, and nutrient concentrations; and the presence of an appropriate microbial population are necessary to enhance the decomposition of organic compounds. The biodegradation process may be thermophilic. If so, microorganisms that occur naturally in the decaying organic matter may biodegrade the contaminants of concern. However, the elevated temperatures associated with thermophilic biodegradation may limit the activity of non-thermophilic indigenous and exogenous organisms. Although biodegradation is usually the mechanism through which contaminant reduction is sought, some contaminants (e.g., nitroaromatics) or their degradation products may be strongly adsorbed on humic materials with covalent bonds, limiting their environmental mobility and thus reducing the potential for exposure.[20][29][34]

The three basic types of composting are open windrow systems, static windrow systems, and in-vessel (reactor) systems. In the open windrow system, the compost is stacked into elongated piles (Figure 2-6). Aeration is accomplished by tearing down and rebuilding the piles. In the static windrow system, piles of compost can be aerated by a forced air system (the piles are built on top of a grid of perforated pipes). The in-vessel system involves placing the compost into a closed reactor. Aeration is accomplished by tumbling, stirring, and forced aeration. Like soil heap bioremediation and solid-phase bioremediation, pH, microbial, and nutrient supplementation, as well as fugitive emission control, may be needed depending on the types and concentrations of contaminants present in the soil.

Slurry-Phase Bioremediation (Liquid/ Solids Treatment)

In slurry-phase bioremediation, excavated contaminated soil is typically combined with water and then placed in an onsite, stirred-tank reactor(s) where the soil is combined with water to form a slurry. The solids content of the slurry depends on the type of soil, the type of mixing and aeration equipment available, and the rates of contaminant removal that need to be achieved. Contaminated surface or groundwater may be used as makeup water, enabling slurry-phase units to alleviate water and soil contamination problems simultaneously. Depending on the characteristics of the soil, it may be directly fed into the slurry system, pretreated to remove contaminants not amenable to biodegradation, or pretreated using soil washing to achieve a significant reduction in the volume of material requiring treatment. If required, nutrients, pH amendments, and/or microbial supplements may be added to the slurry. The slurry is then aerated and/or agitated to facilitate the aerobic biodegradation of the contaminants. This encourages efficient biodegradation by promoting contact between contaminants, microbes, nutrients, carbon sources, water, and electron acceptors.

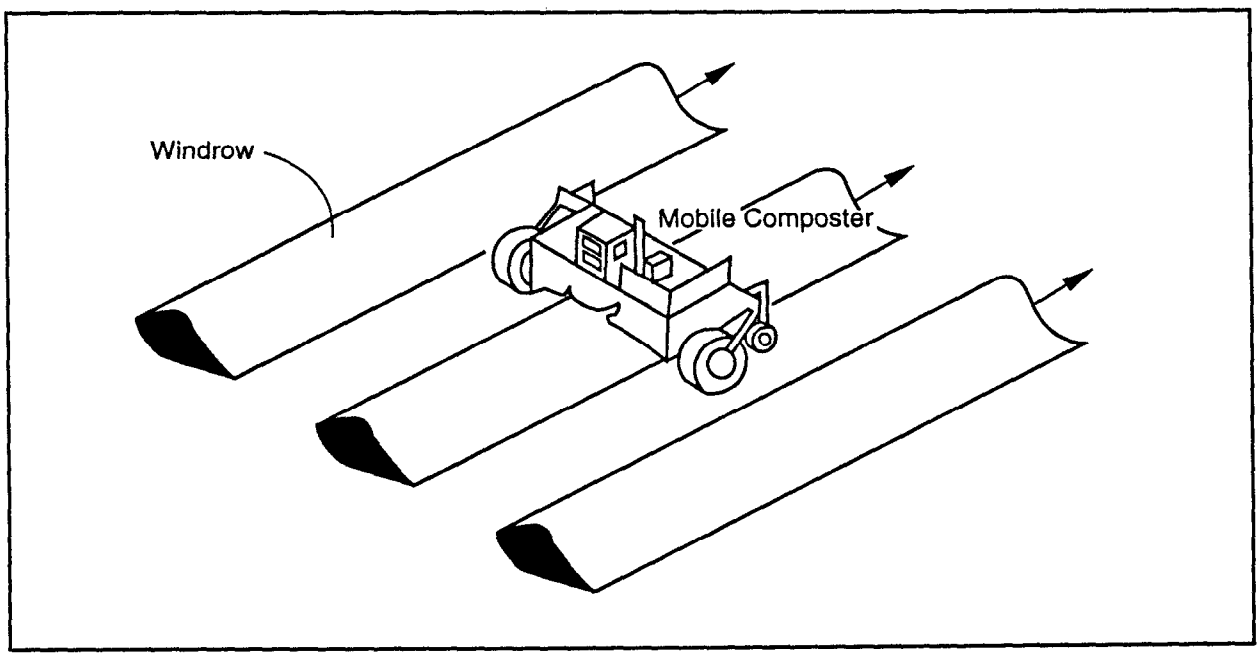


Figure 2-6. Open windrow composting.

The process can be operated in either a batch or a continuous mode (Figure 2-7).

As with solid-phase bioremediation, the process can be designed to contain and treat VOCs. Additionally, slurry-phase bioremediation systems may be used to treat sludges and sediments in existing lagoons and impoundments, thus eliminating the need for excavation (Figure 2-8). In such systems, an impermeable layer should be present under the slurry-phase system to prevent contaminant migration.

Biofilters

Microorganisms can also be used to treat organic vapors by employing biofilters. Biofilters operate in a manner similar to processes used to biologically treat wastewater (e.g., trickling filters). As with these processes, biofilters provide bacteria with a surface on which to grow. Oxygen concentrations, temperature, nutrient concentrations, moisture levels, pH, and carbon levels are adjusted to optimize contaminant degradation, resulting in significant vapor phase contaminant reductions. The primary components of biofilters are: an air blower, an air distribution system, a moisturizing system, filter media, and a drainage system (See Figures 2-9 and 2-10). The technology is considered very effective in removing light aliphatic compounds (e.g., propane and isobutane) with removal efficiencies in the range of 95 to 99 percent. Chlorinated aliphatic and aromatic compounds can also be removed using biofilters, however somewhat lower removal rates have been reported.[16]

2.1.3 Anaerobic Bioremediation Applications

The in situ and ex situ technologies described in the previous subsections normally function under aerobic conditions. However, anaerobic biological processes can be applied to either in situ or ex situ technologies. The application of nutrients and moisture and the control of pH are common elements of anaerobic and aerobic systems. Anaerobic systems use chemical oxygen sources as electron acceptors. Oxygen is normally limited for in situ systems either by natural conditions or by artificial means (surface flooding or other surface barriers). If oxygen penetration is limited and a readily degradable substrate is present, indigenous microorganisms will rapidly deplete the available oxygen. The effectiveness of such oxygen barriers will be limited until the oxygen content of the soil or groundwater is depleted. Limiting oxygen levels is easier to accomplish in ex situ (e.g., slurry reactors) than in situ applications. Please note, however, that some processes may be micro-aerophilic and will not work under strict anaerobic conditions. A number of papers are available describing anaerobic slurry phase processes.[10][28]

Anaerobic organisms can be facultative (organisms which can grow either in the presence or the absence of oxygen) or obligate (organisms which grow only under anaerobic conditions). Denitrifying bacteria are typically aerobic organisms which utilize nitrate as an electron acceptor in the absence of oxygen. Sulfate-reducing bacteria are strict anaerobes which utilize as electron acceptors either eletion

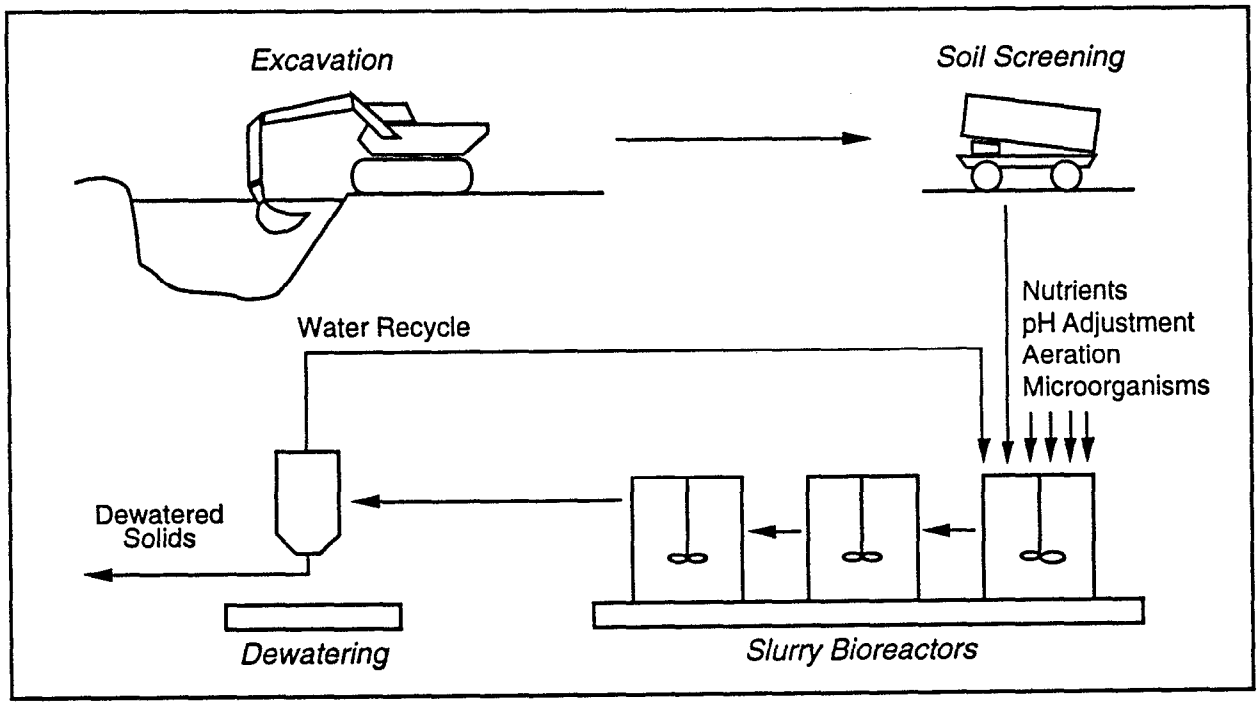


Figure 2-7. Above-ground slurry-phase bioremediation.

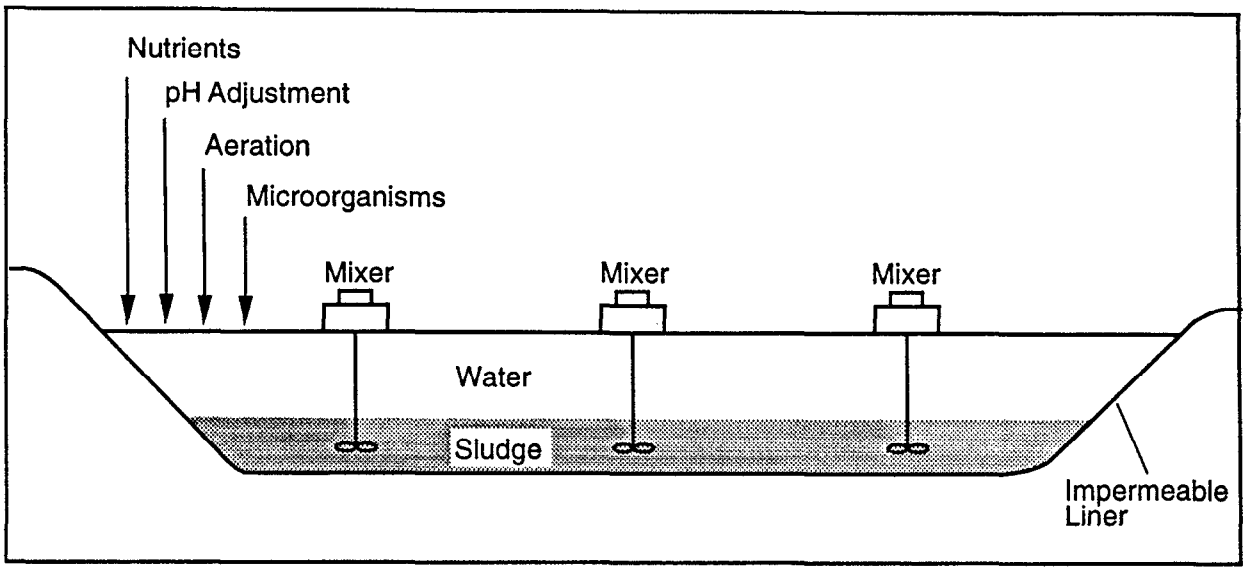


Figure 2-8. Slurry-phase bioremediation in existing lagoon.

mental sulfur or sulfur compounds. Methanogenic bacteria are obligate anaerobes which utilize carbon sources and produce methane gas. The following paragraphs discuss each of these groups of anaerobic organisms.

Facultative anaerobic microorganisms have the ability to grow in the presence or absence of oxygen. In the presence of oxygen, the organisms are able to use oxygen as the terminal electron acceptor. In the absence of oxygen, an alternative electron acceptor is utilized. Growth rates, biomass production, and metabolic rates are lower under anaerobic conditions. Alternative electron acceptors may be organic acids or inorganic molecules such as nitrate (in the case of the denitrifying bacteria). Generally, these organisms are heterotrophic in nature, and able to utilize a wide variety of carbon sources under aerobic or under anaerobic conditions. However, the pathways used and metabolic intermediates produced may differ under aerobic and anaerobic conditions.

Denitrifying bacteria utilize nitrate as an electron acceptor in the absence of oxygen.[62] The majority of these organisms are classified as aerobic bacteria, since they are primarily found in oxygen-containing environments. The ability of the denitrifying bacteria to grow under essentially anaerobic conditions allows the use of an additional pool of metabolic activities for bioremediation. These microorganisms express alternative pathways, in many instances, for the degradation of organic compounds under denitrifying conditions. For example, under either aerobic or denitrifying conditions, a species of Pseudomonas was able to utilize o-, m-, p-phthalates; benzoate; cyclohex-1-ene carboxylate; and cyclohex-3-carboxylate. However, m-hydroxybenzoate and p-hydroxybenzoate could only be utilized under denitrifying conditions. This allows the consideration of reactors that use both aerobic and denitrifying strategies to expand the range of compounds that are degradable by a given microbial consortium.

Sulfate-reducing bacteria utilize sulfate, elemental sulfur, or reduced sulfur compounds as electron acceptors. The product of these energy reactions is hydrogen sulfide (H_2S). The typical environments are mud and sediments, which are anaerobic, as well as the internal tracts of humans. These organisms utilize a variety of carbon sources, but many are not degraded to CO_2; that is, very few are mineralized. The potential value of these organisms may be in their ability to attack sulfur-containing compounds or in the treatment of sulfate- or sulfur-containing wastes.

The methanogenic bacteria are obligate anaerobic bacteria. They have been utilized by the waste treatment industry for a number of years. One group of these organisms is capable of using hydrogen and CO_2 for the production of methane. Another group can utilize acetate for the formation of methane. Generally, the methanogenic bacteria are found as part of a consortium composed of heterotrophic organisms, hydrogen-producing organisms, and the methanogens. The heterotrophic anaerobes degrade available organic carbon sources to CO_2 or acetate through a series of reactions involving a number of bacteria. This includes organisms that will reduce organic acids to CO_2 and acetate. The hydrogen- producing bacteria are essential to, and generally occur in close association with, the methanogens. The activity of the methanogens has been closely studied over the years because of the value as a fuel source of the methane produced.

2.2 PRELIMINARY SCREENING AND TECHNOLOGY LIMITATIONS

The determination of the need for and the appropriate level of treatability studies is dependent on available literature, expert technical judgment, and site-specific factors. The first two elements, the literature search and expert consultation, are critical to determining if adequate data are available or if a treatability study is needed for decision-making.

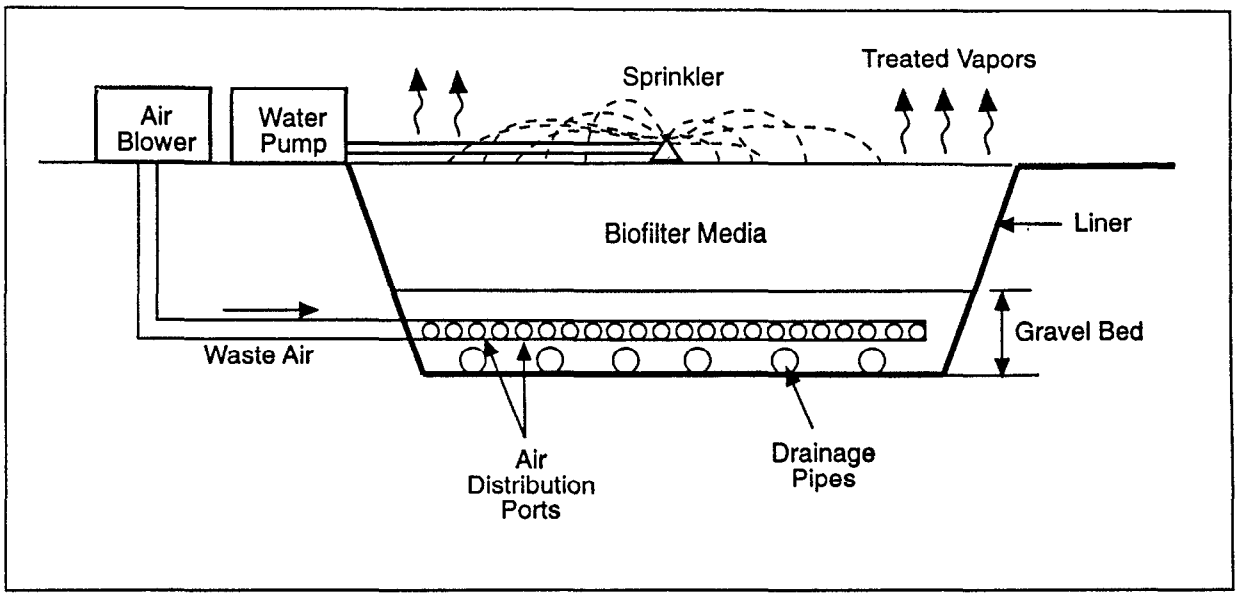


Figure 2-9. Earth biofilter treatment.

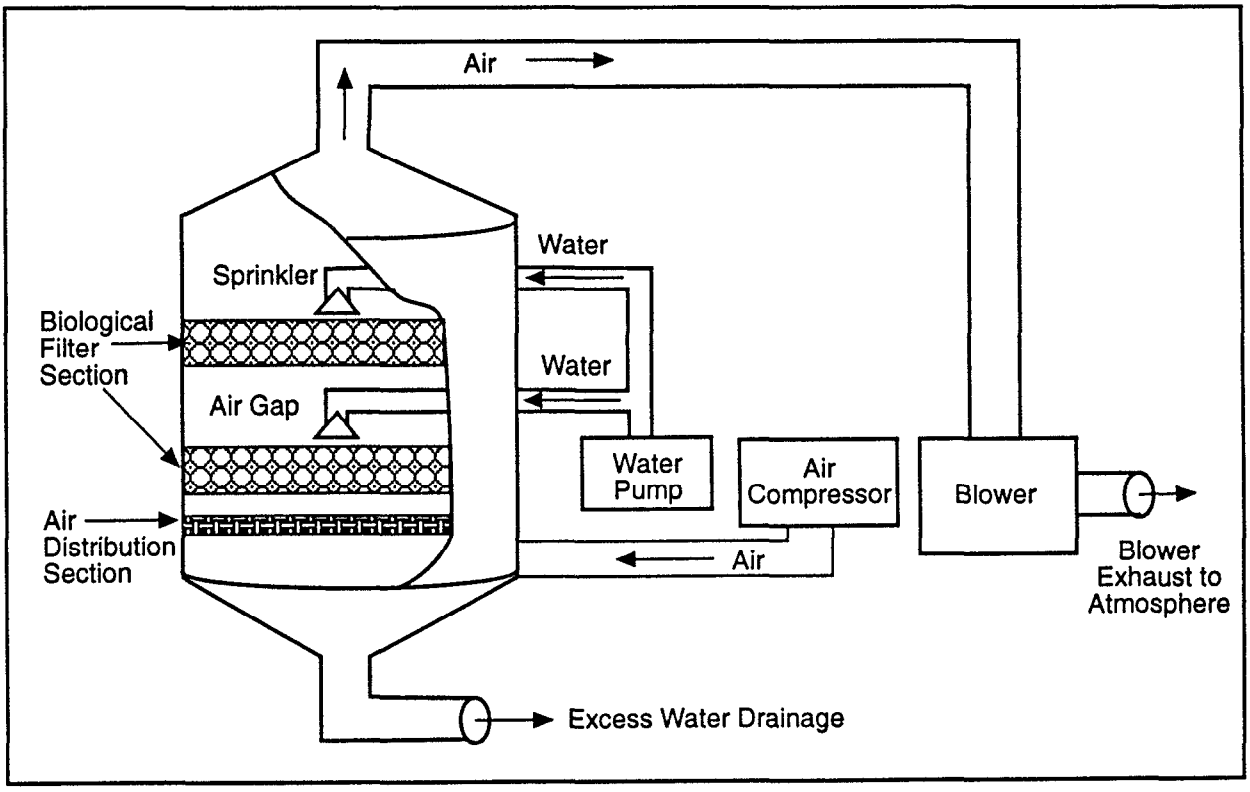


Figure 2-10. Biofilter/Biotower treatment.

2.2.1 Literature/Database Review

The following reports and electronic databases can be consulted when planning and conducting treatability studies and when prescreening bioremediation for use at a specific site. Existing reports include:

- Guide for Conducting Treatability Studies Under CERCLA, Final. U.S. Environmental Protection Agency. EPA/540/R-92/071a, October 1992.[52]
- Guidance for Conducting Remedial Investigations and Feasibility Studies Under CERCLA, Interim Final. U.S. Environmental Protection Agency. EPA/540/G-89/004, October 1988.[51]
- Guide for Conducting Treatability Studies Under CERCLA: Aerobic Biodegradation Remedy Screening, Interim Guidance. U.S. Environmental Protection Agency. EPA/540/2-91/013A, July 1991.[53]
- Superfund Treatability Clearinghouse Abstracts. U.S. Environmental Protection Agency. EPA/540/2-89/001, March 1989.[72]
- The Superfund Innovative Technology Evaluation Program: Technology Profiles Fifth Edition. U.S. Environmental Protection Agency. EPA/540/R-92/077, December 1992.[74]
- Summary of Treatment Technology Effectiveness for Contaminated Soil. U.S. Environmental Protection Agency. EPA/540/8-89/053, June 1990.[71]
- Inventory of Treatability Study Vendors, Volumes I and II. U.S. Environmental Protection Agency. EPA/540/2-90/003a and b, March 1990.[61]
- Bioremediation in the Field. U.S. Environmental Protection Agency. EPA/540/N-92/004. (Published Quarterly)[41]
- Bioremediation of Contaminated Surface Soils. U.S. Environmental Protection Agency. EPA/600/2-89/073, August 1989.[42]
- Innovative Treatment Technologies: Semi-Annual Status Report. U.S. Environmental Protection Agency. EPA/542/R-92/011, October 1992.[59]
- User's Guide for Land Treatment-Compound Property Processor and Air Emissions Estimator (LAND7). U.S. Environmental Protection Agency. EPA/540/3-87/026, November 1989.[76]
- Hazardous Waste Treatment, Storage, and Disposal Facilities (TSDF) - Air Emission Models. U.S. Environmental Protection Agency. EPA/450/3-87/026, November 1989.[56]
- Innovative Hazardous Waste Treatment Technologies: A Developer's Guide to Support Services, Second Edition. U.S. Environmental Protection Agency. EPA/540/2-91/012, June 1992.[57]
- Federal Remediation Technologies Roundtable. Federal Publications on Alternative and Innovative Treatment Technologies for Corrective Action and Site Remediation, Second Edition. U.S. Environmental Protection Agency. EPA/542/B-92/001, August 1992.[49]
- Innovative Treatment Technologies: Overview and Guide to Information Sources. U.S. Environmental Protection Agency. EPA/540/9-91/002, October 1991.[58]

Currently, RREL in Cincinnati, Ohio is expanding the RREL Treatability Data Base. This expanded database contains data from soil treatability studies. In addition, a repository for the treatability study reports will be maintained at the Water and Hazardous Waste Research Division of RREL in Cincinnati. Contact Glenn Shaul in the Toxics Control Branch of RREL at (513) 569-7408 regarding this database.

Robert S. Kerr Environmental Research Laboratory (RSKERL) in Ada, Oklahoma is presently developing the Subsurface Remediation Technology (SRT) Database, which will provide site-specific information concerning subsurface contamination and remediation activities currently being proposed or conducted at hazardous waste sites throughout the United States. The SRT Database will be available in early 1993 by way of an electronic bulletin board system (BBS) operated by RSKERL or via the local area networks (LANs) at the EPA Regional Offices. RSKERL has also developed a Soil Transport and Fate (STF) Database which presents information concerning the behavior of organic and inorganic chemicals in soil environments. This database is packaged with a Model Management System, which consists of the Vadose Zone Interactive Processes (VIP) Model and the Regulatory and Investigative Treatment Zone (RITZ) Model. Additional information on the SRT and STF Databases as well as the Model Management System can be obtained by calling Dr. David Burden at (405) 456-8500. His office is located in RSKERL's Center for Subsurface Modeling Support (CSMoS) of the Application and Assistance Branch.

The Office of Solid Waste and Emergency Response (OSWER) maintains the Cleanup Information (CLU-IN) BBS for communicating ideas and disseminating information and to serve as a gateway to other OSWER electronic databases. Currently, the CLU-IN BBS has eight different components, including news and mail services and conferences and publications on specific technical areas. The contact is Dan Powell, (703) 308-8827, of OSWER's Technology Innovation Office (TIO).

TIO has also developed the Vendor Information System for Innovative Treatment Technologies (VISITT) Database. This database contains information provided to TIO by technology developers, manufacturers, and suppliers regarding innovative technologies for hazardous waste site remediation. To obtain technical assistance or a copy of VISITT, call the VISITT Hotline at (800) 245-4505 or (703) 883-8448.

The Office of Research and Development (ORD) headquarters maintains the Alternative Treatment Technology Information Center (ATTIC), which is a compendium of information from many available databases. Data relevant to the use of treatment technologies in Superfund actions are collected and stored in ATTIC. ATTIC searches other information systems and databases and integrates the information into responses. It also includes a pointer system that refers the user to individual experts in EPA. The system currently encompasses technical summaries for the Superfund Innovative Tech-

nology Evaluation (SITE) Program, treatment technology demonstration projects, industrial project results, and international program data. Contact the ATTIC System Operator at (301) 670-6294 or access the database with a modem by calling (301) 670-3808.

Several other databases also provide information that may be useful during bioremediation remedy selection treatability studies. The Hazardous Waste Superfund Collection Data Base (HWSFD) contains bibliographic references and abstracts pertaining to the documents in the Hazardous Waste Superfund Collection at the EPA Headquarters Library. User support for this database can be obtained by calling (800) 334-2405. The National Technical Information Service (NTIS) Bibliographic Data Base is the largest single source for public access to federally-produced information. This database is available to the public through a number of commercial vendors including the following: BRS, (800) 345-4277; CISTI, in Canada, (613) 993-1210; DATA-STAR, (800) 221-7754; DIALOG, (800) 334-2564; ORBIT, in Virginia, (703) 442-0900, and in the rest of the U.S., (800) 456-7248; and STN International, (800) 848-6533. The Records of Decision System (RODS) is an online database containing the full text of the Superfund RODs for National Priorities List (NPL) sites nationwide. Contact the RODS Help Line (202) 260-3770 for assistance.[48]

Finally, the RREL Technical Support Branch is supporting a variety of treatability-related activities, including development of this guide and other technology-specific guidance documents, preparation of engineering bulletins, compilation of a list of vendors who perform treatability studies, and performance of treatability studies for EPA Regions.

2.2.2 Technical Assistance

Technical assistance can be obtained from the Technical Support Project (TSP) Team, which is made up of a number of Technical Support Centers (TSCs). It is a joint service of OSWER, ORD, and EPA Regions. The TSP offers direct, site-specific technical assistance to OSCs and RPMs and develops technology workshops, issue papers, and other information for EPA Region staff. The TSP:

- Reviews contractor work plans, evaluates remedial alternatives, reviews RI/FS reports, and assists in selection and design of a final remedy
- Offers modeling assistance, data analysis, and data interpretation
- Assists in developing and evaluating sampling plans
- Conducts field studies (soil gas, hydrogeology, site characterization)
- Develops technical workshops and training, issue papers on groundwater topics, and generic protocols
- Assists in performance of treatability studies

For further information on the TSP, contact:

Technology Innovation Office
Contact: Richard Steimle
(703) 308-8846

The following support centers provide technical information and advice related to biodegradation and treatability studies:

1. **Ground-Water Fate and Transport Technical Support Center**
Robert S. Kerr Environmental Research Laboratory
Ada, OK
Contact: Don Draper
(405) 332-8800

RSKERL in Ada, Oklahoma, is EPA's center for fate and transport research, focusing its efforts on transport and fate of contaminants in the vadose and saturated zones of the subsurface, methodologies relevant to protection and restoration of groundwater quality, and evaluation of subsurface processes for the treatment of hazardous waste. RSKERL provides technical assistance such as evaluating remedial alternatives, reviewing RI/FS and RD/RA Work Plans, and providing technical information and advice.

2. **Engineering Technical Support Center (ETSC)**
Risk Reduction Engineering Laboratory
Cincinnati, OH
Contact: Ben Blaney or Joan Colson
(513) 569-7406 or (513) 569-7501

ETSC provides technical information and advice related to treatability studies. The ETSC is sponsored by OSWER but operated by RREL; it handles site-specific remediation engineering problems. Access to this support center is available through the EPA site Project Managers.

RREL offers expertise in contaminant source control structures; materials handling and decontamination; treatment of soils, sludges, and sediments; and treatment of aqueous and organic liquids. The following are examples of the technical assistance that can be obtained through the ETSC:

- Review of the treatability aspects of the RI/FS
- Review of RI/FS treatability study Work Plans and final reports
- Oversight of RI/FS treatability studies
- Identification of alternative remedies
- Assistance with studies of innovative technologies
- Assistance in full-scale design and startup

3. **Emergency Response Team (ERT)**
Technical Support Center (TSC)
Office of Emergency and Remedial Response (OERR) Branch
Edison, NJ
Contact: Joseph Lafornara
(908) 321-6740

The ERT TSC is located at the OERR Environmental Response Branch in Edison, New Jersey. ERT provides technical expertise for the development and implementation of innovative treatment technologies through its Alternative Technology Section. The following are examples of the types of technical assistance that can be obtained through ERT:

- Consultation on water and air quality criteria, ecological risk assessment, and treatability study test objectives
- Development and implementation of site-specific health and safety programs
- Performance of in-house remedy screening and remedy selection treatability studies of chemical, physical, and biological treatment technologies
- Sampling and analysis of air, water, and soil
- Provision of onsite analytical support
- Oversight of treatability study performance
- Interpretation and evaluation of treatability study data

In addition to the TSCs, the Gulf Breeze Environmental Research Laboratory in Gulf Breeze, Florida provides technical assistance to EPA Regions. Research interests include biodegradation and bioremediation of pesticides, petroleum hydrocarbons, PAHs, and chlorinated solvents. Contact Rick Cripe at (904) 934-9261 for further information.

2.2.3 Prescreening Characteristics

Before a treatability study is conducted, a literature search should be performed to confirm whether the compounds of interest are known to be amenable to biological treatment. Evidence of biodegradation under dissimilar conditions, as well as data relating to compounds of similar structure, should be considered. If preliminary research indicates that bioremediation is a poor candidate for selection, further research may be warranted. Expert recommendations regarding the technology's potential should be obtained before eliminating bioremediation from further consideration. Caution should also be employed when reviewing studies demonstrating the degradation of pure chemicals. Chemical interactions or inhibitory effects of contaminants can alter the biodegradability of chemicals in the complex mixtures frequently found at Superfund sites. Particular attention should also be paid to degradation products, since they may be as toxic or more toxic than the parent compound. Studies reporting the disappearance of a specific compound as a measure of biodegradation can be misleading since in some instances disappearance may occur concomitantly with transformation to a more toxic compound. An example is the conversion of the relatively non-toxic herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) to the mutagenic compound 2,4,-dichlorophenol by a genetically engineered soil organism.[31]

Several documents and review articles that present detailed information on contaminant biodegradability are listed in Section 7, References. However, discretion should be exercised when using these reference materials, since conditions that allow the biodegradation of compounds traditionally considered nonbiodegradable are continually being discovered through ongoing research and development efforts. Examples of classes of compounds that are amenable to bioremediation include:[11][14][21][25][27][65]

- Petroleum hydrocarbons (e.g., gasoline and diesel fuel)
- Nonchlorinated solvents (e.g., acetone, ketones, and alcohols)
- Wood-treating wastes (e.g., creosote and pentachlorophenol)
- Aromatic compounds (e.g., benzene, toluene, xylenes, and phenols)
- Some chlorinated aromatic compounds (e.g., chlorobenzenes, biphenyl with fewer than five chlorines per molecule)

The literature search should also investigate the chemical and physical properties of the contaminants, particularly contaminant volatility, solubility, and biological availability (i.e., how strongly the contaminant is sorbed to the soil) in order to assess their impacts on contaminant removal. Information regarding site conditions and soil properties should be compiled. A partial list of site and soil characteristics that can impact bioremediation are presented in Table 2-2.[42] The physical/chemical parameters of the media should also be determined; these include, salinity, total organic carbon (TOC), oxygen availability, moisture content, temperature, available electron acceptors, and the presence and chemical state of metals, especially iron. When possible, data should be gathered from previous site characterization efforts. If the quality of these data is questionable, it may be necessary to perform preliminary testing. The utility of the data must be balanced against the testing costs and time considerations.

Furthermore, since biodegradation may not be able to reduce contamination to target levels within practical time frames, alternative technologies may be required to supplement biological treatment as part of a treatment train.

There is no steadfast rule that specifies when to proceed with remedy screening, when to eliminate biodegradation as a treatment technology, or when to proceed to remedy selection testing based on a preliminary screening analysis. An analysis of the existing literature coupled with the site characterization will provide the information required to make an educated decision. However, when in doubt, a remedy screening study is recommended. Several guidance documents are available to aid in determining the key contaminant and matrix characteristics that are needed to prescreen various technologies.[37][52][54] Example 1 is a hypothetical literature search provided to illustrate some of the complexities of this analysis.

Table 2-2. Site and Soil Characteristics Identified as Important in Biological Treatment

	In situ	Ex situ
Soil type	X	X
Extent of contamination	X	X
Soil profile properties		
Boundary characteristics	X	
Depth of contamination	X	
Texture*	X	
Structure	X	
Bulk density*	X	X
Clay content	X	
Type of clay	X	X
Cation exchange	X	X
Organic matter content*	X	X
pH*	X	X
Redox potential*	X	X
Hydraulic properties and conditions		
Soil water characteristic curve	X	
Field capacity/permanent wilting point	X	X
Water holding capacity*	X	X
Permeability* (under saturated and a range of unsaturated conditions)	X	
Infiltration rates*	X	
Depth to impermeable layer or bedrock	X	
Depth to groundwater, including seasonal variations*	X	
Flooding frequency	X	
Runoff potential*	X	
Geological and hydrogeological factors		
Subsurface geological features	X	
Groundwater flow patterns and characteristics	X	
Meterological and climatological data		
Wind velocity and direction		X
Temperature	X	X
Precipitation	X	X
Water budget	X	

* Factors that may be managed to enhance soil treatment.

2.2.4 Technology Limitations

Many factors impact the feasibility of biodegradation. These factors should be addressed prior to the selection of biodegradation and prior to the investment of time and funds in further testing. Some of these factors are discussed in this section. A detailed discussion of these factors is beyond the scope of this document. The reader should consult references 37, 42, 45, and 46, and others, for more information.

The physical form in which the contaminants are distributed within the media, as well as the amount, location, and extent of the contamination, can have a profound impact on the viability of bioremediation. In general, contaminants may be dissolved in the groundwater, adsorbed onto the soil, absorbed into the soil, or, depending on contaminant solubility and density, distributed as "free product" or non-aqueous phase liquid (NAPL). NAPLs can occur either on the top of the water table [e.g., light non-aqueous phase liquids (LNAPLs) or "floaters"] or at the bottom of the aquifer, against the bedrock or some other impervious geologic structure [e.g., dense non-aqueous phase liquids (DNAPLs) or "sinkers"]. The distribution of contaminant into these different phases is ultimately a function of their physical and chemical prop-

Example 1

Soil and groundwater at a chemical manufacture production plant are contaminated with trichloroethylene (TCE) beneath buildings and roadways at depths of 25 to 50 feet. The TCE plume is 600 yds in length, and TCE concentrations are between 100 and 6,000 parts per billion (ppb). The drinking water standard is 5 ppb. A literature review was performed to determine, whether biological treatment can reduce TCE to these levels.

Numerous papers in the academic literature show that TCE can be degraded in the presence of various co-metabolites; aerobically in the presence of aromatic compounds like phenol or gaseous alkanes like methane and propane, and anaerobically in the presence of various simple organic compounds like acetate or benzoate. In the papers, which appear to have adequate QA/QC, biological treatment has accounted for losses ranging from 30 to 99.5 percent in 2- to 60-day tests.

Although no full scale cleanups are on record, two well documented in situ pilot tests were found, one by a major university in conjunction with EPA and the other by a large environmental engineering firm. Both indicate positive results and recommend full-scale treatment as a viable option for those sites. For these reasons, the RPM decided that a remedy screening study to assess the feasibility of using biological treatment at this site was warranted. The RPM contacted several of the people involved in the first pilot test (the EPA oversight officer and the professor at the university) to seek suggestions on how to proceed.

erties and the hydrogeological and geochemical characteristics of the formation.

Variabilities in waste composition can cause inconsistent bacterial activity and, ultimately, inconsistent degradation. Heterogeneities such as debris, fill material, and geological anomalies (e.g., large clay lenses, rocks, and cavities) will influence air, water, contaminant movement, and excavation requirements. These formations can significantly impede in situ bioremediation activities by obstructing the transport of nutrients or oxygen to the contaminated media. Groundwater levels, contaminant depth, and the soil bearing capacity (as related to the soil's ability to support equipment) can also impact biological treatment. In combination, these parameters can determine whether the media requiring treatment are amenable to either in situ or ex situ bioremediation.

Soil characteristics, such as nonuniform particle size distribution, soil type, moisture content, hydraulic conductivity, and permeability, can also significantly affect biodegradation. Since organic contaminants tend to adsorb to fine particles such as silts and clays, variations in media composition and contaminant concentrations can lead to variations in biological activity and inconsistent degradation rates. The presence of significant quantities of organic matter (humus, peat, nonregulated anthropomorphic compounds, etc.) may also cause high oxygen uptake rates, resulting in depleted oxygen supplies during in situ applications. Low soil permeability can hinder the movement of water, nutrients, and oxygen through the contamination zone. Low percolation rates may cause amendments to be assimilated by soils immediately surrounding application points, preventing them from reaching areas that are more remote either vertically or horizontally. Often only ex situ remedial technologies are applicable to sites that contain low-permeability soils. This is true for both biological and nonbiological applications. Monitoring can be used to determine amendment fate. Amendment concentrations and application frequencies can be adjusted to compensate for physical/chemical depletion and/or high microbial demand. If these modifications fail to compensate for microbial demand, remediation may occur by a sequential deepening and widening of the active treatment layer (i.e., as the contaminant is degraded in areas near the amendment addition points, and microbial activity decreases due to the reduced substrate, the amendments move farther, increasing microbial activity in those areas).

Even in relatively permeable soils, ion exchange and filtration mechanisms can limit the movement and therefore the effectiveness of microbial and nutrient amendments. It may be necessary during treatment to improve the transport of water, electron acceptors, mineral nutrients, co-substrates, and microorganisms by controlled pumping or by other means. Care must be taken when performing the concomitant addition of electron acceptors and donors through injection wells. Excessive microbial growth or high concentrations of iron or manganese may cause clogging in the well screen or the soil pores in the immediate vicinity of the well screen.[60] Soils prone to oxygen transport limitations may be most appropriately treated using above-ground land treatment or reactor approaches. Although the above-soil characteristics significantly impact in situ treatment, they can also influence the viability of ex situ treatment, specifically materials handling and mixing requirements.

The presence of either an indigenous or introduced microbial population capable of degrading the contaminants of concern is essential to the success of biological processes. Although acclimated microbes have been known to tolerate very high concentrations of metals given long-term exposure, elevated levels of heavy metals, pesticides, highly chlorinated organics, and some inorganic salts may inhibit microbial activity. Other parameters such as contaminant concentration, pH, and temperature also affect microbial activity. In some instances, these characteristics can be controlled or modified through engineering practices. Metals may be leached or complexed to reduce microbial toxicity and improve the potential for treatment. Toxic effects may be addressed by dilution, pH control, metals control, (e.g.,

immobilization, volatilization, chelation, and washing), sequential treatment, or by employing microbial strains resistant to the toxicants. Physicochemical factors limiting biodegradation such as temperature, pH, water activity, electron acceptors, nutrients, and toxicity, must be addressed by either ameliorating the problem or by employing appropriate strains resistant to adverse conditions.

In general, the effectiveness of these engineering practices must be assessed on a site-by-site basis. Generally, system operation can be easier to control and sampling simpler to perform during ex situ applications. Particular attention should be paid to any negative side-effects that may occur. Examples of problems that may be encountered include the following:

- Surface active agents may be added during bioremediation to increase the bioavailability of poorly water-soluble or sorbed organic pollutants. If the soil-water partition coefficient of the target contaminant is less than 10, modifying the soil's capacity to retain water may cause soluble compounds to leach into the groundwater.
- Excessive nitrate formation, which may leach into the soil-water, may result from nitrogen addition.
- Some nitrogen fertilizers tend to change soil pH, necessitating further pH adjustment.
- By adding a carbon source to encourage the co-metabolic degradation of a specific compound, preferential degradation of the added substrate may inhibit the degradation of the compounds of interest.

Please note that each contaminant has a range of concentrations at which the potential for biodegradation is maximized. Below this range microbial activity may not occur without the addition of a co-substrate. Above this range microbial activity may be inhibited and, once toxic concentrations are reached, eventually arrested. During inhibition, contaminant degradation generally occurs at a reduced rate. In contrast, at toxic concentrations, contaminant degradation does not occur. The concentrations at which microbial growth is either supported, inhibited, or arrested vary with the contaminant, medium, and microbial species.

Contaminant volatility is particularly important, especially in stirred and/or aerated reactors where the contaminants can volatilize before being degraded. Example 2 illustrates how contaminant volatility impacts treatability testing and potentially limits the application of a biological technology.

Contaminant solubility should also be determined, since highly soluble compounds can leach from the soil before being degraded. Attention should be paid to contaminant mixtures that will behave differently from pure compounds. Interactions between the contaminants and the soil may affect the reported solubility, volatility, and partition coefficients of the pure compounds. Contaminant weathering may lead to binding in soil pores, which can limit availability even of reportedly soluble compounds.

Although preliminary data may indicate that the technology is capable of reducing contamination levels to acceptable limits, researchers are cautioned against stopping a study before site cleanup goals are met. Although the initial rate of removal after a potential lag period is generally rapid, with time this rate decreases to a near-zero value. As shown in Figure 2-11, the concentration (the asymptote) at which the contaminant removal rate is essentially zero represents, for all intents and purposes, the lowest cleanup concentration that be achieved during a remedial action. While additional contaminant removal may occur over a very long period of time, this typically non-zero concentration is the bioremediation end point from a practical perspective. Typically, this asymptote is a function of the following:

- Soil type - asymptote concentrations are higher in fine grained soils.
- Initial contaminant concentration - the higher the initial concentration, the higher the end point tends to be.

Example 2

A site contains soil contaminated with a mixture of VOCs and semivolatile organic compounds (SVOCs). A remedy screening shake flask study measured greater than 90 percent biodegradation of the VOCs and SVOCs. Solid-phase bioremediation was being considered for full-scale application at the site. However, concerns were raised regarding organic carbon volatilization during solid-phase treatment.

A remedy selection study was performed to determine the relative contribution of volatilization and biodegradation to the removal of the organic compounds. The study demonstrated that volatilization was the predominant mechanism for the removal of the VOCs and the low molecular weight SVOCs. Air stripping removed 99 percent of VOCs within 21 days. Biodegradation was the major process for destruction of the high molecular weight organic compounds and removed 88 percent of SVOCS within 100 days.

Based on the results of the remedy selection study, an RD/RA study of a slurry-phase process was scheduled. Biotreatment was selected to maximize biodegradation of both the VOCs and SVOCs using a slurry-phase process that included off-gas collection and recycling.

- The "age" of the contaminated soil - the longer the soil has been contaminated, the more "irreversible" the contaminant partitioning, the lower the contaminant bioavailability, and the higher the endpoint.

Since the asymptote is difficult to predict and is sometimes greater than cleanup criteria, treatability testing must be continued until either the removal goals have been met, the asymptote has been identified, or the allowable treatment time has been exceeded.

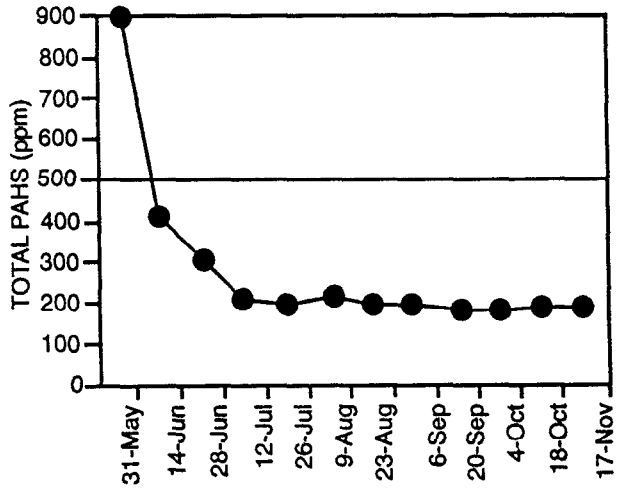


Figure 2-11. A graphic representation of the contaminant removal asymptote.

SECTION 3
THE USE OF TREATABILITY STUDIES IN REMEDY EVALUATION

This section presents an overview of the use of treatability studies in confirming the selection of biodegradation as the technology remedy under CERCLA. It also provides a decision tree that defines the tiered approach to the overall treatability study program with examples of the application of treatability studies to the RI/FS and remedy selection testing processes. Subsection 3.1 presents an overview of the general process of conducting treatability tests. Subsection 3.2 defines the applicability of each tier of testing, based on the information obtained, to assess, evaluate, and confirm biodegradation as the selected remedy. Subsection 3.3 provides an expanded description of the tiered approach to biodegradation treatability testing.

3.1 PROCESS OF TREATABILITY TESTING IN SELECTING A REMEDY

Treatability studies should be performed in a systematic fashion to ensure that the data generated can support the remedy evaluation process. This section describes a general approach that should be followed by RPMs, potentially responsible parties (PRPs), and contractors during all levels of treatability testing. This approach may include some or all of the following:

- Selecting a contracting mechanism *
- Issuing the Work Assignment *
- Establishing data quality objectives
- Preparing the Work Plan
- Preparing the SAP
- Preparing the Health and Safety Plan
- Conducting community relations activities
- Complying with regulatory requirements
- Executing the study
- Analyzing and interpreting the data
- Reporting the results

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* Tasks not performed by contractors.

These elements are described in detail in the generic guide, which provides information applicable to all treatability studies. It also presents information specific to remedy screening, remedy selection, and RD/RA testing.[52]

Treatability studies for a particular site will often entail multiple tiers of testing. Duplication of effort can be avoided by recognizing this possibility in the early planning phases of the project. The Work Assignment, Work Plan, and other supporting documents should include all anticipated activities.

There are three levels or tiers of treatability studies: remedy screening, remedy selection, and RD/RA testing. Some or all of the levels may be needed on a case-by-case basis. By balancing the time and cost necessary to perform the testing with the risks inherent in the decision (i.e., selection of an inappropriate treatment alternative), the level of treatability testing required can be determined. These decisions are based on the quantity and quality of data available and on other decision factors (e.g., State and community acceptance of the remedy and new site data). The flow diagram for the tiered approach, Figure 3-1, traces the data review process and the decision points and factors to be considered step by step.

Technologies are generally evaluated first at the remedy screening level and progress through remedy selection testing to the RD/RA tier. A technology may enter the process, however, at whatever level is appropriate based on available data on the technology and site-specific factors. For example, if the technology under study has been successfully applied at a similar site, a remedy screening study may not be needed to demonstrate potential applicability. Rather, treatability studies may progress directly to remedy selection testing to verify that performance standards can be met. It should be noted, however, that treatability studies, at some level, will normally be needed to ensure that the site target cleanup goals can be achieved. Figure 3-2 shows the relationship of the three levels of treatability study to each other and to the RI/FS process.

3.2 APPLICATION OF TREATABILITY TESTS

Before conducting treatability studies, the objectives of each tier of testing must be established. Biodegradation treatability study objectives are based upon the specific needs of the RI/FS. There are nine evaluation criteria specified in the document, Guidance for Conducting

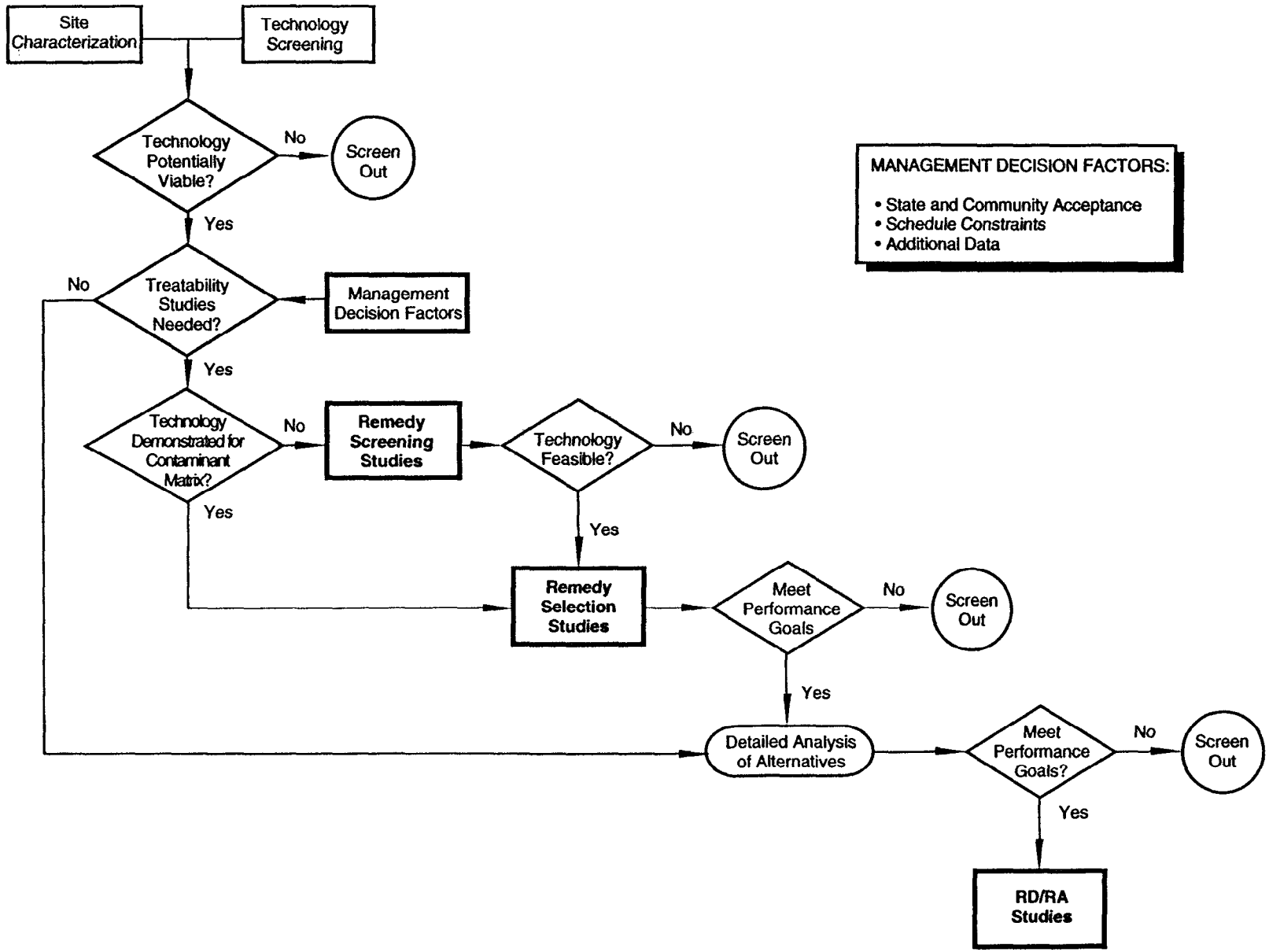

Figure 3-1. Flow diagram of the tiered approach to conducting treatability studies.

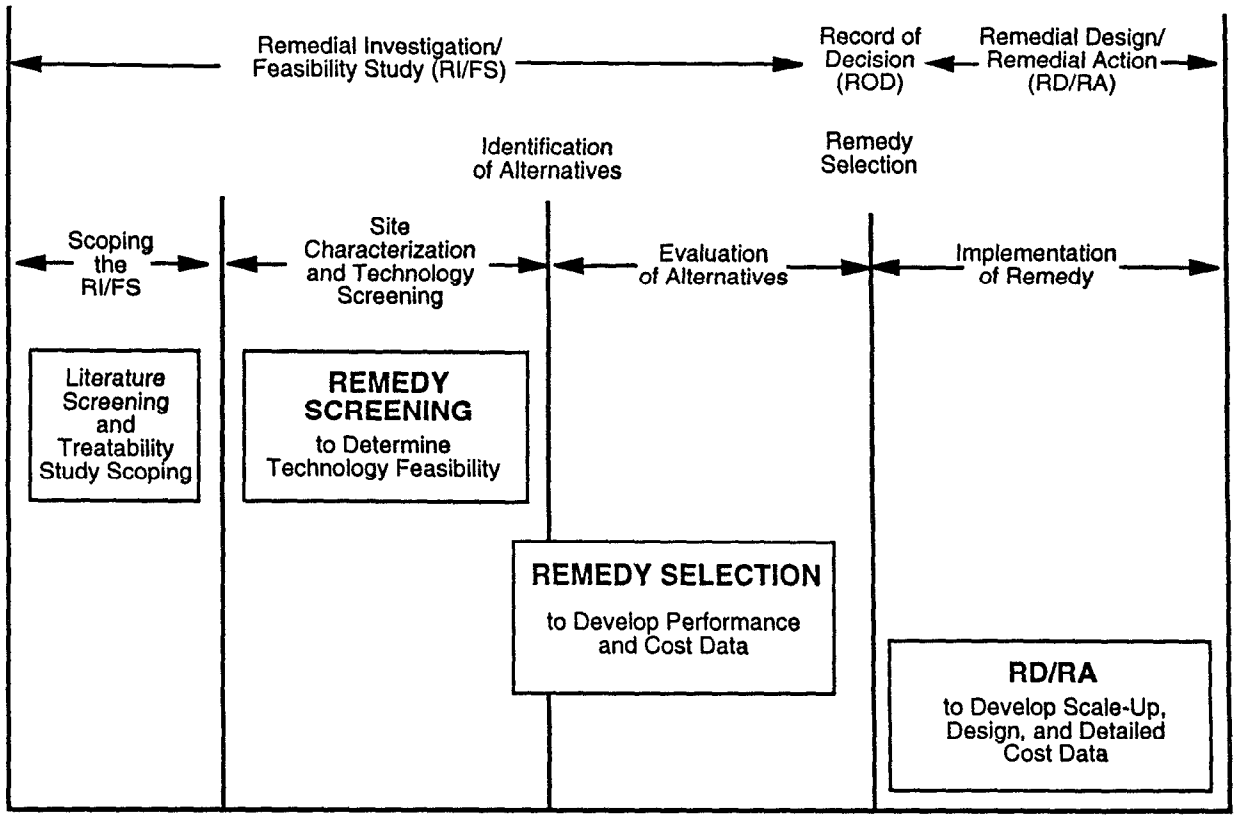

Figure 3-2. The role of treatability studies in the RI/FS and RD/RA processes.

Remedial Investigations and Feasibility Studies Under CERCLA (Interim Final).[51] A detailed analysis of different remedial alternatives using the nine CERCLA criteria is essential. Treatability studies provide data for as many as seven of these criteria. These seven criteria are:

- Overall protection of human health and the environment
- Compliance with applicable or relevant and appropriate requirements (ARARs)
- Long-term effectiveness and permanence
- Reduction of toxicity, mobility, or volume through treatment
- Short-term effectiveness
- Implementability
- Cost

The two remaining CERCLA criteria, State and community acceptance, are based in part on the preferences and concerns of the State and community regarding alternative technologies. A viable remediation technology may be eliminated from consideration if the State or community objects to its use. Although these criteria cannot be targeted for assessment during the treatability study, process data may be produced that address State and community concerns. Potentially this data may be used to change the State's or community's perception of the technology under study. The remainder of this subsection discusses the seven applicable criteria.

The first criterion, overall protection of human health and the environment, is used to evaluate how a technology, as a whole, can be used to protect human health and the environment. In previous years, cleanup goals often reflected background site conditions. Attaining background cleanup levels through treatment has proved impractical in many situations. The present trend is toward the development of site-specific cleanup levels that are risk-based rather than background-based. In situations where unique cleanup criteria have been designated as part of a site-specific risk assessment, the evaluation of a technology's ability to provide for "overall protection of human health and the environment" may be limited to assessing the technology's ability to attain targeted contaminant reductions. Often, however, the evaluation of this criterion draws on the technology's compliance with the other evaluation criteria, specifically long-term effectiveness and permanence; short-term effectiveness; compliance with ARARs; and reduction of toxicity, mobility, and volume through treatment. To assess whether the technology is capable of protecting human health and the environment, treatability study data from remedy selection testing must be obtained to help to answer the following questions:

- What will be the maximum remaining contaminant concentrations?
- Will the residual contaminant levels be sufficiently low to meet the established ARARs or the risk-based contaminant cleanup levels?

- What are the contaminant concentrations and physical and chemical differences between the untreated and the treated fractions (e.g., have contaminant toxicity, mobility, and volume been reduced)?

The second criterion, compliance with ARARs, ensures that the selected technology meets all of the relevant Federal and State ARARs (as defined in CERCLA Section 121) that have been identified in previous stages of the RI/FS process. ARARs may be categorized as chemical-specific requirements that define acceptable exposure levels and thus preliminary remediation goals (i.e., maximum contaminant levels); as location-specific requirements that set restrictions on activities within specific locations, such as floodplains, wetlands, or historic sites; and as action-specific requirements that set controls or restrictions for particular treatment and disposal activities related to the management of hazardous wastes (i.e., RCRA minimum technology standards).[51] Subsequent text addresses in detail limitation that two of the most common ARARs, LDRs and TSCA rulings have on the application and testing of biological technologies.

The LDRs for Newly Listed Wastes and Contaminated Debris Rule, promulgated on August 18, 1992, may limit the applicability of certain bioremediation technologies to certain sites. LDRs apply to hazardous wastes regulated by RCRA that are intended for land disposal. For each category of hazardous waste, the LDRs establish treatment standards that are either concentration-based (hazardous constituents must be reduced to a set concentration before the material is eligible for land disposal) or technology-based (material containing the listed hazardous waste must be treated by the designated Best Demonstrated Available Technology, or BDAT). LDRs generally become applicable as soon as hazardous waste is excavated. For RCRA corrective actions and CERCLA remediations, however, LDRs would not apply if the hazardous waste is treated in its original Corrective Action Management Unit (CAMU) or in a temporary unit (such as a bioreactor), which will be removed from the site following treatment.[40][41][47][51] In situ treatment (i.e., non-excavated) of hazardous waste also does not trigger LDRs.

The LDRs for Newly Listed Wastes and Contaminated Debris Rule was Phase 1 of a three-part regulation. Phase 2, LDRs for Newly Listed Waste and Contaminated Soil, is scheduled for issue in late summer 1993, while Phase 3 is scheduled for issue in March 1994. It is possible that bioremediation will be the BDAT standard for soils contaminated with certain chemicals, but until Phase 2 is promulgated, all treatment must either comply with the existing LDRs or seek compliance alternatives. The available compliance alternatives include the following:[41]

- Treatability variances (for wastes that are considered more difficult to treat than the waste on which the standard was based)
- No-migration petitions (which require demonstrating that the waste cannot migrate from the disposal location for as long as it will remain hazardous)
- BDAT exemption for groundwater reinjection (groundwater can be exempt from the LDRs if it is just pumped to the surface, amended, and reinjected)

Additional information can be obtained from the following sources:[41]

OSWER's TIO
Contact: Michael Forlini
703-308-8825

RCRA/Superfund Hotline
800-424-9346 or
703-920-9810 (from Washington, D.C.)

TSCA may also apply to certain applications of bioremediation. Genetically-modified microorganisms are currently regulated under TSCA Section 5 (1986), which is part of an interagency Coordinated Framework for Biotechnology.[9] Microorganisms are subject to premanufacture notification (PMN) reporting under TSCA Section 5 when they are intended for TSCA uses, which include bioremediation and other commercial applications.[40]

There are numerous circumstances under which microorganisms are exempt from regulation under TSCA Section 5. PMN reporting is required only for new microorganisms and does not apply to naturally-occurring microorganisms. In the 1986 policy statement, new microorganisms were defined as microorganisms that contain genetic material from organisms of different genera.[40]

New draft TSCA biotechnology rules entered EPA's Red Border review process on December 27, 1991. The draft rules propose that the definition of new microorganisms be changed to include only those microorganisms that possess deliberately-modified hereditary traits and are likely to exhibit new behaviors. The draft rules also propose exemptions for new microorganisms that fall into one or more of the following categories:[40]

- Test marketing.
- Common microorganisms that have a history of safe use.
- Microorganisms that are listed in the regulations and have met specific criteria regarding introduced genetic material and containment practices. This category includes Tier I exemptions (one-time certification of compliance must be obtained before the first use of the microorganism) and Tier II exemptions (request must be filed 45 days before the microorganism is manufactured or imported).
- Research and development activities in which the microorganisms are contained in a structure such as a greenhouse, a bioreactor, etc.

These new rules are still in draft form and are not likely to take effect before 1995.[40]

Future risks to human health and the environment are evaluated when determining the third criterion, the long-term effectiveness of a remedial action. The magnitude of any residual risk and the adequacy and reliability of controls must be evaluated. Residual risk, as applied to biodegradation, assesses the risks associated with the residual contaminants and metabolites or byproducts in the treated soil and groundwater at the conclusion of all remedial activities. When relatively toxic compounds or compounds with potential to be transformed into toxic

byproducts require treatment, a mass balance to assess mineralization using a radiolabeled compound may be appropriate. The volume, toxicity, and mobility of the residuals, as well as their propensity to bioaccumulate, should be determined during testing.

Since mineralization studies can provide evidence indicating that a biological process is capable of transforming the contaminants into benign endproducts, logically speaking, toxicity testing should not be considered unless the mineralization data demonstrate that the biological process is incapable of actually mineralizing the target compounds. The potential for long-term release of adsorbed contaminants from the treated soil matrix should also be addressed during biological treatability testing. If controls are needed to manage the residuals, data should be compiled during testing to help determine both the type and degree of long-term management to be employed. Long-term operation, maintenance, and monitoring requirements, as well as difficulties and risks associated with long-term application of a control, will need to be obtained in order to assess whether a control is suitable for long-term application. Attention should be paid to future site access restrictions and monitoring requirements. Such assessments are usually beyond the scope of a remedy selection treatability study, but may be marginally addressed based on remedy selection testing results.

During the assessment of the fourth criterion, reduction of toxicity, mobility, or volume through treatment, specific numerical data requirements are targeted, including (where applicable):

- What mass/volume of media was treated during the test?
- What were the contaminant removals experienced during treatment? What percentage can be attributed to biological removal mechanisms? How do these data compare to background levels for biological and nonbioloical removal mechanisms?
- Have the mass and mobility of the toxic contaminants been reduced, and if so, by how much? How do the mobility and toxicity of the leachate from the treated soil compare to the leachate from the untreated soil?
- Has the volume of toxic material been reduced, and if so, by how much?
- What residual contaminants and/or byproducts are left in the soil following treatment? What are the quantities and characteristics of these residuals? What are the risks associated with these contaminants?

Toxicity studies may need to be conducted on the treated and untreated media to determine toxicity reduction. Since toxicity studies measure a substance's effect on living organisms, they can also provide information regarding two other CERCLA criteria: overall protection of human health and the environment and long-term effectiveness and permanence. Toxicity studies are typically separated into two categories: environmental effects testing [77][82] and health effects testing.[80] Environmental effects testing measures toxicity to certain plants and animals, while the health effects testing is used to estimate toxicity to humans based on existing data and tests conducted on single and multicellular organisms. Several specific toxicity tests are briefly described in the compendium of tools presented in Appendix A. The design and interpretation of toxicity studies require consultation with trained professionals because of the inability to measure human toxicity directly and because a substance that is toxic to one organism might be more or less toxic to another.

The fifth criterion, short-term effectiveness, addresses the effects of the treatment technology from remedy design and construction through implementation and completion of response objectives. An estimated cleanup date may be projected from data obtained regarding residual contaminant concentrations in the soil. Risks faced by the community, workers, and the environment during the remedial action (e.g., uncontrolled contaminant volatilization during slurry bioreactor treatment) must be identified and appropriate controls evaluated.

The sixth criterion, implementability, evaluates the technical and administrative feasibility of an alternative. This relates to the availability of required goods and services as well as the technical feasibility of biodegradation at the site. Determining whether the contaminated soil is chemically and physically amenable to biological treatment under site-specific conditions is essential. The following questions must be answered in order to address the implementability of a bioremediation technology:

- What are the oxygen sources (i.e., electron acceptors) and nutrient availabilities of the site soils? Is supplementation possible? What are the costs and benefits associated with supplementation?
- Is in situ treatment practical, in view of site and soil characteristics, or do heterogeneities exist that would inhibit in situ biodegradation? If so, can the media be safely excavated for ex situ biodegradation?
- What is the water infiltration rate? Soil permeability? Ion exchange capacity? Is contaminant migration (through the air or groundwater) likely? To what depth does the vadose zone extend? Can issues regarding these parameters be resolved or addressed?
- What are the characteristics and quantities of contaminants that will remain after biodegradation? Is this concentration within project goals? Will an additional treatment mechanism need to be employed to meet project goals?
- What is the administrative feasibility associated with using this technology? Has it been used before within the Region? How quickly can it be approved for use? Will the State and local governments approve its use? Can existing time constraints be met?

Additionally, the implementability criterion evaluates whether vendors and process equipment are available to perform the remediation, if adequate space exists to perform treatment operations, and what materials handling problems might be encountered if soil must be excavated.

The final EPA evaluation criterion that can specifically be addressed during a treatability study is cost. RD/RA

treatability studies provide data to estimate the following important cost factors:

- The initial design of the full-scale unit
- The estimated capital, operating, and maintenance costs
- Initial estimate of the time required to achieve target cleanup levels, as reflected by operation and maintenance costs.

In some cases, remedy selection treatability studies can provide preliminary estimates of the same cost and schedule factors. However, in order to evaluate this criterion adequately, a conceptual design of the bioremediation system is needed and tradeoffs between capital and operating costs must be made. Additional treatment and disposal costs must also be considered. A properly designed biological treatment technology should produce either CO_2 and water or other relatively innocuous degradation products, thus reducing the possibility that process residuals will require additional treatment and disposal as hazardous or regulated wastes. However, certain technologies, particularly ex situ technologies, can be expected to generate residuals requiring some level of treatment and disposal. For example, aqueous and slurry-phase technologies frequently generate excess sludge (e.g., biomass), which requires treatment, dewatering, and disposal.

In general, most smaller-scale remedy selection studies only show that biodegradation can meet the required target concentrations under experimental conditions. The results of successful smaller-scale laboratory selection studies must be combined with soil characterization data and performance data from similar sites to evaluate the implementability of the technology at a specific site. Even after these steps are taken, there may be a high degree of uncertainty as to the ability of the technology to reach the contaminant target levels under field conditions in a reasonable time. As a result, larger-scale field studies are often recommended, particularly during the evaluation of an in situ bioremediation technology.

Table 3-1 shows how remedy selection treatability studies address seven of the nine criteria. The experimental parameters monitored during the study are chosen to provide data on the ability of the test to meet the study goals. Remedy selection treatability study goals and experimental parameters are discussed in Subsections 4.1 and 4.2, respectively.

3.3 BIODEGRADATION TREATABILITY TESTS

The following subsections describe the tiered approach to biodegradation treatability testing. Basic elements of each tier of testing are provided. A detailed discussion of remedy selection testing may be found in Section 4. Since this document is intended as guidance for remedy selection studies only, a more thorough description of the remedy screening and RD/RA studies is beyond the scope of this document.

It is important to note, that as more information is gathered regarding the application of a specific technology to certain types of contaminants, testing requirements will decrease.

3.3.1 Remedy Screening

Remedy screening is the first level of testing. It is used to determine whether biodegradation is possible with the site-specific waste material in question. These studies are generally low in cost (e.g., $10,000 to $50,000) and usually require 1 week to several months to complete. Additional time must be allowed for project planning, chemical analyses, interpretation of test data, and report writing. Only limited quality control (QC) is required. Remedy screening studies yield data indicating a technology's potential to meet performance goals. They generate little, if any, design or cost data and should not be used as the sole basis for selection of a remedy.

Typically, aerobic biological remedy screening studies are performed in test reactors containing saturated soil, unsaturated soil, soil slurries, and aqueous solutions. Studies employing simple shake flasks, soil pans, or slurry reactors are usually employed.[21][81] Normally pH, contaminant loading rates, and oxygen and nutrient availability are adjusted to increase the chances of success. These reactors may be small sacrificial batch reactors (approximately 40 mL to 1 L in size) or larger microcosms (1 to 10 L) that are subsampled. (Only a portion of the contents are removed at each sampling time to monitor the progress of biodegradation.) The microbial population can be either indigenous (e.g., acclimated or nonacclimated) to the site, selectively cultured, a proprietary mixture provided by a vendor, or any combination of the preceding. Inhibited controls are employed to account for abiotic removal during treatment. As an alternative, abiotic losses can be monitored directly. The goal of a screening level study is to determine whether biodegradation can occur. Since the ability of a technology to meet treatment goals is not the issue, it is usually not necessary to establish complete removal of the contaminant of interest. Thus, a reduction in contaminant concentration over a 3- to 6-week period of 20 percent (minimum) to 50 or 60 percent (corrected for nonbiological losses through photodecomposition, volatilization, adsorption, etc.) would indicate that biological treatment may be feasible.

Contaminant reductions and other criteria used to evaluate treatability study tiers are listed in Table 3-2. The information required to determine the success of each level of treatability study is also presented. While the criteria listed are not all-inclusive, they provide readers with a "yardstick" with which they can compare proposed treatability studies and verify that the appropriate tier is being investigated.

Example 3 illustrates the type of information that might result from a remedy screening study and the conclusions that might be drawn from that information. For more detailed information on remedy screening, please consult the biodegradation screening guide and EPA's Center Hill facility staff in Cincinnati, Ohio. RREL has recently developed a protocol for performing biological remedy screening studies at this facility. Information regarding these treatability studies may be obtained from Eugene Harris at (513) 569-7862.[69]

Table 3-1. Ability of Remedy Selection Treatability Studies To Address RI/FS Criteria

Study goals	Experimental parameters	RI/FS criteria*
Compare performance, cost, etc., of different treatment systems at a specific site	Dependent on type of treatment systems compared	• Overall protection of human health and the environment • Compliance with ARARs • Long-term effectiveness and permanence • Reduction of toxicity, mobility, and volume through treatment • Short-term effectiveness • Implementability • Cost
Measure the initial and final contaminant concentrations, and calculate the percentage of contaminant removal from the soil, sludge, or water through biodegradation	Contaminant concentration	• Overall protection of human health and the environment • Compliance with ARARs • Long-term effectiveness and permanence • Reduction of toxicity, mobility, and volume through treatment
Estimate the type and concentration of residual contaminants and /or byproducts left in the soil after treatment	Contaminant/byproduct concentration	• Overall protection of human health and the environment • Compliance with ARARs • Long-term effectiveness and permanence
Develop estimates for reductions in contaminant toxicity, volume, or mobility	Contaminant concentration, toxicity testing	• Reduction of toxicity, mobility, and volume through treatment
Identify contaminant fate and the relative removals due to biological and nonbiological removal mechanisms	Contaminant concentrations present in solid, liquid, and gaseous phases taken from test and control reactors, oxygen uptake/CO_2 evolution	• Overall protection of human health and the environment • Long-term effectiveness and permanence • Reduction of toxicity, mobility, and volume through treatment • Short-term effectiveness
Produce design information required for next level of testing	Temperature, pH, moisture, nutrient concentrations and delivery, concentration and delivery of electron donors and acceptors, microbial composition, soil characteristics, test duration, nonbiological removal processes	• Implementability • Cost
Develop preliminary cost and time estimates for full-scale remediation	Treatability study cost (i.e., material and energy inputs, residuals quality and production, O&M costs, where appropriate), test duration, time required to meet performance goals	• Short-term effectiveness • Implementability • Cost
Evaluate need for pretreatment and requirements for long-term operation, maintenance, and monitoring	Soil characteristics, contaminant concentration/toxicity	• Compliance with ARARs • Long-term effectiveness and permanence • Short-term effectiveness • Implementability • Cost
Evaluate need for additional steps within treatment train	Soil characteristics, contaminant concentration, nonbiological removal processes, residual quality (relative to further treatment and/or disposal requirements)	• Overall protection of human health and the environment • Long-term effectiveness and permanence • Implementability • Cost
Assess ability of bioremediation to meet site-specific cleanup levels	Contaminant concentration	• Overall protection of human health and the environment • Compliance with ARARs • Long-term effectiveness and permanence • Reduction of toxicity, mobility, and volume through treatment
Determine optimal conditions for biodegradation and evaluate steps needed to stimulate biodegradation	Temperature, pH, nutrient concentrations and delivery, concentration and delivery of electron donors and acceptors, microbial composition, soil characteristics, test duration, contaminant concentration	• Short-term effectiveness • Implementability • Cost

* Depending on specific components of the remedy selection treatability study, additional criteria may be applicable.

Table 3-2. Biodegradation Criteria for Each Treatability Study Tier

Criteria	Remedy screening	Remedy selection	Remedy design
Biodegradation of most-resistant contaminants of concern(COCs)	>20% net removal compared to removal in inhibited control	Meets cleanup standards under test conditions	Meets cleanup standards under site conditions
Initial contaminant concentration	Optimal for technology	Maximum concentration expected during remediation	Actual range of concentrations expected during remediation
Environmental conditions	Optimal for technology (include site conditions if possible)	Simulate expected site treatment conditions	Actual site treatment conditions for the specific technology
Extent of biodegradation	Estimate*	Quantify	Quantify
Biodegradation rate	Crude estimate*	Defensible estimate	Quantify
Estimate time to reach cleanup standards	NA	Estimate	Refined estimate
Mass balance	Crude*	Closure or defensible explanation	Closure or defensible explanation
Toxic byproducts	Detect*	Test for if appropriate*	Test for if appropriate
Process control and reliability	NA	Assess potential	Demonstrate
Microbial activity	Crude measure*	Verify/quantify*	Quantify/monitor*
Process optimization	NA	Estimate*	Refined estimate
Cost estimate for full-scale	NA	Rough, -30%, +50%	Detailed/refined
Bid specifications	NA	NA	Nearly complete
Experimental scale	Usually bench-scale	Either bench- or pilot-scale	Usually pilot- or full-scale

* Not required, although sometimes possible to address significantly.

Example 3

A former agricultural distributorship contained approximately 12,000 cubic yards of pesticide-contaminated soil, having combined concentrations of less than 200 parts per million (ppm) for 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyacetic acid (MCPA). The average combined concentration of 2,4-D and MCPA in the soil was 86 ppm. Regulatory cleanup requirements for the site were 10 ppm. A remedy screening study was performed to determine whether significant biodegradation could be achieved with a solid-phase bioremediation process. Soil microcosms, designed to simulate a full-scale solid-phase bioremediation system, were established to evaluate biodegradation. Initial and final contaminant concentrations, as well as microbial plate counts, were analyzed to assess performance.

The soil microcosm studies demonstrated that the naturally-occurring microorganisms in the soil could biodegrade the 2,4-D and MCPA, provided nutrient concentrations and moisture content were maintained within ranges conducive to biodegradation. The average combined 2,4-D and MCPA concentrations decreased from 86 to 5 ppm in 12 weeks, a decrease of 94 percent. The concentration of pesticides in the inhibited controls was reduced by 10 percent, indicating that biodegradation was the predominant removal mechanism. Based on the positive results from the remedy screening studies, a remedy-selection field-scale test was designed to determine whether bioremediation was capable of achieving the site cleanup levels under field conditions.

Please note that the biodegradation screening guide does not address anaerobic treatability testing. To accomplish this the reader must possess a basic understanding of anaerobic processes. (Note: anaerobic processes occur in an environment lacking in free oxygen but possessing alternative electron acceptors such as nitrate, carbonate, or sulfate.[43] Anaerobic conditions may either occur naturally or be established by modifying site or media characteristics. Further information on these processes can be obtained from various sources.)[68] Some important characteristics of anaerobic applications and testing are mentioned in Subsection 2.1.3 and Section 4 of this document. Experimental designs for anaerobic remedy screening studies can be developed by applying these considerations to the experimental designs described in the biodegradation screening guide. Other references may be consulted that provide additional information on anaerobic treatment and treatability studies.[38][43][68]

3.3.2 Remedy Selection

Remedy selection testing is the second level of testing. To the maximum extent practical, remedy selection tests should simulate site conditions during treatment, allowing researchers to identify the technology's performance on a waste-specific basis for an operable unit. These studies are generally of moderate cost (e.g., $50,000 to $300,000) and may require several weeks to 2 years to complete. They yield data that identify whether the technology is likely to meet expected cleanup goals and can provide information in support of the detailed analysis of the alternative (i.e., seven of the nine evaluation criteria). Toxicity testing of residual contaminants and intermediate degradation products may be necessary. Slurry-phase reactors, soil pans, or contained soil treatment systems are generally used to evaluate ex situ bioremediation technologies, while soil plots and soil columns (both within the laboratory and field) may be used to evaluate in situ technologies.

Throughout this document, the phrase "contained soil treatment" is used to describe treatability studies conducted on excavated soil in a treatment cell. These studies are typically larger-scale representations of compost piles, soil heaps, or land treatment systems. Contained soil treatment systems are constructed on a larger scale than soil pans but may be similar to soil pans in other respects. The other testing methods discussed in this document are considered self-explanatory. Further information on the basic characteristics of contained soil treatment and the other test methods is provided in Table 4-2.

Smaller-scale treatability studies using soil pans, small soil columns, and small slurry-phase reactors, are generally performed in the laboratory and may last from 1 week to 6 months, depending on the type of study employed. The media (i.e., soil, sediments) treated during these studies should be taken from the contaminated site. Due to the relatively small amounts of media tested during these treatability studies (refer to Table 4-2), operating parameters are relatively easy to control. While this makes it easier for researchers to approximate ideal operating conditions, it unfortunately makes it less likely that these studies will simulate actual site conditions during full-scale treatment, especially when evaluating an in situ technology. Studies performed to evaluate slurry reactors are the exception. These smaller-scale studies should be designed to achieve mass balance closure. In reality, results providing at least a semi-quantitative mass balance are usually acceptable for remedy selection. In general, they are less expensive than larger-scale field studies and typically cost from $50,000 to $150,000.

Larger-scale treatability studies using soil plots and contained soil systems are generally performed in the field and last from 2 months to 2 years. These studies typically cost $100,000 to $300,000 and are particularly appropriate for complex sites where in situ biodegradation is being considered. Generally, these studies are conducted onsite, preferably on a small portion of the area requiring remediation. Large soil column studies, on the other hand, are often performed in the laboratory. However, techniques to assess biodegradation using soil columns within the field are being developed. These buried columns will be able to examine microbial activity at isolated depths using remote sensing instrumentation.[66] Steps are often taken to isolate the media physically from the environment, thereby preventing possible contaminant migration. Although the design of treatability studies depends on the characteristics of the specific technology under analysis, these studies typically use techniques and equipment that are similar or identical to those used during full-scale remediation, enabling these studies to approximate full-scale treatment closely. These studies often provide detailed information that may be used to supplement RD/RA studies and can be used in the design of the full-scale treatment system.

Table 3-2 lists the type of information needed to determine the success of a remedy selection treatability study. Example 4 describes the type of information collected during a hypothetical remedy selection treatability study as well as the conclusions and interpretations made from that information.

3.3.3 Remedial Design/Remedial Action

RD/RA testing is the third level of testing. By operating a field unit under conditions similar to those expected during full-scale remediation, RD/RA testing can be used to:

- Provide the data required for final full-scale design
- Develop more accurate cost and time estimates for full-scale remediation
- Confirm biodegradation rates and cleanup levels determined during remedy selection
- Optimize unit operating parameters

These studies are of moderate to high cost (e.g., $100,000 to $500,000) and may require several months or more to complete. They should be performed during the remedy implementation phase of a site cleanup.

RD/RA tests usually consist of bringing a mobile treatment unit onto the site or constructing a small-scale unit for nonmobile technologies. The size and scope of the RD/RA test may be determined by several factors including the complexity of the process and the availability of equipment, test material, funds, and time. It is also critical that the RD/RA test equipment be sized so that realistic scale-up factors can be used for the transition to

Example 4

An abandoned refinery NPL site contains numerous pits holding approximately 60,000 cubic yards of waste contaminated with styrene tar and other organic materials. The site contains rubble and debris in the pits, posing significant materials handling problems. The contaminant of particular concern, phenanthrene, was detected at 500 ppm, significantly above the acceptable limit of less than 1 ppm. VOCs were detected at 300 ppm. Average initial contaminant concentrations in the soil treated during the treatability study were 36.3 ppm and 26.0 ppm for phenanthrene and VOCs, respectively. Although styrene tar is traditionally remediated by incineration, public resistance prompted an investigation into biological alternatives. Following a laboratory screening study demonstrating phenanthrene biodegradability, a remedy selection field demonstration was initiated. Final concentrations of less than 260 ppb and 5,800 ppb for VOCs and phenanthrene, respectively, were targeted. Site cleanup goals for phenanthrene were set at less than 1 ppm.

A pilot-scale, solid-phase air stripping and biological treatment facility was constructed to demonstrate the feasibility of bioremediating contaminated soils and organic residues. The treatment facility consisted of an enclosed, lined treatment bed containing 200 cubic yards of contaminated soil from a backfilled storage lagoon at the former refinery site. The liner was an 80-mil high density polyethylene (HDPE) synthetic membrane with heat-welded seams. A sand drainage layer was placed on top of the liner and a 6-inch layer of contaminated soil was placed on top of the sand. Nutrients were applied to the treatment bed through an overhead spray system. The treatment bed was tilled daily to increase soil surface area and provide aeration. Volatile emissions from the treatment bed were contained by a plastic-film greenhouse and routed to carbon adsorption units. Aerobic heterotrophic and phenanthrene degrading microorganisms were periodically assessed to determine microbial activity.

Sampling after 21 days of operation indicated that greater than 99 percent of the VOCs had been removed by air stripping. Samples collected after 94 days of operation demonstrated that an average of 89 percent of the SVOCs had been biodegraded. Phenanthrene concentrations were reduced an average of 84 percent. Phenanthrene had a half-life of 33 days, corresponding to approximately 130 days to reach the concentration approaching the analytical detection limit for phenanthrene (using EPA-approved procedures). This was a significant improvement in degradation rate over the 69 and 298 day half-lives reported in two previous studies, which were identified during the literature search. (These studies were performed at two different sites.) The data indicated that approximately 131 days would be required for the phenanthrene concentration to reach the analytical detection limit using the EPA-approved procedures. The study demonstrated that soils could be remediated using a combination of air stripping and bioremediation. Based on performance during testing, additional testing was recommended.

full-scale operation. If possible, the RD/RA equipment should be designed so that it can be readily converted to the full-scale remediation system. In some cases, RD/RA tests may be a continuation of remedy selection tests using the same apparatus. A complete mass balance, including all nonbiological pathways, should be performed at this level of testing. Typical testing periods are from 2 to 6 months. For more complex sites, for example sites with different types of contaminants in different areas or with different geological structures in different areas, longer testing periods may be required.

Given the limited availability of peer-reviewed published data on full-scale applications using innovative technologies, RD/RA testing will generally be necessary. Table 3-2 lists the type of information needed to assess the success of an RD/RA treatability study. Example 5 illustrates the type of information that might be collected during a hypothetical RD/RA study as well as the conclusions and interpretations made from that information.

Example 5

The manufacture and handling of explosives at U.S. Army industrial facilities has resulted in significant soil contamination. Previous remedy selection testing demonstrated the feasibility of using composting to remediate soils and sediments that had been contaminated with explosives [2,4,6-trinitrotoluene (TNT); hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX); and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetraazocine (HMX)] over a period of years. RD/RA testing was performed on soils from an Army depot site with 1,500 cubic yards of explosives-contaminated soil. Initial contaminant concentrations within the soil ranged from 200 to 3,700 ppm for TNT, RDX, and HMX combined. The average combined concentration in the soil/sediment treated during RD/RA testing was 1,700 ppm. A site cleanup goal of 100 ppm for total explosives was targeted. The study was designed to determine the maximum soil/sediment loading level, optimal amendments and process parameters, and the feasibility of using mechanically-agitated compost pile technologies. Individual laboratory studies were conducted for amendment selection and sample homogenization.

Four pilot-scale compost piles consisting of soiled livestock bedding material, livestock feed, hay, fertilizers, and explosives-contaminated sediments were constructed onsite. A process control/monitoring system was designed to control and record temperature, provide oxygen, and sample and analyze exhaust gas from each reactor for moisture and oxygen. Periodic sampling to determine explosives concentrations was also performed. Data were fed to a computer located in the site trailer. Two amendment selection tests and two soil loading tests were conducted. Different soil loadings were employed within each pile. Relatively small amounts of material were treated.

Results to date indicate extensive removal of TNT, HMX, and RDX at soil loading levels high enough to justify full-scale implementation. During treatability testing, bioassays were also conducted in addition to compound-specific analyses. These assays indicated that the toxicity reductions generally parallel TNT and RDX reductions. It is known that intermediates are formed in the degradation of explosives but the bioassays indicate that the intermediates are significantly less toxic than the parent compounds.

A mixture of 10 percent contaminated soil and 90 percent amendments proved optimal; a combined explosives concentration of 75 ppm was obtained, reflecting a removal of 96 percent. However, effective composting was achieved at soil loading rates up to 40 percent by volume. Materials handling requirements, operation and maintenance costs, material costs, and overall analytical requirements were evaluated. At full scale, it is estimated that treatment costs would be $250 per ton of contaminated soil. Materials handling requirements, operation and maintenance costs, materials costs, and overall analytical requirements were analyzed.

SECTION 4
TREATABILITY STUDY WORK PLAN

Section 4 of this document is written assuming that an RPM is requesting treatability studies through a Work Assignment/Work Plan mechanism. Although the discussion focuses on this mechanism, it can also apply to situations where other contracting mechanisms are used.

This section focuses on specific elements of the Work Plan for bioremediation treatability studies. These include test goals, experimental design and procedures, equipment and materials, sampling and analysis, data analysis and interpretation, reports, schedule, management and staffing, and budget. These elements are described in Subsections 4.1 through 4.9. Complementing these subsections are Section 5, Sampling and Analysis Plan, and Section 6, Treatability Data Interpretation, which address the sampling and data analysis elements of the Work Plan in greater detail. Table 4-1 lists all of the Work Plan elements.

Carefully planned treatability studies are necessary to ensure that the data generated are useful for evaluating the validity or performance of a technology. The Work Plan, prepared by the contractor when the Work Assignment is in place, sets forth the contractor's proposed technical approach for completing the tasks outlined in the Work Assignment. It assigns responsibilities and establishes the project schedule. It may also establish costs, although vendor costs may be considered confidential. The Work Plan must be approved by the RPM before initiating subsequent tasks. For more information on each of these sections, refer to the generic guide.[52]

Table 4-1. Suggested Organization of Biodegradation Treatability Study Work Plan

No.	Work plan elements	Subsection
1.	Project technology description	
2.	Remedial technology description	
3.	Test goals	4.1
4.	Experimental design and procedures	4.2
5.	Equipment and materials	4.3
6.	Sampling and analysis	4.4
7.	Data management	
8.	Data analysis and interpretation	4.5
9.	Health and safety	
10.	Residuals management	
11.	Community relations	
12.	Reports	4.6
13.	Schedule	4.7
14.	Management and staffing	4.8
15.	Budget	4.9

4.1 TEST GOALS

Setting goals for the treatability study is critical to the ultimate utility of the data generated. Goals appropriate to the tier of study must be defined before starting the treatability study. It is essential to consider how the different tiers of testing relate to and build upon each other when defining the study goals. Typically, remedy screening tests are used to determine if bioremediation is feasible with the site-specific waste material in question. The ability of a technology to meet treatment goals is not the issue during remedy screening. Since it is not usually necessary to establish complete removal of the contaminant of interest, data compiled at this level of testing are normally used to assess contaminant biodegradability. Remedy selection tests, on the other hand, are used to answer the questions, "Will biodegradation reduce contaminant concentrations to meet cleanup goals?" and "Can the contaminant be treated in a cost-effective manner?" As a result, remedy selection test goals are typically site-specific and may be based on cleanup levels, risk assessments, or other criteria. RD/RA testing is used to develop detailed design and cost data and to confirm the applicability of full-scale performance. Test goals at this tier emphasize process optimization, cost minimization, and the collection of specific design data.

Brief descriptions of remedy screening and RD/RA study goals were presented in Subsections 3.3.1 and 3.3.3, respectively. For an in-depth discussion of remedy screening goals, consult the biodegradation screening guide.[53]

4.1.1 Remedy Selection Treatability Study Goals

Remedy selection treatability study goals are based on current site contaminant levels and cleanup goals for soils, sludges, and water at the site. The ideal goals for a remedy selection treatability test are the cleanup criteria for the site. In previous years, cleanup goals often reflected background site conditions. Attaining background cleanup levels through treatment has proved impractical in many situations. The present trend is toward the development of site-specific cleanup levels that are risk-based rather than background-based.

For several reasons, such as ongoing waste analysis and ARARs determination, cleanup criteria are sometimes not finalized until the ROD is signed, long after treatability studies must be initiated. Nevertheless, treatability study goals need to be established before the study has begun in order to assess the study's success. In many instances, this may entail an "educated guess" as to what the final cleanup levels will be. In the absence of set cleanup levels, the RPM can estimate goals for the treatability studies based on the first four criteria listed at the beginning of Subsection 3.2. Previous treatability study results may provide the basis for an estimate of the treatability study goals when site cleanup goals have not been set. Cleanup goals can be based on regulatory requirements that do not account for the risk present at the specific site. Meeting standards can be expensive and time consuming. Studies can help project the time required to achieve the various target levels being considered.

Cleanup criteria directly relate to the final management of the material. They may dictate the need for complementary treatment processes to remediate the entire waste stream. For example, while biodegradation may be used to treat organics, a follow-on or pretreatment technology may be needed to treat metals and inorganics. Such combinations must be considered when planning the treatability studies and during the overall remedy evaluation phase. The development of graduated goals for contaminant reduction may fully address these complex needs. For example, if biodegradation can reduce soil contaminant levels to 100 ppm, no further treatment may be necessary. If, however, biodegradation can only reduce the contaminant level to 1,000 ppm, treatment with another technology may be required.

Data obtained during remedy selection testing should be used to assess whether a technology can meet site-specific cleanup levels. Consequently, testing should last until the contaminant concentration falls below the study cleanup goal or contaminant removal has leveled off and contaminant reductions cease to occur at a reasonable rate (i.e., the "asymptote"). To accomplish this, it may be necessary to extend the length of the treatability study. Often the removal asymptote associated with a specific matrix and technology is a function of the starting concentration. Therefore, in most cases,a soil sample containing the highest level of contamination expected at the site in question should be employed during remedy selection testing. It is important that the contaminant concentration not be so high that microbial activity is inhibited. In the event the maximum concentration is representative of only a minor portion of the media being treated, treatability studies using soils with "average" concentrations may be more appropriate. Treatability studies using average-concentration soils are also appropriate if the soil will be diluted during treatment (i.e., slurry treatment).

Ideally, a preliminary full-scale design and cost analysis will be conducted prior to the remedy selection treatability study. This preliminary analysis will indicate the parameters of particular importance in the optimization and evaluation of the technology. The degree to which the study "mimics" the proposed technology, the quality and reliability of the data and its interpretation will be significantly impacted. Thus, studies that closely simulate field conditions will provide the most reliable information about a technology. Specific goals of the remedy selection tier of testing are:

- Measure the initial and final contaminant concentrations in the media and calculate the percentage of contaminant removal from the soil, sludge, or water attributed to biodegradation
- Determine the type and concentrations of residual contaminants and/or byproducts left in the soil after treatment
- Estimate reductions in contaminant toxicity, volume, or mobility
- Identify contaminant fate and the relative removals due to biological and nonbiological removal mechanisms
- Produce the design information required for the next level of testing, in the event RD/RA studies are warranted
- Develop preliminary cost and time estimates for full-scale remediation
- Evaluate the need for pretreatment prior to biological treatment (e.g., add bulking agents prior to composting or remove oversize particles prior to slurry-phase treatment), as well as long-term operation, maintenance, and monitoring requirements
- Evaluate the need for additional steps within the treatment train (e.g., soil washing to remove metals, soil vapor extraction to remove VOCs prior to ex situ bioremediation)
- Assess the ability of bioremediation to meet the cleanup levels for a specific site
- Determine optimal conditions for biodegradation and evaluate the steps needed to stimulate biodegradation (e.g., nutrient addition, surfactant addition, cultured microbial populations)
- Compare the performance, cost, feasibility, timeliness, permitting requirements, etc., of different treatment systems at a specific site

Toxicity reduction may also be an important goal in some remedy selection treatability studies, especially if this parameter has been identified as a cleanup criterion for the site. Toxicity reduction can be demonstrated by performing of toxicity tests on the treated and untreated media. Toxicity testing may also be used to establish test goals. Information on specific toxicity tests is provided in Appendix A.

Example 6 is provided to demonstrate typical goals for a remedy selection study as well as the type of decision that can be made when these goals are achieved.

4.2 EXPERIMENTAL DESIGN AND PROCEDURES

Careful planning during the design of a treatability study is required to ensure that appropriate data are obtained. The experimental design must identify the critical parameters and determine the required number of replicate tests. This subsection discusses the different elements remedy selection treatability study design. A brief description of remedy screening and RD/RA studies, addressing goals, design, and purpose, can be found in Subsections 3.3.1 and 3.3.3, respectively.

The information presented in this subsection is intended merely as a guideline or starting point. Because remedy selection treatability studies are site- and contaminant-specific, this information should be modified, as necessary, for a given site. Subsection 4.2.1 presents an overview of remedy selection experimental design. It is beyond the scope of this document to go into great detail on statistical experimental design, but useful texts on the subject are available.[6][24]

A number of factors commonly influence the basic design and operation of biological studies. These factors have a profound impact on both treatability study operation and utility. Important factors to be considered when designing a biological treatability study include the following:

- Moisture
- Nutrients
- Electron acceptors (e.g., oxygen, nitrate, sulfate)
- Microorganisms
- Duration of test
- Inhibitory compounds and their control
- Impact of nonbiological removal processes (e.g., volatilization, sorption, photodecomposition, leaching)
- Toxicity testing
- Bioavailability

Readers are referred to Subsection 4.1 for a discussion of treatability study objectives and specific removal goals. Brief discussions of other factors important for the design and operation of biological studies are included in Subsections 4.2.2 through 4.2.13. Within these subsections references are made to optimizing study parameters in order to maximize performance. It is important to stress that the intent is to maximize performance under achievable field conditions in a cost-effective manner in order to achieve intended results. Subsections 4.2.14 and 4.2.15 discuss design and operational parameters unique to treatability studies for in situ and ex situ technologies, respectively. Although each method is mentioned singly, using a combination of different testing methods at the laboratory and/or field scale may provide a more accurate, cost-effective assessment of the technology's capabilities at the remedy selection level. For example, although large-scale field applications reliably mimic full-scale applications, it may be easier and more cost-effective to use laboratory-scale testing to determine the effects mixing patterns, treatment coverage, transport processes, temperature, and pH have on biodegradation rates. Furthermore, depending on the technology and site under study, one study alone may not be able to provide sufficient information to select a technology reliably.

The guidance provided in the referenced subsections is primarily designed for aerobic treatability studies; however, with some modifications, this guidance can also be applied to anaerobic treatability studies. Subsection 2.1.3 provides an overview of common types of anaerobic organisms encountered.

Example 6

A remedy-selection laboratory study was performed to determine whether biodegradation could be used to remediate PCP-contaminated soil from a wood treatment facility (i.e., a pole yard). Since PCP is known to be amenable to biotreatment at concentrations less than 500 ppm, the RPM was able to bypass remedy screening testing. The object of the remedy selection study was to determine the rate and extent of PCP biodegradation achievable using solid- and slurry-phase treatment processes. Small soil pan and slurry phase studies designed to simulate full-scale processes were established, as were inhibited controls to measure the effect of abiotic processes on PCP removal. The average PCP concentration in the soil was 100 ppm, which was representative of site conditions.

The studies demonstrated that both solid- and slurry-phase processes could be used to biodegrade the PCP effectively. However, the rate and extent of biodegradation achievable was greater with slurry- rather than solid-phase processes. Ninety percent of the PCP was removed within 4 weeks with the slurry-phase processes. Sixty percent of the PCP was removed within 12 weeks with the solid-phase process. An additional 8 weeks was needed to remove 90 percent of the PCP during the solid-phase study. Abiotic processes did not contribute significantly to the removal of PCP.

Based on the results of the remedy selection study and the need for rapid cleanup, RD/RA slurry-phase testing was performed to provide the data required to design and implement a full-scale slurry-phase remediation process.

4.2.1 Remedy Selection Experimental Design

In formulating an experimental design, the total number of samples taken depends on the desired difference in concentrations that the experimenter wishes to detect, the measurement variability (the analytical coefficient of variation), and Type I and II error probabilities. The probability associated with a Type I error reflects the chance that the experiment will indicate that there is a statistically significant treatment effect when, in reality, none exists (false positive). Conversely, the Type II error probability is the chance of not detecting a significant treatment effect when, in reality, the treatment is effective (false negative). Traditionally, experimental designs have been constructed so that these error probabilities are on the order of 5 percent (e.g., 95 percent confidence levels).

Replicate systems or replicate subsamples (at least duplicate and preferably triplicate) are recommended for all remedy selection treatability studies to ensure reliable data. Replicate samples are used to measure overall analytical precision and should be performed for approximately 10 percent of the samples analyzed. Matrix spikes are used to assess the accuracy (the agreement between the analytical result and the actual compound concentration) of analytical data. Matrix spikes are known concentrations of target analytes added to a sample of soil, water, sediment, or air (the sample matrix) prior to sample preparation and analysis. Matrix spikes are used to evaluate sample bias (the effect the matrix has on the ability to detect the target analytes accurately). Surrogate spikes (compounds similar to the target analytes in chemical composition and behavior, but not normally found in the environmental samples) are also used to measure accuracy during organic compound analyses.

Equipment rinsate, trip, and method blanks are used to assess the potential for sample contamination from equipment during sample collection/preparation, during sample handling and shipping, and arising from sample processing during analytical testing, respectively. Further information on quality assurance can be found in Test Methods for Evaluating Solid Waste[73] and Data Quality Objectives for Remedial Response Actions.[44] In general, the analytical variability associated with soil and sludge sampling and analysis can be quite high (on the order of 20 to 50 percent). Therefore, a sufficient number of samples must be taken for statistically significant effects to be observed. Additional information on sample size selection is available in many statistics textbooks.[6][18][24]

Remedy selection treatability studies range from small laboratory studies employing soil pans, slurry-phase reactors, or soil columns, to relatively large field applications utilizing small plots of land (field plots) or contained soil systems. Generally, slurry-phase reactors, soil pans, and contained soil treatment systems are used to evaluate ex situ bioremediation technologies, while soil columns and field plots are more commonly used to evaluate in situ technologies. Ultimately, remedy selection studies should strive to simulate the conditions encountered during full-scale applications of the technology under study.

The size of equipment used in remedy selection testing is influenced by a number of factors, including the following:

- The amount of time and money available for testing
- The uncertainty associated with the technology
- The number of technologies being tested (as related to space, cost, and time restrictions)

The test system used during remedy selection testing can consist of a single large reactor or multiple small reactors. Studies that employ large reactors include field studies, large flask studies, and soil pan studies. Multiple reactors consisting of serum bottles, small slurry reactors, and small soil reactors may be set up in place of a single large system. It is typically expensive and time-consuming to use field-scale equipment to conduct remedy selection testing, particularly if numerous technologies are being considered. It may also be easier to examine the effects of mixing patterns, transport processes, temperature, pH, and nutrient addition in laboratory-scale equipment. Field studies, however, usually provide the best approximation of full-scale performance. These studies can also estimate the environmental impact and cost with a higher level of certainty. All of these considerations will influence the size and scale of the system selected for a remedy selection study.

4.2.2 pH

Most microorganisms thrive within a neutral range (pH between 6.5 and 8.5). However, many acidic or alkaline soils support a viable microbial population capable of degrading the contaminants of interest. The indigenous microbes within these soils may have evolved to the point where they cannot survive or are inhibited at a different pH. If pH adjustment is required to optimize a particular microbial population, additives such as hydrochloric acid, potassium hydroxide, lime, or buffer solutions may be used during treatability testing. The amount of acid or base added to a soil sample during testing varies with the buffering capacity of the soil. Care must be taken to ensure that the addition of amendments does not inhibit biological activity. Furthermore, the pH has a profound effect on abiotic contaminant reactions within the soil. Depending on the specific characteristics of the soil, changes may cause materials (i.e., metals) within the soil to precipitate and may increase the mobility of hazardous contaminants present in the soil. Alternatively, a change in pH may cause the contaminant to become strongly sorbed to the soil, thus inhibiting degradation. Consequently, although a neutral pH will generally enhance microbial activity, pH adjustment should not be employed unless an associated increase in the biodegradation rate is first demonstrated, and only if the pH control is deemed feasible during remediation. In situations where biodegradation is limited by an extreme pH (i.e., less than 2), additives may be used to adjust the medium's pH.

4.2.3 Soil Characteristics

Soil and contaminant heterogeneity can significantly impact the quality of the data generated and therefore must

be considered when designing a study. In general, as long as the test results are not compromised, the media may be homogenized to address heterogeneous characteristics. However, it may not be appropriate to use homogenized media when obtaining specific types of data pertaining to in situ biodegradation. Alternatively, the number of replicate samples taken may be increased to account for soil heterogeneity. For small reactors, where the entire contents are sacrificed at a sampling time, more replicate reactors should be prepared. For large reactors, where only a portion of the contents are removed at each sampling time, multiple samples from the reactor should be taken. Large reactors must be sized accordingly, so that removal of multiple samples does not adversely affect the processes taking place in the reactor.

4.2.4 Temperature

The temperature of the medium should be routinely monitored during testing in order to assess its impact on system performance (e.g., removal rates). Depending on the type of study being performed and the technology under consideration, temperature control may be required in order to optimize biodegradation. The optimum temperature for biodegradation depends on the microorganisms present but is usually between 15° and 30° C for aerobic processes and 25° to 35° C for anaerobic processes. Temperature control may be difficult in large-scale treatability studies, particularly those utilizing in situ systems. Although groundwater and subsurface soil temperatures do not significantly change throughout the year, some in situ studies performed on contaminated media above the frost zone may experience marked decreases in removal rates during the colder seasons. Temperature control techniques utilized during in situ treatability studies include covering the treatment area, blowing heated air through tunnels in the treatment area, installing in-ground heaters, and percolating heated water through the media. Vegetation can provide a cover to prevent the surface soil from heating in the summer and to act as insulation to reduce heat loss in the winter.[54]

4.2.5 Moisture

Moisture levels are also routinely monitored and modified during treatability testing in order to assess the impact moisture content has on system performance. It is generally desirable to maintain the soil moisture level between 40 to 80 percent of field capacity for solid-phase aerobic treatability studies; however, the actual range employed during testing depends on the nature of the medium under treatment and the operational characteristics of the technology under study. During solid-phase anaerobic treatability studies, the treatment area may be flooded to help to maintain anaerobic conditions. Moisture availability is not a concern for slurry-phase treatment, since surplus water is available.

4.2.6 Nutrients

Nutrient availability is frequently a limiting factor during biological treatment. As a result, nutrient amendments are commonly employed during bioremediation and biological treatability studies. The nutrients most frequently added are nitrogen (e.g., ammonia nitrogen) and phosphorus (e.g., phosphate). Organic nitrogen may be required by some organisms. Protein supplementation has also been shown to increase the degradation of heavy oils. Nitrogen must be added cautiously in order to avoid changing the soil pH and to prevent groundwater contamination due to excessive nitrate formation. Supplemental carbon sources (glucose, acetate, citrate, and corn starch solutions), inorganics (micronutrients, mineral salt, and ammonia salt solutions, etc.), and/or vitamins may also be provided. Agricultural fertilizers and products, such as alfalfa, blood meal, wild rice hulls, and manure, are also common. Carbon to nitrogen ratios may range from 100:0.5 to 100:7.0, while carbon to phosphorus ratios may range from 100:0.1 to 100:1.0. Depending on the site and technology under consideration, nutrient ratios may be determined based on initial TOC as an indication of carbon content. (Note: accurate carbon mass determinations are difficult to obtain with highly heterogeneous soils.) These ranges are merely guidelines; optimum nutrient conditions are site-specific. In general, nutrient concentrations should be monitored and maintained at some reasonably moderate but steady state concentration determined experimentally. Biodegradation in one or more systems with nutrient addition can be compared to the biodegradation in one or more systems without nutrient addition.

Soil water can be monitored for ammonia (NH_3), phosphate (PO_4), nitrate (NO_3), and nitrite (NO_2) in order to determine whether additional augmentation is required. Alternatively, amendments can be added when biological activity slows down. During soil plot studies, it may be beneficial to monitor nutrient concentrations in groundwater obtained from both up-gradient and down-gradient locations.

4.2.7 Electron Acceptors

Oxygen is the most common terminal electron acceptor for aerobic microorganisms. Oxygen availability is also a common limiting factor for biological treatment. Oxygen addition methods vary widely, particularly between different types of treatability studies. During small-scale slurry-phase studies, oxygen is typically transferred from the headspace into the slurry by shaking or mixing. Oxygen addition in larger slurry-phase systems typically utilizes diffusers or aerating mixers. Ex situ solid-phase systems (soil pans or contained soil treatment systems) typically obtain oxygen from mixing or tilling. In situ systems are typically provided with oxygen through the injection of liquids such as water with high dissolved oxygen (DO) levels or hydrogen peroxide or through forced aeration systems such as bioventing. Air, oxygen, hydrogen peroxide, and nitrate amendments may be employed. Gas injection or infiltration of water containing these oxygen sources may be further enhanced by introducing microscopic bubbles of gas (gas aphrons) into the soil at levels greater than their solubility limits. Treatability study data demonstrate that the soil retains the gas aphrons longer than air or other gases directly injected into the soil; however, studies pertaining to the full-scale application of aphrons have not been identified. Gas aphrons are best suited for sandy soils.[54]

Oxygen requirements cannot be calculated from the con-

taminant concentrations because naturally-occurring organics and inorganics will also be degraded and will therefore contribute to the oxygen demand. Oxygen uptake rates and the oxygen content within soil pore water (i.e., DO) should be monitored to assess oxygen requirements. Oxygen consumption data collected during remedy selection testing will be used to design the oxygen supply system. Generally, oxygen consumption is easier to monitor in closed reactor systems.

When evaluating whether to employ percolation techniques to introduce aqueous amendments to the vadose zone during the aerobic biodegradation of contaminated surface water, groundwater, or soil, it is important to estimate the amount of oxygenated water that will be required to mineralize the contaminants (and other carbon sources) at the site. Rough calculations can be made by remembering the following relationships: 1) the maximum solubility of oxygen in water is approximately 8 mg/L at 20°C; and 2) the complete mineralization of one pound of hydrocarbon (e.g., hexane) stoichiometrically requires approximately 3 pounds of oxygen. The resulting estimate can be used to verify whether sufficient oxygen will be present, similar to estimates of BOD or COD.

Non-oxygen electron acceptors, such as nitrate, sulfate, or carbonate, can be used singly or in combination to enhance anaerobic biodegradation. The type of electron acceptor employed depends on the class of anaerobe responsible for contaminant degradation (facultative anaerobic, sulfate-reducing, methanogenic, and denitrifying bacteria). Subsection 4.2.16 lists a number of common electron acceptors according to types of anaerobes that utilize them.

4.2.8 Microorganisms

Nutrient addition, temperature control, pH control, etc., are generally performed in order to encourage the growth of either an indigenous or introduced microbial population capable of biologically degrading the contaminants of concern. Usually an indigenous population exists in the medium, which has already developed the ability to utilize the contaminants of concern. The purpose of biological testing and remediation is to modify any conditions that have impeded the growth of these microbes and maximize their ability to degrade the contaminants of concern. The metabolic diversity of the naturally-occurring microbial community should be determined. Microbes capable of using a wide range of organic substrates, as well as specific substrate degraders capable of degrading certain compounds of interest, should be evaluated. Bioassays using target species may need to be performed. Parallel testing to evaluate the degradation attributed to introduced and indigenous bacteria should be performed. Bacteria should be enumerated at the beginning and end of each experiment at a minimum. Intermediate analyses may be appropriate since biological activity can be measured relative to oxygen uptake rates and microbial plate counts.

If a microbiological characterization of the medium indicates that the naturally-occurring microbial activity is insufficient to achieve the required rates of biodegradation, even after environmental conditions have been enhanced, inoculation can be evaluated. Commercially-available cultures reported to biodegrade the contaminants of concern or microorganisms enriched from site samples may be used. Researchers are cautioned against employing microbial supplements without first assessing the relative advantages associated with their use, as well as potential competition that may occur between the indigenous and introduced organisms. Generally, this evaluation may be accomplished by inoculating one of two groups of identical test cells. Care must be taken during testing to ensure that samples are not contaminated with airborne microbes. During the evaluation of in situ technologies, the impact of site conditions such as climate, precipitation, soil properties, and carbon levels, should be evaluated in order to assess their impact on microbial movement from the injection point to the contamination location. Potential competition with other microorganisms, the ability of the microbes to survive in a foreign and possibly hostile (i.e., toxic) environment, as well as the microbes' ability to metabolize a wide range of substrates should be evaluated. Additionally, when choosing a commercially-marketed microbial supplement, the RPM should ensure that there are independent, peer-reviewed data supporting its applicability.

4.2.9 Test Duration

The duration of the treatability study must be considered in order to allocate personnel and funding properly, as well as plan for appropriate monitoring efforts over time. In general, at least three or four time periods should be studied, including the time-zero (T_0) analysis. However, if the study goals are met prior to the completion of all time periods, it is not necessary to continue sampling at additional time periods.

Researchers are cautioned against stopping a study before the site cleanup goals are met, since initially high removal rates can decrease to near zero values at concentrations above the site cleanup goals (see Subsection 2.2.4 for expanded description of this phenomena). For all practical purposes this asymptotic behavior defines the bioremediation end point.

4.2.10 Chemical Inhibition

Although acclimated microbes have been known to tolerate very high concentrations of contaminants and metals given long-term exposure, elevated concentrations may inhibit microbial activity. Studies may be performed to determine whether biological activity is inhibited by a given chemical or combination of chemicals present in the soil. These tests should determine contaminant concentrations at which microbial growth is supported, inhibited, or arrested. Inhibitory concentrations may be estimated by monitoring reductions in the number of actively degrading microorganisms present as contaminant concentrations increase. Toxic effects may be addressed by dilution, pH control, metals control (e.g., immobilization, volatilization, chelation, and washing) sequential treatment, or by employing microbial strains resistant to toxicants. Inhibition is typically studied in soil pans or small slurry-phase reactors rather than larger-scale systems.

4.2.11 Nonbiological Removal Processes

Remedy selection treatability tests should also include controls to measure the impact of nonbiological processes, such as volatilization, sorption, chemical degradation, migration, and photodecomposition. Inhibited controls can be established by adding formaldehyde, mercuric chloride (during non-EPA studies), sulfuric acid (added to lower the pH to 2 or below), or sodium azide to retard microbial activity. The media may also be autoclaved in order to inhibit microbial activity. (Note: considerable difficulty has been reported using some chemicals to inhibit microbial processes in soils.) Contaminant concentrations are measured in both the test reactors and the control reactors at the beginning of the study (T_o), at intermediate times, and at the end of the study. The mean contaminant concentrations in both the control and test reactors at the end of the test can be compared to their initial concentrations to see if a statistically significant change in concentration has occurred. The decrease in the control reactors may be attributed to abiotic mechanisms, while the decrease in the test reactors would be a result of abiotic and biotic processes. The difference in mean contaminant concentrations between the test reactors and the inhibited control reactors at each time interval sampled will show whether there is a statistically significant reduction in contaminant concentration due to microbial activity. Care should be taken to assess the effects that the different sterilizing agents can have on the chemical behavior of the soil-contaminant system. For example, formaldehyde has the potential to act as an electron donor, while sulfuric acid addition will impact pH. Sodium azide can, under certain circumstances, promote spontaneous explosive reactions, while mercuric chloride may complex certain petroleum hydrocarbons, leading to artificially low hydrocarbon concentrations. Placing the media in an autoclave may result in the desorption of volatile contaminants. Finally, sterilization agents may modify soil structure.

Complete sterilization of soils can be difficult to accomplish. Incomplete mixing of sterilization agents with soils can result in pockets of surviving microbes in soil pores. In some cases, microbial populations can transform and detoxify sterilizing agents. Additional sterilizing agents can be provided during the test to maintain reduced biological activity. The effectiveness of sterilizing agents can be measured by techniques such as microbial enumeration, respirometry, and enzyme analysis. Unless these or similar techniques show no microbial activity, it may not be possible to distinguish between removal of contaminants by abiotic and biological processes in the control reactors. However, complete sterilization of the control is not necessary provided biological activity is inhibited sufficiently so that a statistically significant difference between the test and control means can be determined. If sterilization is not complete, substantial degradation in the control can mask the occurrence of biodegradation in the test reactor. Both during and at the end of the study, plate cultures can be performed to determine whether controls were adequately sterilized.

In addition to employing controls, a number of methods exist that can be used to assess system performance. Oxygen uptake and/or carbon dioxide evolution can be monitored to assess the biological activity in a closed system.[21X81] Oxygen uptake measurements are useful indicators of biological activity in both the test and control reactors. Volatilization may also be estimated by establishing a closed system and monitoring offgases for VOCs and SVOCs.[21X32X81] For smaller-scale studies, organic traps and collection systems for media analysis may be used to evaluate more precisely both biological and abiotic removal mechanisms. Alternatively, an independent vapor extraction simulation may be used to assess the maximum amount of VOCs in the matrix. This will provide an estimate of the maximum amount of abiotic loss due to VOCs. If significant VOC losses are experienced (i.e., greater than 25 percent), VOCs should be quantitated directly.

Ideally, performance should be assessed using a mass balance approach capable of accounting for mineralization, transformation, volatilization, and residual concentrations. Samples of the solid, liquid, and gaseous phases should be analyzed when appropriate. The concentrations of contaminants, as well as any added substrates, metabolites, electron acceptors, radiolabeled compounds, and nondegradable tracers generated by or introduced to the test media should be determined. Radiolabeling may be employed to help to evaluate the fate of the contaminants and to perform a mass balance calculation. Due to the relatively high cost associated with purchasing radiolabeled compounds, this technique should be used only when a less expensive method for calculating mass balance is unavailable. In general, the cost of the labeled compounds is usually proportional to the complexity of the compound. Mineralization studies using ^{14}C labeling may be particularly appropriate for studies involving either relatively toxic compounds or compounds with the potential to be transformed into toxic byproducts.

4.2.12 Toxicity Testing

Toxicity testing that examines environmental and health effects can be used to determine whether the risk posed by the medium under study is adequately reduced by bioremediation. Examples of common toxicity testing techniques can be found in Appendix A, "Compendium of Tools." Toxicity tests may also be conducted for one or more of the time periods studied and may be used to determine whether treatment is complete.

4.2.13 Bioavailability

In order for biodegradation to occur, the microorganisms responsible for contaminant degradation must have access to the contaminants requiring treatment. The biological availability, or bioavailability, of a contaminant is a function of the contaminant's solubility in water and its tendency to adsorb on the surface of the soil. Adsorption is the major mechanism affecting the fate and transport of most organic and inorganic compounds in soils. The tendency of organic molecules to adsorb on the soil is determined by both the contaminant's and soil's physical and chemical characteristics. Important contaminant properties that affect adsorption include: chemical struc-

ture; contaminant acidity or basicity (pKa or pKb); water solubility; permanent charge; polarity; and molecule size. In general, the leaching potential of a chemical is proportional to the magnitude of its adsorption (partitioning) coefficient in the soil. The bioavailability of poorly-water soluble or sorbed organic pollutants may be improved by using surface active agents or surfactants.

4.2.14 Experimental Design of In Situ Systems

The following subsections contain experimental design information specific to soil column and field plot treatability studies. These studies are traditionally used to evaluate in situ technologies. Table 4-2 outlines some of the basic characteristics of the different testing methods employed and should be referred to when reading these subsections.

Soil Column Treatability Studies

Soil columns may be composed of soil, sediment, sand, or stone and can vary in size from 0.01 to 3,200 cubic feet. As outlined in Table 4-2, these studies last from 1 week to 6 months and may be performed in both the laboratory and field.

EPA's RREL is currently performing studies using in situ columns that are 9 inches in diameter and approximately 8 inches in length. These columns are isolated from the surrounding medium by a cylinder that is gently driven into the soil, sediment, or sand. The columns are open at the bottom and have a top through which temperature and carbon dioxide measurements can be taken. They can be installed at any excavatable depth and covered with the excavated soil, providing data on subsurface biodegradation. Future research will include the addition of amendments to the in situ soil columns.[1][12][66]

Alternatively, the column of contaminated medium may be relocated to a laboratory for the treatability study. In order to simulate in situ conditions more closely, the soil is often disturbed as little as possible. Degradation rates determined using soil columns filled with homogenized soil may, however, be more representative of an entire site than those using undisturbed soil cores. There are other advantages and disadvantages associated with soil columns filled with homogenized soil: they can be sampled without disrupting the integrity of the system but they do not provide an accurate representation of the hydraulic conductivity or nutrient transport of the undisturbed soil. To maximize the applicability of the tests to in situ treatment, soil columns filled with homogenized soil can be used to determine degradation rates and undisturbed soil cores can be used to estimate hydraulic conductivity and other parameters that do not require soil sampling.[8] When soil columns filled with homogenized soil are used, the representativeness of the study can often be improved by compacting the soil until its transport properties are similar to those of the undisturbed soil. Depending on the size of the columns and the desired number of sampling points, replicate soil columns or replicate samples from a single column may be used.

In addition to providing information on nutrient adsorption, hydrogen peroxide decomposition (aerobic systems), and "plugging" potential within the soil, soil column treatability studies can provide information relative to the degree of biodegradation that can be expected at various depths. These studies may also be designed to assess vertical movement of bacteria within contaminated soil and the utility of alternative oxygen sources. It should be noted, however, that other factors influencing the effectiveness of bioremediation are not examined in undisturbed soil column studies. These factors include lateral infiltration of air, water, and contaminants, and the effects of groundwater pumping on soil characteristics.

As with most other treatability studies, pH, moisture, nutrient addition, oxygen availability, and temperature

Table 4-2. Remedy Selection Treatability Study Characteristics

Type of study	Applicability	Scale	Size	Duration
Field plots	In situ bioremediation	Field-scale	1 to 1,111 yd_2 plot of land*	2 months to 2 years
Soil columns	In situ bioremediation	Lab- and field-scale	0.01 - 3,200 ft_3 of soil, sand, sediment, or stone	1 week to 6 months
Soil pans	Solid-phase treatment	Lab-scale	2 to 100 lbs of soil	1 to 6 months
Slurry-phase reactors	Slurry-phase and solid-phase (occasionally) treatment	Field-scale	Greater than 20 gallons of slurried media	2 to 3 months
		Lab-scale	1 fluid oz to 20 gallons	1 to 8 weeks
Contained soil systems	Composting, soil heap bioremediation, and solid-phase treatment	Lab- and field-scale	7 ft_3 to 3,900 yds_3 of soil	10 days to 10 months

* Field plot sizes are given as areas rather than volumes because treatment depths are frequently undefined.

are often monitored and modified. Moisture monitoring (daily or weekly) and nutrient addition are typical. Sprinkler systems and upflow percolation systems are commonly used. In order to encourage biodegradation beyond the initial layer of soil, oxygen is almost always supplied, frequently by injecting a liquid oxygen source (i.e., hydrogen peroxide or aerated water) directly into the column or by inducing airflow through the unsaturated soil. If volatilization is a concern, an airtight soil column equipped for offgas monitoring using organic traps may be needed.

Field Plot Treatability Studies

Field plots may provide the closest approximation to full-scale in situ treatment. These treatability studies, which last from 2 months to 2 years, are typically conducted on plots ranging in size from 1 to 1,111 square yards (i.e., one-fourth of an acre). These plots are usually located within a portion of the area requiring remediation. (Note: plot sizes are given as areas rather than volumes because treatment depths are frequently undefined.) Because field plots are relatively large, field plot treatability studies typically use replicate sampling.

Field plots often use techniques and equipment that are similar or identical to those used in full-scale remediation. These studies can closely approximate many aspects of full-scale treatment. The data obtained from these studies can often be used to:

- Develop the design for full-scale treatment
- Optimize specific operating parameters (e.g., nutrient and oxygen addition rates)
- Develop cost and schedule estimates for the full-scale system

Field plot treatability studies frequently employ pH monitoring and adjustment (using lime or phosphoric acid). The soil moisture is also frequently monitored and adjusted during field plot treatability studies. Infiltration and irrigation systems are commonly used to add water to a field plot.

The nutrient addition methods chosen for treatability studies that utilize field plots are similar to those chosen for full-scale treatment. Nutrient addition alternatives include the following:

- Addition of chemical nutrients to the water being applied to the soil
- Application of agricultural fertilizer

Regular nutrient monitoring is also recommended to ensure that nutrient addition rates are sufficient but not excessive. One logical scheme consists of groundwater monitoring both up-gradient and down-gradient of the nutrient injection points. Typical analytes include nitrate (NO_3^{-1}), nitrite (NO_2^{-1}), kjeldahl nitrogen, ammonia(NH_3), and phosphate (PO_4^{-3}); less common analytes include sulfate (SO_4^{-2}) and iron.

The oxygen addition techniques chosen for treatability studies that utilize field plots are similar to those chosen for full-scale treatment. Oxygen addition alternatives typically used at this scale include forced aeration/bioventing and hydrogen peroxide injection. Oxygen availability should be monitored routinely to ensure that it is adequate.

Temperature should be considered in the design of treatability studies utilizing field plots since the ground temperature above the frost line naturally varies with the season and climate and can significantly impact biodegradation rates. Temperature control (typically a heating system) may be helpful for some studies. However, studies are generally timed to occur during those seasons with the most favorable weather and temperature conditions. During those studies in which temperature variations are expected to impact biodegradation processes, temperature monitoring should be employed to assess its impact on the biodegradation rate. In some studies, it may be helpful to monitor the temperature of both the soil and the groundwater.

Instead of using inhibited controls, soil plot studies have traditionally used control plots that are monitored and sampled in an identical fashion to normal test plots, but do not receive enhancement. The data obtained from test and control plots are compared to determine whether any amendments (e.g., nutrients, oxygen) added to the test plots actually enhanced biological activity.

Specific concerns regarding contaminant volatilization or migration may require the application of different types of controls. If volatilization is a concern, the plots may be enclosed in airtight covers and the air monitored for volatile contaminants. If migration is a concern, the test plots and all but one control plot should be isolated from the surrounding soil. The results will indicate whether it will be necessary to take steps to limit volatilization or migration during full-scale treatment. A leachate collection system may be required to obtain a mass balance closure and to prevent contamination of surrounding areas. Leachate and underbedding material may be sampled to assess the potential for contaminant migration. Specialized volatilization sampling devices may be employed to measure contaminants emitted to the atmosphere.

4.2.15 Experimental Design of Ex Situ Systems

Three ex situ experimental designs are covered in this subsection: soil pans, contained soil treatment studies, and slurry-phase tests. These studies are generally shorter in duration than in situ studies and place less emphasis on evaluating and accounting for specific site characteristics (e.g., soil permeability). Table 4-2 outlines some of the basic characteristics of the different testing methods employed and should be referred to when reading these subsections.

Soil Pan Treatability Studies

As outlined in Table 4-2, soil pan studies are generally short-term studies (1 to 6 months in duration) performed in the laboratory within shallow pans capable of holding between 2 and 100 pounds of soil. The medium (i.e., soil, sediments) treated during these studies will usually be taken from the site and should possess contamination levels which are representative of the site. Because soil

pans are typically small, operating parameters (e.g., nutrient availability, pH, moisture, oxygen, and temperature) are relatively easy to control and study costs are relatively low (refer to Table 4-2). Generally oxygen addition can be provided by tilling or mixing the soil one to three times per week, while moisture is monitored and amended either daily or weekly. Since conditions are usually so easy to control, these studies are more likely to reflect ideal operating conditions rather than the less-than-perfect conditions typically experienced during field applications.

Because soil pan studies are typically small, replicate systems are recommended. These additional systems eliminate many of the data quality problems associated with collecting replicated samples from a single soil pan without generating substantial cost. When designing a soil pan treatability study, sampling requirements must be considered. If an entire soil pan is to be sacrificed at each sampling time, substantially more replicates need to be prepared. The volume of material in each pan, however, can be significantly smaller. If subsampling is employed, fewer replicates are generally required. The volume of soil in each pan, however, must be sufficient to allow removal of sample aliquots without adversely affecting the continued use of the pan for the study. During each sampling effort, a minimum of three samples (pans or aliquots) from the test group and two samples from the control group are recommended.

An abiotic control can be prepared for soil pan treatability studies in order to assess contaminant reduction due to nonbiological mechanisms. Depending on previous testing initiatives, inhibition testing may also need to be included during the remedy selection treatability study. During other types of treatability studies, inhibition tests may not be necessary. If volatilization is a concern, the soil pans may be tested in a closed (i.e., airtight) system and monitored for volatile contaminants. Alternatively, organic traps may be employed to assess volatilization in closed systems.

Example 7 describes a simple experimental design for a remedy selection treatability study utilizing a soil pan.

Contained Soil Treatment Experiments

Contained soil treatability studies are frequently used to assess the effectiveness of composting, soil heaping, and other solid-phase biotreatment technologies. Although they can be performed within a laboratory setting, the majority of these studies take place in the field using larger-scale systems. As outlined in Table 4-2, these studies typically last from 10 days to 10 months and handle moderate to very large volumes of soil (7 ft^3 to 3,900 yd^3).

Example 7

Twenty thousand cubic yards of soil were contaminated with creosote during the life of a railroad tie treating plant. Approximately 4 percent of the soil was composed of compounds that were extractable using benzene (i.e., benzene extractables). Average total PAH concentrations were 900 mg/kg. Total PAHs in the soil ranged from 100 to 2,000 ppm, and benzene extractables ranged from 2 to 10 percent by weight. A soil pan study was performed to determine whether cleanup criteria (i.e., 100 ppm for target PAH compounds and 1 percent for benzene extractables) could be achieved using solid-phase biological land treatment.

Testing was conducted using stainless-steel pans (6.0 x 10.0 x 2.5 inches). Each pan contained approximately 2 pounds of material. At the beginning of the study, water was added to obtain a 20 to 25 percent moisture content, a range conducive to microbial activity. The pans were incubated for 8 weeks at ambient temperature. The soil was tilled daily with a hand trowel to optimize aeration and contact between the microorganisms and the contaminants. Pans were covered with polyethylene film to minimize moisture loss during the incubation period without preventing oxygen transfer. Water was added to the pans to maintain the moisture content at 20 to 25 percent. The pH of the pans was monitored at regular intervals to ensure that it remained within the range considered conducive to microbial activity (7.5 to 8.5). Microbial activity was assessed by enumerating the numbers of microorganisms in the pans at regular intervals. The numbers of aerobic heterotrophic microorganisms were determined by standard enumerative techniques with a 1-gram sample removed randomly from each pan initially and after 2, 4, 6, and 8 weeks of incubation (Appendix A - Compendium of Tools). At each sampling point, nine random samples from the entire depth of the pan were removed and composited to provide the sample for chemical analysis. Sampling points were sampled initially, and at 2, 4, 6, and 8 weeks and analyzed for benzene extractables (which was considered a inexpensive indicator of trend). Gas chromatography/mass spectrometry (GC/MS) techniques were used to measure the concentrations of VOCs and SVOCs (PAHs) initially and at 8 weeks. All experiments were performed in triplicate to ensure reliable data.

Analytical data demonstrated that the benzene extractable and total PAH contamination dropped to 1.0 percent and 80 ppm, respectively, during the 8-week study. Based on these removals, as well as other operational data evaluated during parallel testing, researchers estimated that it will take 2 years to achieve the treatment goal of 100 ppm total PAHs and 1 percent benzene extractables at a cost of approximately $40 per cubic yard.

Although the design of contained soil treatment experiments depends on the characteristics of the specific technology under analysis, these studies generally provide detailed information regarding onsite applications of the technology that may be used to supplement RD/RA studies. Polyethylene liners, leachate collection systems, forced aeration systems, soil infiltration systems, mixing equipment, and humidity recorders are among the auxiliary equipment that may be employed during these experiments.

During both aerobic and anaerobic studies, pH control and regular (weekly or biweekly) pH monitoring are recommended. Supplements may be added as needed. Bulking agents may also be required. If inhibition testing reveals that contaminant concentrations are inhibiting microbial activity, the contaminant concentrations may require dilution by the addition of less-contaminated soil to maximize treatment.

Moisture content, rainfall, and pan evaporation rates may be monitored daily or weekly to help to evaluate watering needs. Readings should be taken at several depths to ensure the bottom of the treatment area is not saturated and becoming anaerobic. The soil can be maintained near field moisture capacity by using infiltration systems, water sprays, and irrigation systems.

Nutrient augmentation is often limited to nitrogen or phosphorus addition, but may include potassium and carbon addition. The optimum C:N:P:K ratio is dependent on the amount and type of waste requiring treatment and the microorganisms to be optimized. Commercial fertilizers and manure are two of the more common supplements applied during confined solid-phase treatability studies. Regular (daily or weekly) sampling for nutrient concentrations is recommended, as nutrients are usually added at the beginning of the treatability study and whenever testing indicates that concentrations are below the optimum operating range. Aeration is frequently accomplished using mechanical mixing or forced aeration. Routine (daily or weekly) monitoring is recommended to ensure that adequate oxygen is available.

System temperature should be monitored daily. The optimum temperature range for most aerobic contained soil treatment test plots is similar to the range recommended for other aerobic treatment methods (15° to 30° C). However, certain microorganisms such as white rot fungus achieve optimal degradation at significantly higher temperatures (i.e., 39° C for white rot fungus). The actual operating temperatures are often lower, however, since only a limited number of land treatment studies are performed within a controlled environment. Composting studies generally operate at higher temperatures (approximately 55° to 70° C).

Contaminant reductions associated with volatilization, adsorption, or chemical incorporation (covalent bonding) into the compost matrix, or chemical degradation are rarely evaluated during contained soil studies. Like soil plot studies, emphasis is placed on determining the relative increase in biodegradation caused by enhancing conditions conducive to biodegradation. Thus researchers attempt to determine the net increase in biological degradation experienced by comparing removals in enhanced systems with removals in biologically active, nonenhanced systems.

Slurry-Phase Tests

During slurry-phase studies, contaminated media are suspended within an aqueous solution that is generally 60 to 90 percent liquid. Continuous or intermittent mixing to encourage both aeration and contaminant/nutrient availability is frequently employed. As shown in Table 4-2, the scale of remedy selection slurry-phase experiments may range from 1 fluid ounce vials to sludge ponds with operating volumes of up to 70,000 gallons. More common, intermediate sizes include 0.1 to 0.3 gallon flasks, 5 to 20 gallon reactors, and 0.3 to 130 gallon sequencing batch reactors. Large-scale field studies (greater than 20 gallons) generally provide better information relative to the onsite application of the technology and are often used to supplement RD/RA requirements. Please note, however, that it is extremely difficult to subsample large reactors efficiently over a period of a study, i.e., to remove the same solids-to-liquid ratio at each sampling point. Feed tanks, carbon adsorbers, vapor absorbers, and digesters may be included in the treatment trains used during large-scale field studies.

Large-scale field applications treating volumes of 20 gallons or greater last an average of 2 to 3 months. Studies using sequencing batch reactors are typically much faster, with hydraulic residence times of 1 to 10 days. Small-scale laboratory experiments typically last between 1 and 8 weeks.

Temperature should be monitored daily to assess possible impacts on biodegradation rates. Monitoring instrumentation can range from a thermometer in a shaker-water bath, to a series of thermosensors within the batch reactors. Temperature controls, such as covers or immersion heaters, may be necessary. Laboratory testing is likely to take place at ambient temperatures, while the temperatures in field-scale studies tend to vary with the season and climate.

Since most aerobic slurry-phase treatability tests are continually mixed, the application of chemical oxygen sources is unnecessary. During large-scale testing, floating aerators, downdraft mixer/surface aerator combinations, or diffusers may be employed to provide oxygen. Oxygen uptake or DO content may be measured to determine the degree of biological activity. If inhibition testing reveals that contaminant concentrations are excessive, the samples may require dilution to maximize testing results and treatment. If volatilization is a concern, the slurry reactors may be sealed with airtight covers and monitored for volatile contaminants. Alternatively, organic traps may be employed to assess volatilization.

Example 8 describes a simple experimental design for a remedy selection treatability study utilizing a slurry-phase system.

4.2.16 Anaerobic Studies

During anaerobic treatability studies oxygen availability must be reduced or eliminated. This can be accomplished by consuming the DO in the media (supplying excess electron donors to the microbial population) and by limiting the diffusion of more oxygen into the system (e.g., by flooding the soil or establishing an oxygen-free

gaseous phase above the surface of the medium). An oxygen-free gaseous phase may be established by: 1) evacuating the headspace with a suction pump and refilling the headspace with a non-oxygen containing gas (hydrogen, helium, or nitrogen), or 2) placing the test system (i.e., soil pan) in a glovebox with an oxygen-free atmosphere. Alternatively, a gas pack generator can be used to produce an anaerobic atmosphere. When attempting to establish anaerobic conditions using a hydrogen atmosphere, palladium-coated pellets of aluminum may be employed to promote the chemical binding between hydrogen and the last traces of oxygen. Some anaerobic microbes require CO_2 which is usually readily available in soil systems. In such cases a blend of N_2 and CO_2 can be used. Trace oxygen can be scrubbed from this medium by passing it over hot copper.

Since facultative anaerobic, sulfate-reducing, methanogenic, and denitrifying bacteria typically employ different electron acceptors (nitrate, carbonate, or sulfate), as well as produce dissimilar byproducts and metabolic intermediates, test designs employed during anaerobic testing depend largely on the type of microorganisms used to perform biodegradation.[62] Table 4-3 outlines some of the different electron acceptors used and byproducts produced by the different types of microorganisms.

Many anaerobes fail to grow unless the medium has been prereduced (i.e., poised) to a level at or below a particular redox potential or Eh (usually -150 mV to -350 mV at pH 7). Therefore, poising agents such as cysteine hydrochloride, ascorbic acid, thioglycollate, and starch may need to be added during testing. The precise medium-specific Eh that will support the growth of a given anaerobe depends on the size of the inoculum (ongoing growth tends to lower the Eh of the surrounding medium), the identity of the poising agent, and the specific electron acceptor that is supplied.[54] The redox indicator resazurin may be used to demonstrate that anaerobic conditions are maintained throughout the study.[7] Since the Eh of the media will determine which groups of microorganism are active, the particular physiological group of anaerobes to be stimulated should be identified during remedy screening testing.

Two techniques commonly used during anaerobic testing, the McIntosh and Fildes' anaerobic jar and the roll tube technique are listed in Appendix A, Compendium of Tools. Further information on anaerobic processes can be obtained from various sources.[66]

4.3 EQUIPMENT AND MATERIALS

Standard laboratory equipment such as mixing flasks and sample collection bottles should be available for all treatability studies. Additional equipment and material re-

Example 8

An refinery impoundment was used for 40 years as a settling pond for oily waste streams. Following refinery shutdown, a total volume of 25,000 cubic yards of oily sludge was identified. A characterization of the sludge revealed that the material was 15 percent oil and grease (O&G) and 50 percent solids. Average PAH and carcinogenic PAH (CPAH) concentrations of 1,180 and 98 ppm were also identified. A laboratory slurry-phase study was performed to evaluate the feasibility of using slurry bioremediation technology to remediate the oily sludge. Data were also sought regarding the impact that pH, surfactant addition, O&G concentrations, and total solid loadings can have on treatment efficiency. Site cleanup goals of 2 percent for O&G, 100 ppm for PAHs, and 10 ppm for CPAHs were targeted.

The slurry-phase study was conducted in 4 L stainless-steel tanks with spargers located on the bottom for aeration. The slurry was continually mixed with a rotating impeller located in the middle of the reactor. Sludge was combined with deionized water and nutrients as required. Dried sludge was obtained by air-drying at room temperature prior to make-up of the slurry. The bioreactors were incubated at ambient temperature for 4 weeks. Tank volume was monitored daily; pH and DO were monitored daily. Triplicate samples were taken on days 0, 1, 3, 5, 7, 14, 21, and 28 to determine O&G concentrations, PAH concentrations, and microbial activity. O&G concentrations were used to measure the rate of biodegradation of the contaminants in the soil sludge. Microbial activity was assessed by both microscopic examination using a phase contrast microscope and standard enumeration techniques. The numbers of aerobic heterotrophic microorganisms and phenanthrene-degrading microorganisms were determined (refer to Appendix A, Compendium of Tools). Increases in microbial populations in conjunction with losses in contaminant indicated enhanced biodegradation. Triplicate samples were also removed initially and at the end of the experiment for determination of VOC and SVOC concentrations by GC/MS. All experiments were performed in triplicate to ensure reliable data. Past experience with oily wastes ruled out the need for toxicity testing.

Based on mass balance data obtained from the study, O&G, PAH, and CPAH contamination were reduced by 89, 93, and 95 percent, respectively. Corresponding final contamination levels within the sludge residuals from the reactors were 1.7 percent for O&G, 87 ppm for PAHs, and 5 ppm for CPAHs. Total solids was reduced by 15 percent. Preliminary estimates place treatment costs for the site at approximately $125 per cubic yard. Based on these data, a large-scale tank study was proposed to evaluate the technology further.

Table 4-3. Characteristics of Anaerobes Classified According to Physiological Nature

Bacteria type	Electron acceptors	Byproducts
Denitrifying	Nitrate and organic nitrogen, in the presence or absence of oxygen	Excluding excess nitrate, unanticipated and undesirable byproducts are unlikely
Facultative anaerobic	Organic acids or inorganic molecules, in the absence of oxygen	Metabolic intermediates differ under aerobic and anaerobic conditions
Sulfate-reducing	Sulfate, elemental sulfur, reduced sulfur compounds	Hydrogen sulfide
Methanogenic	Hydrogen and CO_2, acetate	Methane

quirements specific to the type of study employed (e.g., slurry-phase, soil pan, etc.) are listed in Table 4-4.

4.4 SAMPLING AND ANALYSIS

The Work Plan should address the test's needs for sampling and analysis work, as well as quality assurance (QA) support. The SAP, which will be prepared after Work Plan approval, helps to ensure that the samples are representative and that the quality of the analytical data is generally known. The SAP addresses field sampling, contaminant characterization, and the sampling and analysis during treatability testing. It consists of two parts: the FSP and the QAPP. Further discussion of the FSP and QAPP and specific sampling and analytical tests and protocols are presented in Section 5 and in the generic guide.

4.4.1 Field Sampling

Field samples are taken to provide baseline contaminant concentrations and contaminated material for treatability studies. A sampling plan should be developed that directs the collection of representative samples from the site for the treatability test. The sampling plan should be site-specific and describe the number, location, and volume of samples to be collected. The objective of the sampling plan must be consistent with treatability test objectives. For example, it may be more appropriate to perform testing on a relatively "undisturbed" or intact soil sample when evaluating an in situ technology. This approach is particularly important for determining baseline information, such as hydraulic conductivity and porosity. When consistency between samples is important, as in determining optimized nutrient addition rates, homogenized and subdivided soil samples may be preferred. This approach minimizes initial differences between test samples, increasing the confidence that differences in results are caused by the manipulated parameter. Homogenizations and composite sampling are also preferred if an ex situ technology is being considered, since the characteristics of an intact soil sample (e.g., relative to its ability to mimic permeability, nutrient and contaminant dispersal) are less relevant. The EPA document, Methods for Evaluating the Attainment of Cleanup Standards, provides information on sampling plan design.[64]

Generally, samples representative of conditions typical of the entire site or a defined area (e.g., hot spots) within the site should be collected. The selection of soil sampling locations should be based on knowledge of the site. Information from previous soil samples, soil gas analysis using field instrumentation, and obvious odors or residues are parameters that can be used to specify sample locations. Alternatively, a random, stratified, or systematic sampling plan could be implemented to allow results to be more easily expressed in statistical terms.[64] This approach, which does not use the sampler's knowledge of the site, may increase the likelihood of missing hot spots bioremediation may not be capable of effectively treating. The EPA document, Test Methods for Evaluating Solid Waste, provides a discussion of random, stratified and systematic sampling as well as sample size requirements.[73]

Composited samples representative of the media requiring remediation are ideal samples for treatability studies that do not require intact undisturbed media. Compositing reduces the variability in contaminant concentration and provides more accurate data on soil concentrations before and after testing. Compositing is usually appropriate for soils containing nonvolatile constituents; however, if the target contaminants are volatile, care should be taken to minimize losses during compositing. Compositing samples on ice is a good method of reducing volatile compound losses, as long as the samples are not allowed to freeze. The EPA document, Groundwater Issue: Soil Sampling and Analysis for Volatile Organic Compounds, provides additional information on this topic.[50]

When obtaining media samples to use during biological treatability studies, emphasis should also be placed on maintaining the biological integrity of the samples. Improper handling of soil samples can reduce microbial populations and/or inactivate extracellular enzymes which are functional under normal field conditions. Although changes to the soil are inevitable during handling, it is important to minimize these changes and their impacts on microbial studies. Drastic changes in soil moisture, temperature, etc. should be avoided. To the

Table 4-4. Equipment and Materials

	Soil columns	Field plots	Slurry reactors	Contained soil systems
Test systems	• Lab/field cylinders	• In-ground barriers • Above-ground berms	• Lab reactors • Small tanks (lab/field)	• Lined/bermed area in the field • Soil pans (lab)
Contaminant sampling	• Small coring device	• Split-spoon • Shelby tube	• Bailer • Sample port	• Split spoon • Shelby tube
Moisture control	• Sprinkler • Upflow percolation • Water can	• Sprinkler • Subsurface irrigation	NA	• Sprinkler • Watering can
Temperature measurement	• Temperature probe • Soil thermometer	• Temperature probes	• Thermometer • Temperature probe	• Temperature probe • Soil thermometer
Nutrient addition (Agricultural chemicals or other chemicals)	• Pumps/sprinklers for dissolved nutrients	• Shovel/rake/etc. • Tractor • Spreader or sprayer • Sprinkler/irrigation system for dissolved nutrients	• Metering pump • Mix tank	• Trowel/shovel/rake/etc. • Tractor • Sprinkler/irrigation
Oxygen addition (for aerobic studies)	• Aerator • Oxygenated water injection system • H_2O_2 injection system • H_2O_2	• Tractor and disc garden tiller • Bioventing/forced aeration	• Floating aerators • Diffusers	• Trowel, hand tool, etc.
pH control	• pH probe for soil dissolved in water • Acid • Base	• pH probe for soil dissolved in water • Lime • Phosphoric acid	• pH probe • Acid • Base	• pH probe for soil dissolved in water • Acid • Base

extent possible, samples should be collected using procedures that minimize the addition or transfer of microbes between samples (e.g., steam cleaning sampling equipment between samples) and the introduction of foreign material (i.e., by sampling devices or drilling residues)[4] samples either should be used promptly or placed in thin-walled polyethylene bags or glass containers and stored at 5 to 10° C. The polyethylene bag will reduce moisture losses, while permitting some gas exchange. Since the biological activity of a sample decreases with time, samples held for greater than 48 hours are generally unsuitable for biodegradation studies. Storage at sub-freezing temperatures should not be used to lengthen the acceptable storage period as it alters the characteristics of the microbial community. Furthermore, samples which are allowed to completely air dry will most likely experience an anomalous burst of respiratory activity upon remoisturizing and a selection for the fungal components of the microbial population.[26] [55]

The method of sample collection is site-specific. For example, drill rigs or hand augers can be used to collect samples, depending on the depth of the sample required and the soil characteristics. Soil cores, which preserve the media's structure, are ideal for determining air permeability, as well as for providing data regarding the impact of geological formations, contaminant/depth relationships, and other site-specific media characteristics. Equipment for obtaining soil samples from upper layer soils can be found in Table 4-5.[68]

Regardless of the technique used to collect the sample, an adequate volume of soil sample should be collected from each sampling location to account for replicate treatability tests and analytical QA/QC requirements. Guidelines for statistical sampling procedures are give in the documents Hazardous Waste Land Treatment and Test Meth-

ods for Evaluating Solid Waste (SW-846).[55X73]

4.4.2 Media Analysis During the Treatability Study

Contaminant concentrations should be determined at the beginning of the study and at the sample times chosen in the experimental design. Consult SW-846[73] for the appropriate methods. GC or GC/MS techniques can be used to evaluate the biodegradation of a wide range of components and confirm that the bioremediation process is treating all of the compounds of concern, and not only a limited set of the compounds. When determining VOCs and SVOCs, it may be possible to minimize costs by substituting GC or other appropriate methods (e.g., high-performance liquid chromatography (HPLC)) for GC/MS methods. However, this is not advised for heavily-contaminated soils that contain a significant amount of other "non-priority pollutant" compounds and degradation intermediates. All sampling and analysis should be performed in accordance with the SAP (Section 5). In order to obtain a statistically relevant measure of background contamination levels, it is necessary to take a significant number of replicate samples that are representative of the area being sampled.

The concentrations of some important matrix parameters are determined by using standard analytical chemistry methods (Table 4-6). These parameters are important for the design of remedy selection testing and RD/RA studies and should be determined before the treatability study begins. These methods should not be used as an indication of the inappropriateness of the technology.

Direct microscopy (e.g., fluorescent staining, buried-slide technique), adenosine triphosphate (ATP) analysis, enzyme activity analysis, and culture counts (e.g., plate counts, dilution counts) may be used to monitor microbial activity during testing.[42]

4.4.3 Monitoring and Process Control Measurements

A monitoring program is an essential component of any remedy selection treatability study. Monitoring data can be used to assess degradation rates and to determine if system design or operational changes are needed. During remedy selection testing biodegradation may be assessed by removing samples from the testing system (e.g., reactor, treatment bed), or in the case of smaller-scale, laboratory tests, by sacrificing the entire contents of smaller test systems at predetermined time intervals. Contaminant concentrations should be determined at the beginning, end, and one or more intermediate time points. Toxicity studies may also be conducted if toxicity reduction is included in the test goals. The length of the study will be determined by the biodegradability of the contaminants and the time required to achieve parallel test goals. Measures of microbial activity (CO_2 evolution, oxygen uptake, etc.) may also be used to identify appropriate sampling times.

Process control measurements are also essential. Nutrients, water, and pH are among the most common media parameters measured. Measurement of ambient and soil temperatures is also customary; weather conditions may also be recorded.[68] The effects different operating parameters have on removal efficiency should be determined. Typically, tests are run in triplicate.

Table 4-5. Equipment for Field Collection of Soil Samples

Hand-driven equipment	Power-driven equipment
Screw-type auger	Continuous flight power auger (hollow-stemmed)
Post-hole auger	Core sampler
Barrel auger	Split-spoon sampler
Dutch auger	Bucket auger
Split-spoon sampler	Cable-tool drill rig
Tube-type sampler	Rotary drill rig
Auger/dry-tube corer	

In addition to monitoring contaminant disappearance and process control parameters, it may be necessary to monitor media outside the treatment zone to assess possible contaminant migration. Depending on the scale of study, groundwater, soil, runoff water, and/or air monitoring may be required. By successfully combining these monitoring efforts, an accurate picture of contaminant fate can be achieved. Generally, as the degree of control associated with keeping the media under study separated from the environment decreases, the potential for contaminant migration increases; therefore the need for additional levels of monitoring decreases.

During field studies, particularly large, in situ and contained soil (e.g., landfarming) studies, soil cores and soil-pore liquid monitoring should be used to determine if hazardous constituents are migrating out of the testing area. Soil core samples generally provide information regarding the movement of the slower moving hazardous constituents, while soil-pore liquid samples evaluate the movement of the faster moving contaminants. The number, location, and depth of soil core and soil-pore liquid samples will provide an accurate indication of conditions below the testing area. When determining vertical contaminant migration, contaminant concentration trends below the testing zone need to be monitored. Increasing concentrations are indicative of migration from the testing zones. Steady or decreasing concentrations without indications of increased biological activity (e.g., no increase in microbial counts) are indicative of minimal vertical migration. However, an increase in microbial activity suggests that a previously limiting factor, such as substrate (i.e., the contaminant's) availability, has been removed. Ultimately, contaminant migration out of the testing zone cannot be conclusively demonstrated by citing a decrease in contaminant concentrate along with increased microbial activity.

The frequency and timing of sampling must be based on the frequency, timing, and rate of amendment application, groundwater proximity, soil permeability, and rainfall. The mobility of the contaminant and the impact treatment has on contaminant mobility must be account-

Table 4-6. Commonly Used Analytical Chemistry Methods

Analysis	Liquid	Soil/sludge
Moisture	—	ASTM 2216
Nitrate	SW-846 Method 9200	—
Total organic carbon	SW-846 Method 9060	SW-846 Method 9060
Total kjeldahl nitrogen	U.S. EPA Method 351.2	ASTM E 778
Soluble orthophosphate	U.S. EPA Method 365.1	—
Soluble ammonia	U.S. EPA Method 350.1	—
pH	SW-846 Method 9040	SW-846 Method 9045
TPH by GC	SW-846 Method 8015	SW-846 Method 8015
TPH by IR*	U.S. EPA Method 418.1	SW-846 Method 9071 U.S. EPA Method 418.1
Base, neutral, and acid extractable compounds	SW-846 Method 8270	SW-846 Method 8270
VOCs	SW-846 Method 8240	SW-846 Method 8240
VOCs by GC	SW-846 Method 8010/8020	SW-846 Method 8010/8020
Total O&G (IR Method)*	U.S. EPA Method 413.2	SW-846 Method 9071 U.S. EPA Method 413.2
Total O&G (Gravimetric method)	U.S. EPA Method 413.2	SW-846 Method 9071 U.S. EPA Method 413.2

* infrared spectrometry

ed for. In addition to providing data on the vertical displacement of the contaminant, soil core samples may also be used to provide data on treatment progress in the testing zone.[73] Lysimeters may also be used to evaluate migration potential during in situ field studies. Table 4-7 provides guidance for developing a monitoring program during large field treatability studies.[73]

When necessary, groundwater should be monitored to determine whether contaminants are migrating out of the testing zone. Pressure vacuum lysimeters, trench lysimeters, and vacuum extractors may be used to monitor soil-pore liquids and/or leachates. Generally, groundwater monitoring supplements the unsaturated zone monitoring system. If runoff water analyses are needed a monitoring program should be instituted. The sampling and monitoring approach will vary, depending on whether the water is released as a continuous discharge or as a batch discharge following treatment. Depending on the technology under study and the characteristics and volume of water produced, a National Pollutant Discharge Elimination System (NPDES) permit may be required.

Due to the volatile nature of many contaminants, air monitoring is a essential element of many site monitoring plans. Besides providing data on potential contaminant releases, air monitoring provides a means for evaluating the effectiveness of vapor suppression techniques. Depending on the scale of the study, personal monitoring equipment, perimeter sampling, and upwind/downwind sampling may be needed to ensure the safety of residents and workers. High efficiency particle filter samplers and gas/vapor samplers may be used. Solid sorbent traps may be used to sample volatile organic air pollutants. Continuous air monitoring may also be advisable.[68] If significant emissions are anticipated during treatability testing, RPMs should check with the appropriate regulatory offices to identify potential monitoring, reporting, and permitting requirements. Depending on the technology under study, air monitoring data may help define contaminant fate, particularly during mass balance calculations.

4.4.4 Treatment Product Sampling and Analysis

Biodegradation, especially ex situ bioremediation, is not always a stand-alone process. The treated solids, liquids, and each of the other various waste streams (biological sludges) should be analyzed for the contaminants identi-

Table 4-7. Guidance for an Operational Monitoring Program

Medium to be monitored	Purpose	Parameters to be analyzed
Soil cores (unsaturated zone)	Determine slow moving hazardous constituents	All hazardous constituents in the waste or the principal hazardous constituents, metabolites of hazardous constituents, and nonhazardous constituents of concern
Soil-pore liquid (unsaturated zone)	Determine highly mobile constituents	All hazardous constituents in the waste or the principal hazardous constituents, mobile metabolites of hazardous constituents, and important mobile nonhazardous constituents
Groundwater	Determine mobile constituents	Hazardous constituents and metabolites or select indicators
Vegetation (if grown for food chain use)	Phototoxic and bioaccumulating hazardous constituents (food chain hazards)	Hazardous metals and organics and their metabolites
Runoff water	Soluble or suspended constituents	Discharge permit and background parameters plus hazardous organics
Soil in the treatment zone	Determine degradation, pH, nutrients, and rate- and capacity-limiting constituents	Hazardous constituents, metabolites, pH, N,P, K, moisture, and microbial population and activity
Air	Personnel and population health hazards	Particulates (adsorbed hazardous constituents) and hazardous volatiles

fied in the original soil analyses and their known degradation products to see if additional treatment is needed. In many cases, indicator contaminants, which are representative of a larger group of contaminants, can be analyzed in place of a full scan. Caution must be exercised in using indicator contaminants since biodegradation efficiencies can vary from one contaminant to another. The process efficiency may be either understated or overstated when analyzing for indicator compounds.

4.5 DATA ANALYSIS AND INTERPRETATION

The Work Plan should discuss the techniques to be used in analyzing and interpreting the data. The objective of data analysis and interpretation is to provide sufficient information to the RPM, OSC, and EPA management to assess the feasibility of biodegradation as a remedial technology. After remedy selection testing is complete, the decision must be made whether to proceed to the RD/RA testing tier, to perform a full-scale bioremediation, or to rule out bioremediation as an alternative. The data analysis and interpretation are a critical part of the remedy selection testing process.

The primary goal of the remedy selection biodegradation treatability study is to determine how well the treatment method removes the contaminants. System performance is affected by a variety of process design variables, including contaminant concentration, nutrient and oxygen availability, abiotic losses, pH, microbial acclimation, and temperature. Often one or more of these variables must be adjusted to enhance the remediation process suitably. In order to properly evaluate the impact the various process variables have on testing results, the following data should be reported for each treatability test:

- Concentration of chemicals in samples at the time of sampling (field concentration) and before the samples are added to the reactors (T_0 reactor concentration)
- Amount of soil used in the reactors and a description of all modifications to the reactors
- Quantity of residual chemicals in each of the reactors at each sampling time
- Quantity of residual chemicals lost due to abiotic processes
- Temperature profile over the entire experiment recorded in a written log indicating type, extent, and time of any action
- Any other additions, removals, changes, manipulations, or mishaps that occur during the course of the

experiment should be recorded in a written log indicating type, extent, and time of any action

- All cited analytical and microbiological procedures (recorded in a written log)
- All QC data (e.g., recovery percentage of spikes, contaminant concentrations, if any, in experimental and analytical blanks)

Additional information on the interpretation of treatability study data is presented in Section 6 of this document.

Assessing whether the bioremediation method under study can achieve site cleanup levels within reasonable time limits and under practical engineering conditions is the primary goal of the remedy selection treatability study. Adjustments should be made for the impact of the different design variables (e.g., pH or oxygen availability). Statistical analysis of data that follow a normal distribution can be performed using the analysis of variance (ANOVA) techniques and other statistical methods. In some instances, the use of nonparametric evaluations may be more appropriate. For details on parametric evaluations, refer to the documents entitled Statistical Analysis of Groundwater Data at RCRA Facilities (Interim Final),[70] and Experimental Design and Analysis.[18] Models (conceptual, mathematical, and physical) may be used as a focus for data integration. These models should be capable of bridging laboratory and field applications. A realistic scale-up to full-scale applications is essential. Both stochastic and deterministic models should be used to identify limiting mechanisms and critical parameters. Best- and worst-case scenarios should be used to define the operational parameters. Data obtained from a large field-scale study should be used to validate the model.

4.6 REPORTS

The last step of the treatability study is interpreting and reporting the results. The Work Plan may discuss the organization and content of interim and final reports. Complete, objective, and accurate reporting is critical, because decisions about implementability will be mostly based upon the outcome of the study. The RPM or OSC may not require formal reports at each treatability study tier. Interim reports should be prepared after each tier. Project briefings should be made to interested parties to determine the need for and scope of the next tier of testing. To facilitate the reporting of results and comparisons between treatment alternatives, a suggested table of contents is presented in the generic guide.[52] At the completion of the study, a formal report is always required.

OERR requires that a copy of all treatability study reports be submitted to the Agency's Superfund Treatability Database repository. One copy of each treatability study report must be sent to:

U.S. Environmental Protection Agency
Superfund Treatability Database (MS-445)
ORD/RREL
26 West Martin Luther King Dr.
Cincinnati, Ohio 45268

4.7 SCHEDULE

The Work Plan includes a schedule for completing the treatability study. The schedule gives the anticipated starting date and ending date for each of the tasks described in the Work Plan and shows how the various tasks interface. Listed below are some of the specific tasks that should always be considered when scheduling:

- Data review/literature search
- Work Plan preparation, review, and revision
- SAP preparation
- Sample collection and disposal
- Field sample analysis
- Treatability test (including analyses)
- Disposal of waste material generated during the test
- Data validation
- Report preparation, review, and revision
- Meetings

The treatability test has the greatest potential for time variance. The schedule for this test can vary tremendously depending on whether a small- or large-scale study is being performed. Small laboratory-scale studies typically take from 3 to 6 months, whereas large field-scale studies usually take from 6 to 9 months. Contaminant types and concentrations involved also can impact the test schedule. For example, a laboratory-scale remedy selection treatability test for soils contaminated with benzene, toluene, ethylbenzene, and xylene (BTEX) may be conducted within a 1 or 2 weeks, whereas tests involving PAHs may take several months because of the relative biodegradability of these classes of organic compounds. Sufficient time must be built into the schedule to reach specified cleanup concentrations. The treatability study must continue until either the removal goals have been achieved or the contaminant removal has reached a distinct concentration at which contaminant reductions cease to occur at a reasonable rate.

The time span for each task accounts for the time required to obtain the Work Plan, subcontractor, and other approvals (e.g., disposal approval from a permitted commercial treatment, storage, and disposal facility); sample procurement time, if necessary; analytical turnaround time; data validation intervals; and review and comment periods for reports and other project deliverables. Some contingency should be built into the schedule to accommodate unexpected delays (e.g., bad weather, equipment downtime) without affecting the project completion date. Example schedules for in situ and ex situ remedy selection studies are presented in Figures 4-1 and 4-2, respectively. If the study involves multiple tiers of testing, all tiers should be shown on one schedule. Careful planning before the start of tests is essential. Depending on the review and approval process, planning can take up to several months.

Setup of the laboratory and procurements of necessary equipment and laboratory supplies for treatability studies may take a month or more. Depending on how rapidly laboratory results can be provided, analytical results can be available in less than 30 days. Shorter analytical turnaround time can be requested, but quick turnarounds will normally increase the costs. Turnaround times should be less than the time between sampling points. Results from one sampling point are needed before the next sample is taken because the sampling schedule may be extended if degradation is occurring at a slower rate than anticipated. This is especially important when sacrificial reactors are used for timepoints and a limited number of these reactors were set up at the beginning of the study. For this reason, inexpensive analyses with quick turnaround times are recommended for monitoring treatability studies even if confirmatory analyses (GC/MS) need to be performed at certain points.

The schedules in Figures 4-1 and 4-2 are based on a 30-day analytical turnaround time. In the event 90-day turnarounds are experienced, the schedules in Figures 4-1 and 4-2 would increase to 26 months and 17 months, respectively, reflecting net increases of 2 months. These schedules do not reflect "standard" and/or "average" timelines for treatability testing. The variability inherent in treatability testing would make any attempt at simulating these conditions meaningless.

Interpretation of the results and final report writing usually requires 1 to 2 months, but this is highly dependent on the review process. It is not unusual for the remedy selection phase to take 3 to 9 months before treatability testing and final reporting can be completed.

4.8 MANAGEMENT AND STAFFING

The Work Plan discusses the management and staffing of the remedy selection treatability study and specifically identifies the personnel responsible for executing the treatability study by name and qualifications. Generally, the following expertise is needed for the successful completion of the treatability study:

- Project Manager (Work Assignment Manager)
- QA Manager
- Chemist
- Microbiologist, Environmental Scientist/Engineer, or Bioengineer
- Lab Technician.

Responsibility for various aspects of the project is typically shown in an organization chart such as the example shown in Figure 4-3.

4.9 BUDGET

The Work Plan should discuss the budget for completion of the remedy selection testing tier unless this information is judged to be business-confidential by EPA. The cost of remedy selection testing varies tremendously and is directly related to the type of test (laboratory or field-scale), the technology under study, the method of sample

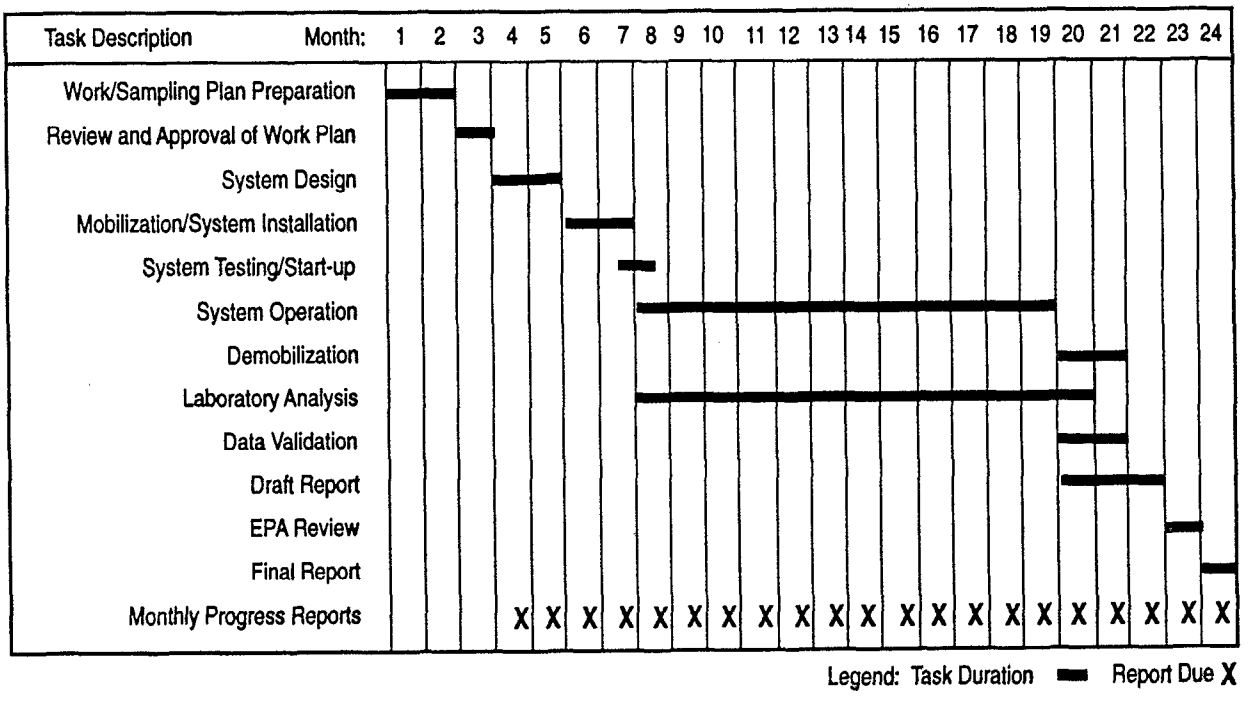

Figure 4-1. Sample treatability testing schedule for remedy selection evaluation of in situ bioremediation.

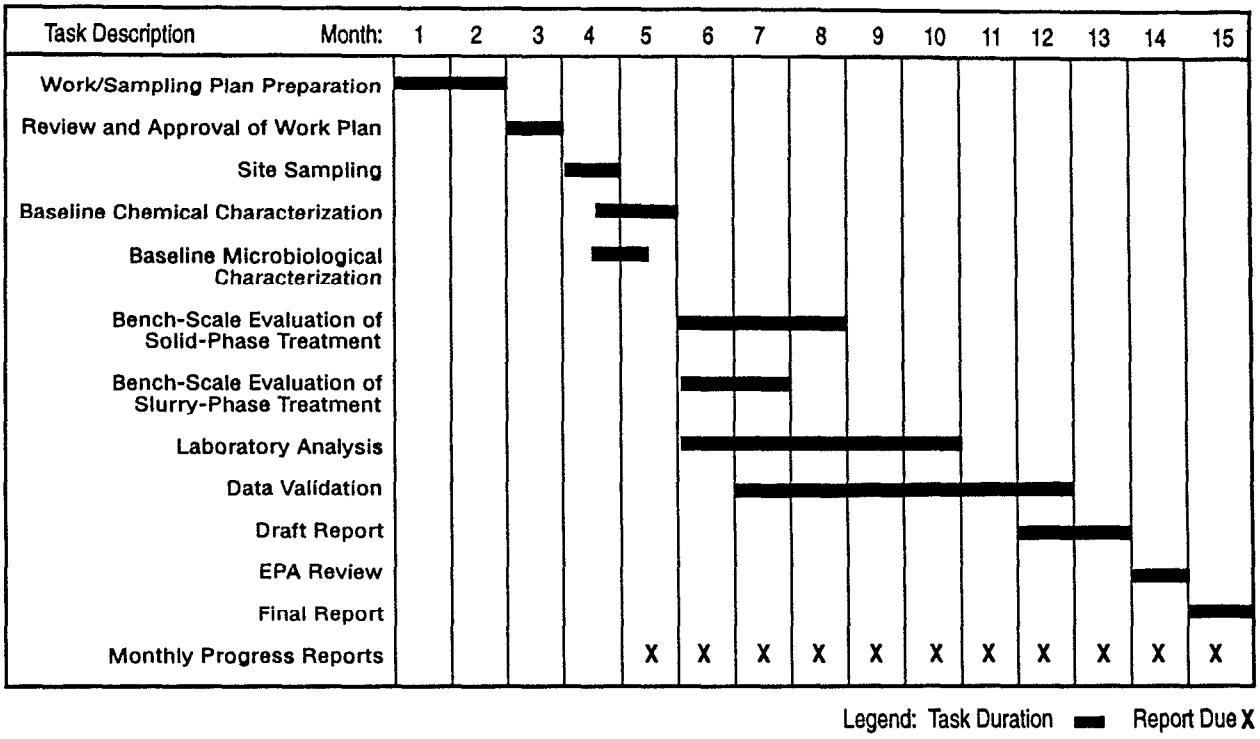

Figure 4-2. Sample project schedule for laboratory remedy selection evaluation of solid- and slurry-phase bioremediation.

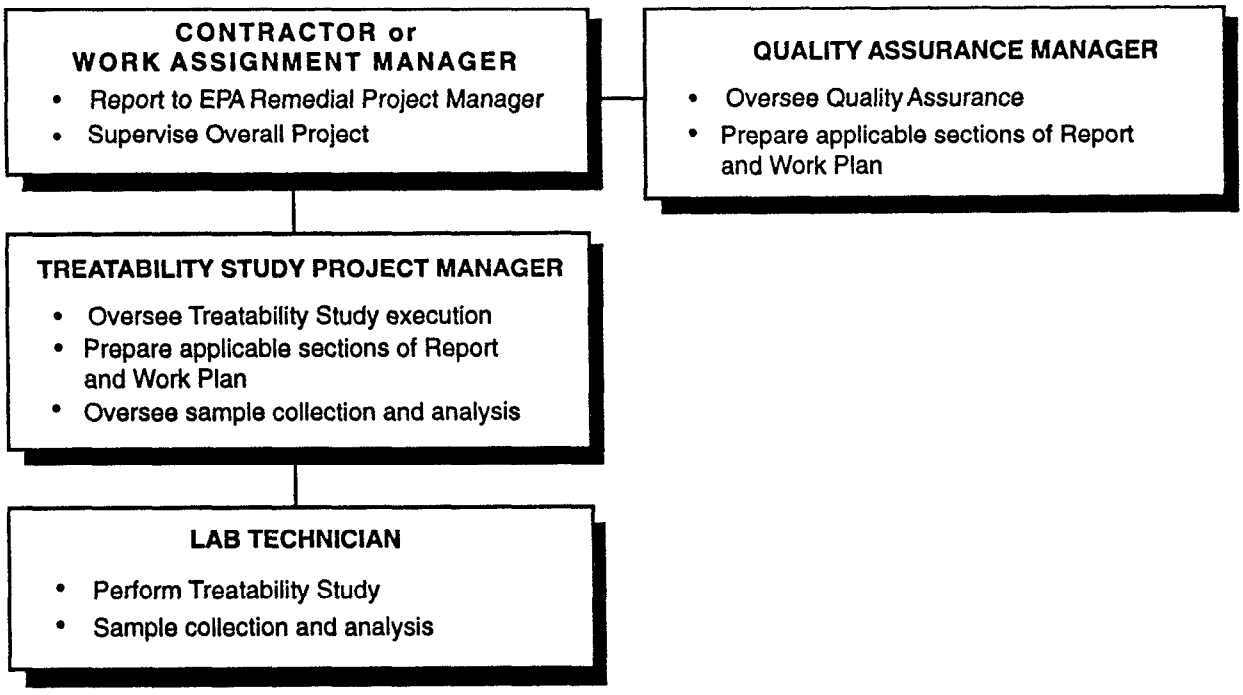

Figure 4-3. Sample organization chart.

collection, the number of samples collected, the type of and number of chemical analyses performed, and the number of replicate tests performed. The factor that most influences the cost of the remedy selection testing phase is whether the test is performed at the laboratory-scale or field-scale level. Larger field-scale studies are more expensive than small laboratory-scale studies because they require field mobilization/demobilization, field crews to run the test, more analytical data, and are usually of longer duration than small-scale tests. The type and number of chemical analyses performed also have a significant impact on the cost of remedy selection testing. Laboratory setup costs also may be inflated due to government requirements. One method to minimize costs is to use an inexpensive analysis as an indicator parameter and to perform a limited number of analyses for the more expensive volatile and semivolatile priority pollutants. Use of GC rather than GC/MS methods, if applicable, should also help to minimize costs. Table 4-8 summarizes the major cost elements associated with remedy selection treatability tests.

Sampling costs will be influenced by the contaminant types and depth of contamination found in the soil, sludge, or sediment. Depending on the depth of contamination and the regulatory requirements, field sampling can cost hundreds of thousands of dollars. The health and safety considerations during sampling activities are more extensive when certain contaminants, (e.g., VOCs) are present. Level B personal protective equipment (PPE) rather than Level D[79] PPE can increase the cost component by an order of magnitude. In general, most laboratory and field-based remedy selection studies will require Level D PPE. Sampling equipment for surface samples is much less complicated than the equipment needed for deep samples. Depending on the number of samples and tests specified, test residuals (e.g., contaminated solvent and water) will require proper treatment and/or disposal. Since effluents and residual materials produced during testing often are treated as a hazardous waste, regardless of whether the contaminant has been degraded, high disposal costs may have to be assumed.

Other factors to consider include report preparation and the availability of essential equipment and laboratory supplies. Generally, an initial draft of the report undergoes internal review prior to the final draft. Depending on the process, final report preparation can be time-consuming as well as costly. Procurement of specialized testing equipment (e.g., reagents and glassware) will also increase the costs.

Table 4-8. Major Cost Elements Associated with Biological Remedy Selection Treatability Studies

Cost element	Approximate cost range (thousands of dollars)
Work plan preparation	2 - 5
SAP preparation	2 - 5
Health and safety plan preparation	1 - 5
Field sample collection	5 - 10
Field sample chemical analysis	5 - 100
Laboratory setup/materials	5 - 10
Treatability test operation	5 - 15
Treatability test chemical analysis	5 - 100
Data presentation/report/ remediation cost estimate	20 - 50
Total cost range	**50 - 300**

The typical costs for the remedy selection testing phase are estimated to range from $50,000 to $300,000. These estimates are highly dependent on the factors discussed previously. Not included in these costs are the cost of government procurement procedures, including soliciting for bids, awarding contracts, etc.

To minimize costs, opportunities for cost savings should be sought actively. For example, during bioventing studies, boreholes used to characterize the site may be converted to bioventing wells.

SECTION 5
SAMPLING AND ANALYSIS PLAN

The SAP consists of two parts: the Field Sampling Plan and the Quality Assurance Project Plan. The purpose of this section is to identify the contents of and aid in the preparation of these plans. The RI/FS requires a SAP for all field activities. The SAP ensures that samples obtained for characterization and testing are representative, and that the quality of the analytical data generated is known and appropriate. The SAP addresses field sampling, waste characterization, and sampling and analysis of the treated wastes and residuals from the testing apparatus or treatment unit. The SAP is usually prepared after Work Plan approval.

5.1 FIELD SAMPLING PLAN

The FSP component of the SAP describes the sampling objectives; the type, location, and number of samples to be collected; the sample numbering system; the equipment and procedures for collecting the samples; the sample chain-of-custody procedures; and the required packaging, labeling, and shipping procedures.

Field samples are taken to provide baseline contaminant concentrations and contaminated material for treatability studies. The sampling objectives must be consistent with the treatability test objectives.

The primary objectives of remedy selection treatability studies are to evaluate the extent to which specific chemicals are removed from soil, sediment, sludge, or water. The primary objectives for collecting samples to be used in remedy selection treatability testing include to following:

- Acquisition of samples representative of conditions typical of the entire site or defined areas within the site. Because a limited mass balance may be required, field sampling plans may be required. However, professional judgment regarding the sampling locations may be exercised to select sampling sites that are typical of the area (pit, lagoon, etc.) or appear to have above-average concentrations of contaminants in the area being considered for the treatability test. This may be difficult because reliable site characterization data may not be available early in the remedial investigation.
- Acquisition of sufficient sample volumes necessary for testing, analysis, and QA/QC. The biodegradation screening guide recommends using about 5 kg of the contaminated medium. During remedy selection testing, the amount of sample will depend on the size of the test and the number of test samples.

From these two primary objectives, more specific objectives are developed. When developing the more detailed objectives, consider the following types of questions:

- Should samples be composited to provide better reproducibility for the treatability test? This question is addressed in Subsection 4.4.1.
- Are there adequate data to determine sampling locations indicative of the more contaminated areas of the site? Have soil gas surveys been conducted? Contaminants may be widespread or isolated in small areas (hot spots). Contaminants may be mixed with other contaminants in one location and appear alone in others. Concentration profiles may vary significantly with depth.
- Are the soils and contaminants homogeneous or heterogeneous? Soil types can vary across a site and will vary with depth. Depending on professional judgement, contaminated samples for various soil types may have to be taken to conduct treatability tests. Variations in soil composition can affect the effectiveness of biodegradation as well as the accuracy of the analyses employed.
- Are contaminants present in the sediment, sludge, or water? Different sampling methods must be used for each of these media. Will media exchange contaminants during treatment? Mass balances may be necessary.
- Is sampling of a "worst-case" scenario warranted? Assessment of this question must be made on a site-by-site basis. Hot spots and contaminants in different media may be difficult to treat. These should be factored into the test plan if they represent a significant portion of the waste site.

After identifying the sampling objectives, an appropriate sampling strategy is described. Specific items that should be discussed briefly and included are listed in Table 5-1.

5.2 QUALITY ASSURANCE PROJECT PLAN

The QAPP consists of 11 sections. Since many of these

Table 5-1. Suggested Organization of the Sampling and Analysis Plan

FSP	
1.	Site background
2.	Sampling objectives
3.	Sample location and frequency Selection Media type Sampling strategy Location map
4.	Sample designation Recording procedures
5.	Sample equipment and procedures Equipment Calibration Sampling procedures
6.	Sample handling and analysis Preservation and holding times Chain-of-custody Transportation
QAPP	
1.	Project description Test goals Critical variables Test matrix Project organization and responsibilities
2.	QA objectives Precision, accuracy, completeness Representativeness and comparability Method detection limits
3.	Sampling procedures and sample custody
4.	Analytical procedures and calibration
5.	Data reduction, validation, and reporting
6.	Internal QC checks
7.	Performance and system audits
8.	Calculation of data quality indicators
9.	Corrective action
10.	QC reports to management
11.	References

sections are generic and applicable to any QAPP and are covered in available documents,[44][67] this guide will discuss only those aspects of the QAPP that are affected by the treatability testing of biodegradation.

5.2.1 Project Description

Section 1 of the QAPP must include an experimental project description that clearly defines the experimental design, the experimental sequence of events, each type of critical measurement to be made, each type of matrix (experimental setup) to be sampled, and each type of system to be monitored. This section may reference Section 4 of the Work Plan. All details of the experimental design not finalized in the Work Plan should be defined in this section.

Items in this section include, but are not limited to, the following:

- Number of samples (areas or locations) to be studied
- Identification of treatment conditions (variables) to be studied for each sample
- Target compounds for each sample
- Number of replicates per treatment condition
- Criteria for technology retention or rejection for each type of remedy selection test.

The Project Description clearly defines and distinguishes the critical measurements from other observations and system conditions (e.g., process controls, operating parameters) routinely monitored. Critical measurements are those measurements or data-generating activities that directly impact the technical objectives of a project. At a minimum, the determination of the target compound (identified previously) in the initial and treated samples will be critical measurements for remedy selection tests. Concentrations of target compounds in all fractions and the oxygen and nutrient availability will be among the critical measurements for RD/RA tests.

5.2.2 Quality Assurance Objectives

Section 2 lists the QA objectives for each critical measurement and sample matrix defined in Section 1. These objectives are presented in terms of the six data quality indicators: precision, accuracy, completeness, representativeness, comparability, and, where applicable, method detection limit.

5.2.3 Sampling Procedures

The procedures used to obtain field samples for the treatability study are described in the FSP. They need not be repeated in this section, but should be incorporated by reference.

Section 3 of the QAPP contains a description of a credible plan for subsampling the material delivered to the laboratory for the treatability study. The methods for aliquoting the material for determination of chemical and physical characteristics, such as bulk density or specific gravity, moisture content, contaminant concentration, etc., must be described.

5.2.4 Analytical Procedures and Calibration

Section 4 describes or references appropriate analytical methods and standard operating procedures to be used for each critical measurement. In addition, the calibration

procedures and frequency of calibration are discussed or referenced for each analytical system, instrument, device, or technique used for each critical measurement.

The methods for analyzing the treatability study samples are the same as those for chemical characterization of field samples. Table 4-6 presents suitable analytical methods. Preference is given to methods in SW-846.[73] Other standard methods may be used as appropriate.[2][3][64] Methods other than GC/MS techniques are recommended to reduce costs, when possible.

5.2.5 Data Reduction, Validation, and Reporting

Section 5 includes, for each critical measurement and each sample matrix, specific presentation of the requirements for data reduction, validation, and reporting. Aspects of these requirements are covered in Subsections 4.5 and 4.6.

5.2.6 Quality Control Reports

Section 10 describes the QA/QC information that will be included in the final project report. At a minimum, reports include:

- Changes to the QAPP
- Limitations or constraints on the applicability of the data
- The status of QA/QC programs, accomplishments, and corrective actions
- Results of technical systems and performance evaluation QC audits
- Assessments of data quality in terms of precision, accuracy, completeness, method detection limits, representativeness, and comparability.

The final report contains all the QA/QC information to support the credibility of the data and the validity of the conclusions. This information may be presented in an appendix to the report. Additional information on data quality objectives[44] and preparation of QAPPs[67] is available in EPA guidance documents.

SECTION 6
TREATABILITY DATA INTERPRETATION

This section is designed to help the site RPM, OSC, or contractor interpret treatability data. The test results and goals for each tier must be evaluated properly to assess the bioremediation potential. Testing results are interpreted in relation to seven of the nine RI/FS evaluation criteria, as appropriate. Subsection 3.2 describes the nine criteria and how they should be addressed for bioremediation.

The remedy screening tier establishes the general applicability of the technology. The remedy selection testing tier demonstrates the applicability of the technology to a specific site. The RD/RA tier provides information in support of the evaluation criteria. The test objectives are based on established cleanup goals or other performance-based specifications (such as removal efficiency).

Subsection 4.6 of this guide discusses the need for the preparation of interim and final reports and refers to a suggested format. In addition to the raw and summary data for the treatability study and associated QC, the treatability report should describe the meaning of the results and how to use these results in the feasibility study for both the screening and selection of alternatives. The report must evaluate the performance of the technology and give an estimate of the costs and schedule for final remediation using the technology.

6.1 TECHNOLOGY EVALUATION

6.1.1 Remedy Screening Phase

Remedy screening treatability studies typically consist of simple laboratory reactor tests. Normally, contaminant concentrations in the matrix are measured before and after treatment. A threshold of greater than 20 percent reduction in contaminant concentration, compared to the abiotic control, indicates that additional treatability studies may be warranted. Before- and after- treatment concentrations normally can be based on duplicate samples at each time period. The mean values are compared to assess the success of the study. A number of statistical texts are available if more information is needed.[5][15][18][70]

When sufficient information is available regarding the contaminant's degradability in the selected media, remedy screening tests may be omitted. This information should be media- and contaminant-specific and may or may not be applicable to other sites.

When the results of a screening study demonstrate that a specific contaminant is biodegradable under laboratory conditions, it should not be assumed that the contaminant will be degraded in a specific soil/site system. Full-scale application, particularly of in situ technologies, requires further site-specific investigation as part of a remedy selection treatability study process.

6.1.2 Remedy Selection Phase

Remedy selection studies should be performed if the results of either the literature review or the remedy screening test indicate that bioremediation is a potential cleanup option. Remedy selection studies are used to identify the technology's performance on a site- and contaminant-specific basis. Costs for these studies generally range from $50,000 to $300,000. Data from remedy selection studies may be used to determine if the technology can meet expected cleanup goals in a reasonable time frame under practical engineering conditions. Data should be used to support the detailed analysis of the alternative with respect to seven of the nine RI/FS evaluation criteria presented in Subsection 3.2. Treatability data analysis during a remedy selection study is demonstrated in Example 9.

When interpreting data relating to contaminant disappearance, RPMs are cautioned against making claims based solely on substrate removal. To accurately assess risk reduction, changes in toxicity, mobility, or volume, and the long-term implications of treatment, RPMs must first determine the extent to which the contaminant has mineralized and the concentration and characteristics of any intermediate byproducts remaining in the media. Ideally, this may be assessed using a mass balance approach. The concentrations of contaminants as well as any added substrate, metabolites, electron acceptors, radiolabeled compounds, and nondegradable tracers generated or introduced to the media should be determined. Data pertaining to initial (baseline), intermediate, and final contaminant and byproduct concentrations should be analyzed. Nonbiological removal mechanisms also must be considered during data interpretation. Section 4.2.11 provides information on the use of biologically inhibited controls to determine the impact of nonbiological removal. During data interpretation, the contaminant concentrations in the test and control cells should be compared. The difference in mean contaminant concentrations between the test and control cells will indicate whether a statistically significant amount of bio-

Example 9

A remedy selection treatability study was performed to evaluate a slurry-phase technology's ability to remediate an impoundment contaminated with petroleum refinery sludges. Surfactants and nutrients were added. Reactor performance was monitored by measuring the oxygen uptake rate and O&G removal. Based on extensive experience with O&G biodegradation, toxicity testing was not performed.

The average initial O&G concentration in the sediment was 41,000 ppm, the maximum concentration expected in the full-scale (70,000 gallon), slurry bioreactor. A cleanup goal of 20,000 ppm O&G was targeted during the study. After 4 weeks the average O&G concentration in the inhibited control was reduced to 39,000 ppm, a reduction of nearly 5 percent. The average O&G concentration in the biologically active system was reduced to 14,000 ppm, a 66 percent reduction in the same time period. The leveling out of O&G concentrations at the end of the experiment indicates that the maximum extent of biodegradation achievable under the test conditions had been reached.

	O&G				
Sample	T_0	T_1	T_2	T_3	T_4
Bioreactor					
Replicate 1	39,000	32,000	21,000	13,000	14,000
Replicate 2	41,000	34,000	24,000	15,000	16,000
Replicate 3	43,000	39,000	24,000	17,000	12,000
Mean Value	**41,000**	**35,000**	**23,000**	**15,000**	**14,000**
Inhibited Control					
Replicate 1	39,000	36,000	37,000	37,000	42,000
Replicate 2	41,000	39,000	40,000	41,000	36,000
Replicate 3	43,000	42,000	40,000	39,000	39,000
Mean Value	**41,000**	**39,000**	**39,000**	**39,000**	**39,000**

The average contaminant concentration in the slurry-phase bioreactor, at each time-point, is compared to the average contaminant concentration in the inhibited control, at the same time-point, to measure the biodegradation at that time-point. The inhibited control accounts for contaminant losses due to volatilization, adsorption to soil particles, and chemical reactions. Some contaminant loss in the control due to biodegradation may occur since total sterilization is difficult to accomplish. However, an O&G analysis of the extract generated from the slurry-phase reactor indicated that abiotic losses were due mainly to adsorption. Since a statistically significant difference between the test and control means exists, O&G reductions in the test bioreactor were attributed to biodegradation.

degradation is occurring. As discussed in Section 4.2.11, the effectiveness and possible side-effects of the sterilizing agents added to the control cells must also be considered during data interpretation.

When the final contaminant concentration is below detection limit, it must be reported as such. For example, if the detection limit is 100 mg/kg and the contaminant was not detected, the final concentration must be reported as "less than 100 kg/mg." If the initial concentration was 200 mg/kg, the removal efficiency must be reported as "greater than 50 percent," even though the actual removal efficiency may be significantly higher than 50 percent. In some cases, it may be possible to avoid this situation by selecting an analytical method with a lower detection limit.

For remedy selection treatability testing, however, the ability of the technology to meet cleanup goals is much more important than the removal efficiency. To provide a decisive evaluation of a technology's ability to reduce a contaminant concentration below the cleanup goal, the final concentration of that contaminant should be analyzed using a method detection limit that is less than or equal to the cleanup goal. If this is not done, a meaningful evaluation of the technology's ability to remediate the site cannot be performed.

In addition to contaminant concentrations, data showing increased microbial counts, oxygen consumption, and carbon dioxide evolution often are considered indicative of contaminant biodegradation. While these data do indicate biological activity, accurate data interpretation must consider the possibility that the bacteria are consuming background carbon rather than the contaminants.

RPMs also are cautioned against attributing improvements in performance to specific characteristics of the treatment process (e.g., microbial supplementation) without first:

- Verifying whether similar removals are experienced in a control cell in which this specific characteristic is varied
- Determining whether other mechanisms, not related to the technique under discussion, were actually responsible for the removal.

Data should be analyzed to determine the impact operating parameters (such as pH, temperature, nutrient and oxygen concentrations, etc.) have on performance (i.e., contaminant and byproduct concentrations, microbial activity, oxygen uptake rates, CO_2 evolution). The resulting information then can be used to refine both time and cost estimates and to identify specific operating parameters for the next level of testing. Potential pretreatment and post-treatment requirements may also be identified.

When evaluating the technology, a rational scale-up from the remedy selection study to full-scale application must be made. Realistic but conservative estimates should be sought for actual treatment efficiencies, times, and schedules. Less than ideal (i.e., laboratory-based) conditions in the field must be identified and compensated for when scaling up from a laboratory-based study to a field study. Best and worst case scenarios should be used to define operational parameters.

A sufficient number of data points and replicates must be obtained in order to perform a valid statistical analysis of the technology. The data must comply with established criteria for precision, accuracy, completeness, method detection limits, representativeness and comparability. An established relative percent difference (RPD) between either the matrix duplicates or between the matrix spike/matrix spike duplicates should be defined in order to assess precision. If QA objectives for precision and accuracy are not met, the precision and/or accuracy of the derived removal efficiency are decreased. Similarly, if completeness objectives are not met (i.e., the ratio of the number of valid measurements to the total number of measurements planned), then the confidence limits associated with the results will be decreased. Strict adherence to the analytical methods and defined calibration procedures is critical to the validity of the generated data. Results generated by an unauthorized method, an unapproved deviation from the standard protocol, or during the operation of uncalibrated or malfunctioning equipment should be rejected. Data lying outside of specified acceptance limits established about the arithmetic mean of the project's entire data set should be identified but not used when determining overall project results.

As mentioned in Subsection 4.5, data following a normal distribution can be analyzed using ANOVA techniques and other statistical methods.[18][70] In some instances nonparametric evaluations may be more appropriate. Models (conceptual, mathematical, and physical) also may be used as a focus for data integration. Both stochastic and deterministic models may be used to identify limiting mechanisms and critical parameters. Zero- and first-order reaction rate models are commonly used to describe the rate of contaminant degradation as a function of contaminant concentration. Zero-order reaction rates are unaffected by the changes in constituent concentration. In contrast, the rate of contaminant transformation during first-order reactions is proportional to the constituent concentration. Generally, the first-order rate model is more widely used because of the model's apparent effectiveness in describing observed results.

Mathematical modeling also can be used to predict the fate and behavior of organic constituents in a contaminated soil system. Modeling results can help identify the potential for air, leachate, or subsoil contamination. The RITZ and VIP models commonly are used. Both models simulate vadose zone processes, including volatilization, degradation, sorption/desorption, advection, and dispersion; however, the VIP model also accounts for the dynamic behavior of organic constituents in unsaturated soil systems under conditions of variable precipitation, temperature, and waste loading. Data regarding physical abiotic loss mechanisms and constituent partitioning within the soil should be developed to ensure that modeling results account for contaminant losses due to both biological and abiotic mechanisms.

Mathematical modeling should not, however, be used to project cleanup levels below those attained during treatability testing. Reaction rates can be used to interpolate data (i.e., to project the time required to reach a contaminant concentration between the initial and final concentrations measured during testing), but should not be used to extrapolate data beyond the final concentration achieved during testing. This recommendation should be strictly observed because, as discussed in Section 2.2.4, biodegradation is an asymptotic process. The concentration at which the contaminant removal rate is very close to zero represents, from a practical perspective, the lowest concentration that can be achieved by the bioremediation technology being tested.

If required, several bioremediation processes can be evaluated simultaneously to determine which process or combination of processes is most appropriate for the cleanup of a given site. For example, if the contaminated materials at a site can be effectively remediated with either a solid-phase or a slurry-phase biological treatment process, both of these processes may be evaluated simultaneously. The biodegradation rates measured during the solid-phase and slurry-phase remedy selection evaluations can then be used to estimate the treatment time, equipment, and land area required by each treatment process. This procedure permits determination of which process or combination of processes can most cost-effectively achieve the required cleanup levels in the required period of time. If sufficient design and cost information are acquired during the remedy selection tests to permit full-scale system design, further RD/RA testing may be unnecessary.

6.1.3 Remedial Design/Remedial Action Phase

RD/RA testing is the third level of testing in the RI/FS process. The cost of these studies generally ranges from $100,000 to $500,000. As discussed in the preceding paragraph, RD/RA studies are not always required. When RD/RA tests are performed, they are typically post-ROD. Therefore, if RD/RA testing is conducted, it should produce the data required for final full-scale RD/RA and costing. The RD/RA testing program is usually conducted on site and should test all equipment and processes so that accurate specifications can be made for the full-scale system.

Example 10 demonstrates the decision process from remedy screening, through remedy selection testing, to the RD/RA. This example is a continuation of Example 6 in Subsection 4.1 of this guide.

The size and scope of the RD/RA testing programs may be decided by several factors, including the quantity of material available for testing, the complexity of the process, the cost, the available time, and the equipment availability. When an RD/RA test is being setup, it is important that the equipment be sized so that realistic scale-up factors can be used for designing a full-scale operation.

In conclusion, technologies generally are evaluated first at the remedy screening level and progress through remedy selection testing to the RD/RA tier. A technology may enter, however, at whatever tier or level is appropriate based on available data about the technology and site-specific factors. For example, a technology that has been studied extensively may not warrant remedy screening testing to determine whether it has the potential to work. Rather, it may go directly to remedy selection testing to determine if the performance standards can be met.

6.2 ESTIMATION OF COSTS

Before considering technologies for RD/RA testing, complete and accurate cost estimates are required. Consequently, when making preliminary cost estimates for full-scale bioremediation, achievable cleanup levels, degradation rates, the concentration and application frequency of various degradation-enhancing supplements (e.g., nutrients, lime, water), contaminant migration controls, and monitoring requirements must be considered. The impact these parameters have on labor, analytical, material and energy costs, as well as the unit's design and possible pre- and post-treatment requirements, also must be considered.

Generally, large-scale field tests can be designed to simulate full-scale performance and costs more accurately than smaller laboratory studies. However, estimating full-scale cost from treatability study data still can be difficult. Given the variability and interaction of factors such as soil temperature, moisture, heterogeneous contaminant concentrations, and optimal amendment concentrations, empirical results may not always depict the range of reasonable bioremediation results. One approach to examining the variability and interaction of these factors is simulation modeling. Simulation models (e.g., Monte Carlo Models) attempt to quantify the probability of a certain set of events or values occurring, based on available empirical data. Using probabilistic simulation methods can produce time and cost estimates for a particular confidence interval and a specific level of certainty (i.e., the researchers can state with 90 percent certainty that the cost of he project will be within +40 percent of the estimate.) Additional information on probabilistic simulations is available in most statistical textbooks.[33]

Example 10

Despite the reduction in PCP concentration during the remedy selection testing tier of treatability testing, the percentage of degradation, as compared to the control, indicated that the process may have been inhibiting microbial activity. The RPM decided to investigate mixing less-contaminated soil with the highly contaminated soil to lower PCP concentrations and stimulate biodegradation. Remedy selection testing, using the design modification suggested by the remedy screening studies, resulted in an average removal of 93 percent of the PCP. RD/RA testing was performed to provide design information for a full-scale system, which was used to remediate the site successfully.

SECTION 7
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APPENDIX A
COMPENDIUM OF TOOLS

There are a number of tools available that can be useful during bioremediation remedy selection treatability studies. Specific tools are briefly described in Tables A-1 through A-6. Additional information on bioremediation testing tools can be found in various references, including the ASTM Standards on Materials and Environmental Microbiology[a]. Other references that may provide further information are listed in Subsection 2.2.1 of this guide. Definitions for unfamiliar technical terms may be found in the Dictionary of Microbiology [b] or the Dictionary of Biotechnology[c]

Table A-1. Tools Used for Toxicity Testing

Tool	Description/Application	Advantages	Disadvantages
Microtox	This automated test measures toxicity to bacteria and can be used to determine whether treatment is reducing the environmental toxicity of leachate from treated soil.	Unit is easy to operate.	Correlation to human and animal toxicity is not clear.
Genotoxicity (40 CFR 798.5100 through 798.5955)[d]	These toxicity tests measure genetic damage to bacteria and other organisms and can be used to determine whether treatment is reducing toxicity to human health.	A good correlation to human and animal toxicity is projected.	An automated testing unit is not yet commercially available.
Seed germination/root elongation (40 CFR 797.2750)[e]	This toxicity test can be used to determine whether treatment is reducing environmental toxicity.	Test is very sensitive to PAHs.	Sensitivities vary for seeds from different plants. Also, this test is not applicable for contaminants that are not water-soluble.
Earthworms	This toxicity test measures impact on earthworm deaths and can be used to determine whether treatment is reducing environmental toxicity.	Applicable for determining soil toxicity.	Not appropriate for determining aquatic toxicity.
Cerio daphnia	This toxicity test measures impact on cerio daphnia and can be used to determine whether treatment is reducing environmental toxicity.	Applicable for determining the toxicity of aqueous media including leachates.	Cannot be used to directly measure soil toxicity.
Fathead minnows	This toxicity test measures impact on fathead minnows and can be used to determine whether treatment is reducing environmental toxicity.	Applicable for determining the toxicity of aqueous media including leachates.	Cannot be used to directly measure soil toxicity.
Genotoxicity (plants)	This toxicity test measures genetic damage to plants and can be used to determine whether treatment is reducing toxicity to the environment.	Extremely sensitive.	Requires lengthy training.

Table A-2. Tools Used to Measure or Describe Biological Activity

Tool	Description/Application	Advantages	Disadvantages
Respirometry	Used to determine biodegradability and reaction kinetics from oxygen consumption or carbon dioxide evolution.	Can be used to test for microbial inhibition; rapid easy to operate.	Oxygen consumption or carbon dioxide evolution due to chemical degradation can yield a false positive.
Fluorocene diacetate	Enzyme-based test used to determine biological activity.		Not readily available.
Resazurin[f]	A redox indicator (can be used to indicate whether anaerobic or aerobic conditions are present).	Easy to use.	---
Microbial assay/enumeration (plate counts, etc.)	Determining the type and number of bacteria present in a sample to determine biological activity.	Standard technique.	May not count the microorganisms of interest.
Epifluorescence microscopy[f]	Determining the total number of active bacteria present in a sample.	---	Measures total bacteria and cannot be used to quantify a certain type of bacteria.[g]
Most probable number (MPN) methods [f]	Estimating the number of microorganisms in a sample that are capable of degrading the contaminants of interest.	Specific to groups of microorganisms with special degradation abilities.	Labor intensive.
Arrhenius equation	Equation used to descibe the temperature dependence of a reaction rate (such as a biodegradation rate).	Allow reaction rate data collected at one temperature to be applied at other temperatures.	Not applicable for all reactions at all temperatures.
Reaction kinetics	Equations used to describe the rate of degradation (or production) of chemical compounds.	Can be used to estimate cleanup times.	Because reactions may be governed by multiple mechanisms or rate-limiting factors, the kinetics may change at low contamination concentrations.

Table A-3. Tools Used to Inhibit Biological Activity

Tool	Description/Application	Advantages	Disadvantages
Formaldehyde	Used to inhibit biological activitity in control cells.	Less hazardous than some inhibitors.	Not always effective; can be degraded by some organisms.
Mercuric chloride	Used to inhibit biological activity in control cells.	Effective.	Use of mercury compounds may be restricted in some laboratories. Not always effective because it can reduce measured petroleum hydrocarbons concentrations.
Sodium azide	Used to inhibit biological activity in control cells (by inhibiting respirometric activity).	Usually effective for aerobic bacteria.	Potentially explosive. Not effective for bacteria that are capable of anaerobic degradation.
Low pH	Used to inhibit biological activity in control cells.	Effective.	---

Table A-4. Tools Used to Develop Mass Balances

Tool	Description/Application	Advantages	Disadvantages
Radiolabeling	Biodegradation studies can be studied using ^{14}C compounds.	Can be used to determine degradation products, removal mechanisms, and mass balances.	Cost can be high.
Liquid scintillation counter	Used to detect radiolabeled compounds.	Can be used to determine degradation products, removal mechanisms, and mass balances.	---

Table A-5. Tools Used for Anaerobic Testing

Tool	Description/Application	Advantages	Disadvantages
McIntosh and Fildes' jar	Closed, flask-like reactor, which employs a gas-pack generator (H_2 and CO_2) and a pallidium catalyst to establish an O_2-free system in which anaerobic testing can be assessed. Can be used to develop an anaerobic culture.	Easy to use.	Subsampling may not be possible.
Roll tube test	Test tubes, containing an agar-like medium and N^2, used to develop an anaerobic culture or to directly assess biodegradation.	Easy to use.	---

Table A-6. Tools Used for Physical Characterization of Soils

Tool	Description/Application
Hydraulic conductivity	A soil property that determines the maxiumum flow rate of water through the soil.
Soil moisture retention[h]	Once determined, the soil moisture retention can be increased or decreased if necessary.
Soil density (ASTM Methods D1556-82 and D2937-83)[j]	Measures soil density.[i]
Particle size analysis of soils (ASTM Method D422-63)[j]	Quantitive determination of the distribution of particle size in soil.
Soil water content (ANSI/ASTM D2216-80 and ASTM D3017-88)[j]	Measures water content of soil.
Specific gravity of soils (ASTM D854-83)[j]	Measures specific gravity of soil.

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