



# Guide for Conducting Treatability Studies Under CERCLA: Biodegradation Remedy Selection

Office of Emergency and Remedial Response  
Hazardous Site Control Division OS-220

**QUICK REFERENCE FACT SHEET**

Section 121(b) of the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) of 1980 mandates EPA to select remedies 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 remedy selection and implementation. They should be performed as soon as it becomes evident that the available information is insufficient to ensure the quality of the decision. Conducting treatability studies early in the remedial investigation/feasibility study (RI/FS) process should reduce uncertainties associated with selecting the remedy and should provide a sound basis for the Record of Decision (ROD). Regional planning should factor in the time and resources required for these studies.

This fact sheet provides a summary of information to facilitate the planning and execution of biodegradation remedy selection treatability studies in support of the RI/FS and the remedial design/remedial action (RD/RA) processes. It is intended to provide Remedial Project Managers (RPMs), On Scene Coordinators (OSCs), Potentially Responsible Parties (PRPs), and other interested persons with enough information to determine whether biodegradation treatability studies may be considered in the remedy selection phase of the RI/FS for the CERCLA site of interest. This fact sheet follows the organization of the "Guide for Conducting Treatability Studies Under CERCLA: Biodegradation Remedy Selection," EPA/540/R-93/514A", 1993. Detailed information on designing and implementing remedy selection treatability studies for biodegradation is provided in the guidance document.

## INTRODUCTION

There are three levels or tiers of treatability studies: remedy screening, remedy selection, and RD/RA testing. Treatability studies conducted during the RI/FS phase (remedy screening and remedy selection) indicate whether the technology can meet the cleanup goals for the site, whereas treatability studies conducted during the 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 remedy selection may also be used to support RD/RA.

Remedy screening studies are designed to provide a quick and relatively inexpensive indication of whether biological degradation is a potentially viable remedial technology. The remedy screening evaluation should provide a preliminary indication that reductions in contaminant concentrations are due to biodegradation and not abiotic processes such as photodecomposition or volatilization.

Remedy selection studies should simulate conditions during bioremediation, allowing researchers to determine the technology's performance on a waste-specific basis. Bench-scale testing is typically used for remedy selection testing; however, it may fall short of providing enough information for remedy selection. Pilot-scale testing also may be appropriate for some sites. Bench-scale studies can, in some cases, provide enough information for full-scale design.

RD/RA testing should provide accurate cost and performance data, confirming that biodegradation rates and cleanup levels determined during remedy selection can be achieved for the site.

This fact sheet and its parent document, the "Guide for Conducting Treatability Studies Under CERCLA: Biodegradation Remedy Selection," EPA/540/R-93/514A primarily focus on the remedy selection tier. These documents also briefly discuss remedy screening and RD/RA testing.

## TECHNOLOGY DESCRIPTION AND PRELIMINARY SCREENING

### Technology Description

Bioremediation generally refers to the breakdown of organic compounds (contaminants) by microorganisms. Bioremediation treatment technologies can be divided into two categories, in situ and ex situ, based upon the location of the contaminated medium during treatment.

- **In Situ**

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.

In situ bioremediation 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. The technology has primarily been used for the treatment of saturated soils. In situ bioremediation is often in conjunction with a groundwater-pumping and soil-flushing system to circulate nutrients and oxygen through a contaminated aquifer and associated soils.

Bioventing is an in situ biological technology predominantly used to treat reasonably permeable, unsaturated soils. Aeration systems, similar to those employed during soil vapor extraction, are used during bioventing to supply oxygen to the soil. An air pump, one or more air injection or vacuum extraction probes, and emissions monitoring at the ground surface are commonly used during bioventing. In order to minimize contaminant volatilization, low air pressures and air flow rates are typically utilized. Some systems, however, utilize higher air flow rates, thereby combining with soil vapor extraction.

- **Ex Situ**

Ex situ biological treatment technologies involve removal of the contaminated media followed by onsite or offsite treatment. Although media handling increases the costs of ex situ treatment, ex situ approaches generally allow greater control of process variables (e.g., pH, nutrient concentrations, temperature, aeration).

Solid-phase bioremediation (sometimes referred to as land treatment or land farming) is a process that treats soils in above-ground treatment systems using conventional soil management practices to enhance microbial degradation of contaminants. Solid-phase bioremediation at CERCLA sites usually involves placing excavating soil in an above-ground soil treatment area. 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.

In slurry-phase bioremediation, excavated contaminated soil is typically placed in an onsite, stirred-tank reactor 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. The water used in the process can be contaminated surfacewater or groundwater, thus facilitating the simultaneous treatment of contaminated soil and water. As with solid-phase bioremediation nutrients and microorganisms may be added to the reactor to facilitate biodegradation.

Soil heap bioremediation involves piling contaminated soil in heaps several meters high. 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 enclosing the soil pile and passing the exhaust from the exhaust from the vacuum through activated carbon biofilters.

Composting involves the storage of biodegradable waste with a bulking (e.g., chopped hay or wood chips). The structurally-firm bulking agent is usually biodegradable. 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 three basic types of composting systems are open windrow

(where the piles are torn down and rebuilt for aeration), static windrow (where air is forced into the piles), and in-vessel (where tumbling, stirring, or forced aeration are used).

Biofilters can be used to treat organic vapors in a manner analogous to the biological treatment of wastewaters. By providing bacteria with a surface on which to grow and optimal oxygen, temperature, nutrients, moisture, and pH conditions, biofilters can significantly reduce vapor phase organic contaminants. The primary components of biofilters are: an air blower, an air distribution system, filter media, and a drainage system. Removal efficiencies in the range of 95 to 99 percent have been reported for light aliphatic compounds, while lower removal efficiencies are common for chlorinated aliphatic and aromatic compounds.

## Technology Status

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

## 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 an unlikely candidate, further research may be warranted. Before discarding biological remediation as an option, expert recommendations regarding the technology's potential should be obtained. The "Guide for Conducting Treatability Studies Under CERCLA: Biodegradation Remedy Selection", EPA/540/R-93/514A, lists references and electronic databases that can be useful when conducting the literature search phase of a bioremediation project. The guide also provides contacts for technical assistance when determining the need or scope of a remedy selection treatability study. One important resource for OSCs and RPMs is the Technical Support Project (TSP) coordinated by EPA's Technology Innovation Office (703-308-8846). The TSP is operated by EPA laboratories and offers technical assistance ranging from review of contractor work plans to assistance in the performance of treatability studies.

The potential biodegradability of the contaminants of concern is an important characteristic to be examined prior to initiating treatability studies. Examples of classes of compounds that are readily amenable to bioremediation are: petroleum hydrocarbons such as gasoline and diesel; wood treating wastes such as creosote and pentachlorophenol; solvents such as acetone, ketones, and alcohols; and aromatic compounds such as benzene, toluene, xylenes, and phenols. Several documents and review articles that present detailed information on the biodegradability of compounds are listed in the reference section of the complete guidance document. However, discretion should be exercised when using these reference materials, as microorganisms that can

biodegrade compounds that have traditionally been considered nonbiodegradable are continually being isolated through ongoing research and development efforts.

Site and soil characteristics that impact bioremediation are listed in Table 1. The potential effects of these factors upon candidate biodegradation technologies should also be considered.

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 may provide the information required to make a decision. However, when in doubt, treatability studies are recommended.

## Technology Limitations

Many factors impact the feasibility of biodegradation. These factors should be addressed prior to the selection of

**Table 1. 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	X
Bulk density*	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 factors	X	
Groundwater flow patterns and characteristics	X	
Meteorological 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.

biodegradation and prior to the investment of time and funds in further testing. Some of these factors that may limit the use of bioremediation technologies include the amount, location, extent, and variability of the contamination. The physical form in which the contaminants are distributed, as well as heterogeneities within the media to be treated, may limit the applicability of biodegradation.

Soil characteristics, such as nonuniform particle size distribution, soil type, moisture content, hydraulic conductivity, and permeability, can also significantly affect biodegradation. Significant quantities of organic matter (humus, peat, non-regulated anthropomorphic compounds, etc.) also may cause high oxygen uptake rates, resulting in depleted oxygen supplies during in situ application. Contaminant volatility is particularly important, especially in stirred or aerated reactors where the contaminants can volatilize before being degraded.

The presence of either an indigenous or introduced microbial population capable of degrading the contaminants of concern is usually essential to the success of biological processes. 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 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, media, and microbial species.

Although preliminary data may be obtained that seem to indicate that the technology is capable of reducing contamination levels to acceptable limits, the rate of contaminant removal from soil during bioremediation exhibits asymptotic characteristics. The initial rate of removal, after a potential lag period, is rapid. With time, the rate decreases to a near-zero value, and the contaminant concentration in the soil approaches a fixed concentration that is typically nonzero (the asymptote). Since the asymptote is difficult to predict and is sometimes greater than the cleanup criteria, treatability testing must be continued until either the removal goals are met or the asymptote is reached.

## THE USE OF TREATABILITY STUDIES IN REMEDY EVALUATION

Treatability studies should be performed in a systematic fashion to ensure that the data generated can support the remedy evaluation and implementation process. A well-designed treatability study can significantly reduce the overall uncertainty associated with the decision, but cannot guarantee that the chosen alternative will be completely successful. Care must be exercised to ensure that the treatability study is representative of the treatment (e.g., the sample is representative of waste to be treated) as it will be employed to minimize uncertainty in the decision.

### Treatability Testing Process

Treatability studies for a particular site will often entail multiple tiers of testing. By balancing the time and cost necessary to perform the testing with the risks inherent in the decision, the level of treatability testing required can be determined. Criteria for measuring the success of each level of treatability study are listed in Table 2.

Remedy screening is the first level of testing. It is used to determine whether biodegradation is possible with the site-specific waste material. These studies are generally low 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 is required. These studies yield data indicating a technology's potential to meet performance goals.

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 two years to complete. They yield data that verify that the technology is likely to meet expected cleanup goals and can provide information in support of the detailed analysis of the alternative.

RD/RA testing is the third level of testing. By operating a field unit under conditions similar to those expected during full-scale remediation, the study can provide data required for final full-scale design and accurate cost and time estimates. Unit operating parameters can be optimized and the ability to

achieve cleanup levels can be confirmed. 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 are performed during the remedy implementation phase of a site cleanup. Figure 1 shows the relationship of the three levels of treatability study to each other and to the RI/FS process.

### Applicability 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 Remedial Investigations and Feasibility Studies Under CERCLA" (EPA/540/6-89/004). A detailed analysis of different remedial alternatives using the nine CERCLA criteria is essential. Treatability studies provide data for up to 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)

**Table 2. Biodegradation Criteria for Each Treatability Study Tier**

Criteria	Remedy Screening	Remedy Selection	Remedy design
Biodegradation of most-resistant contaminants of concern	>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.

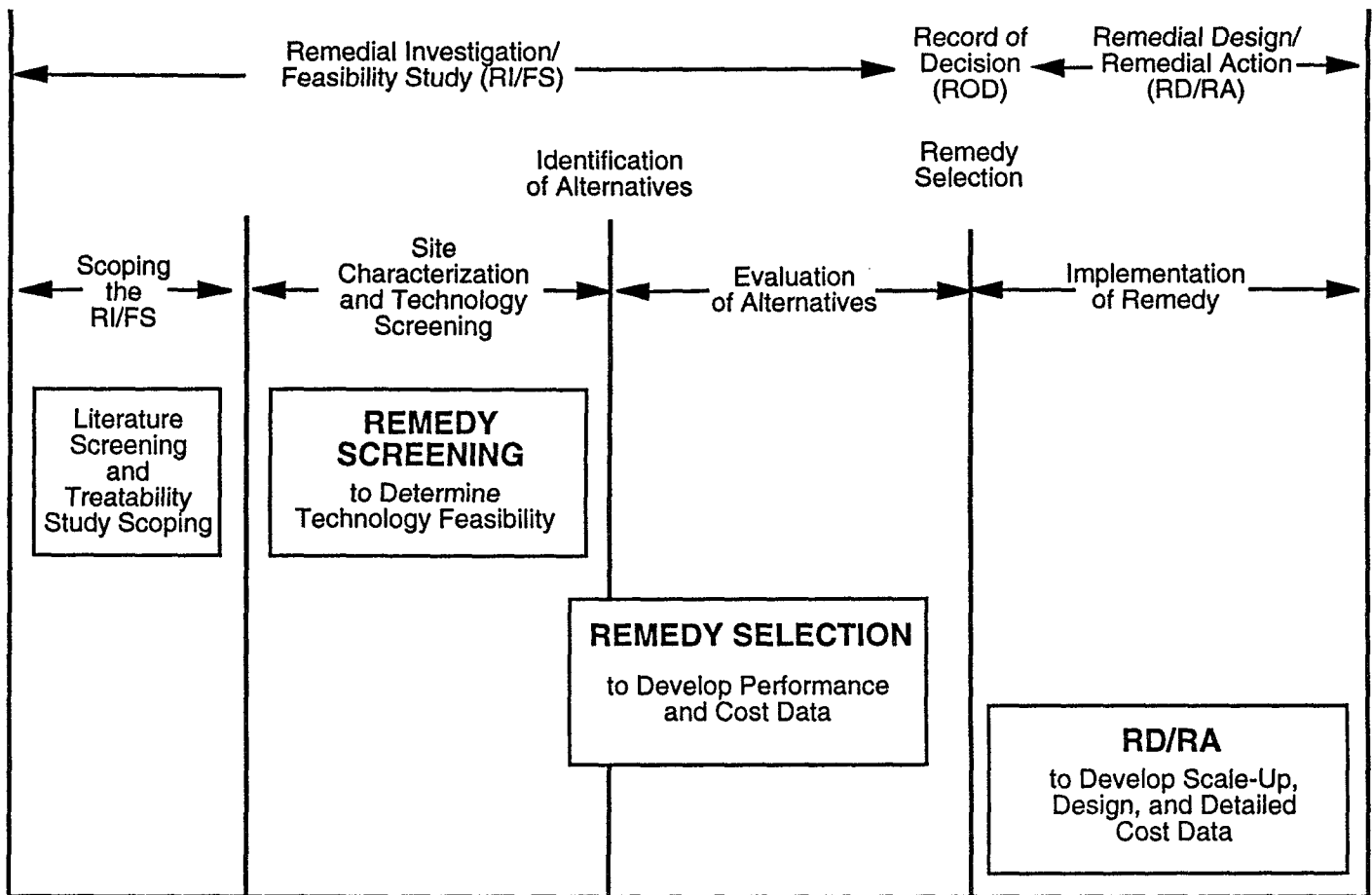


Figure 1. The Role of Treatability Studies in the RI/FS and RD/RA Process.

- ! 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. An available remediation technology may be eliminated from consideration if the state or community objects to its use. Table 3 shows how the study goals of a remedy selection treatability test address RI/FS criteria and the experimental parameters measured to assess the achievement of those goals.

**REMEDY SELECTION TREATABILITY STUDY WORK PLAN**

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 also assigns responsibilities and establishes the project schedule and costs. The Work Plan must be approved by the RPM

before initiating subsequent tasks. A suggested organization of the Work Plan is provided in the "Guide for Conducting Treatability Studies Under CERCLA: Biodegradation Remedy Selection", EPA/540/R-93/514a.

**Test Goals**

Remedy selection treatability goals must consider the existing site contaminant levels and cleanup goals for soils, sludges, and water at the site. The ideal technology performance goals for remedy selection treatability tests are the cleanup criteria for the site. Example remedy selection goals are listed in Table 3. 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 target levels that are risk-based rather than background-based.

**Experimental Design**

Careful planning during treatability study design is required to ensure adequate treatability study data are obtained. Among other requirements, the experiments, the experimental design must identify the critical parameters and determine the required number of replicate tests. Treatability studies can be designed to simulate aerobic conditions, or may be planned to assess biodegradation under anaerobic conditions. Ultimately, remedy selection studies should strive to simulate the conditions encountered during full-scale applications of the technology under study.

**Table 3. 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 <sub>2</sub> 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 study, additional criteria may be applicable.

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

- Overall test objectives (as dictated by site remediation objectives)
- Specific removal goals or desired cleanup levels (as set for a specific site)
- Soil characteristics (soils with higher permeability are more amenable to in situ biodegradation)
- pH (most microbial degraders thrive when the pH is between 6.5 and 8.5)
- Temperature (optimum range is usually between 15EC and 30EC for aerobic processes and 25EC to 35EC for anaerobic processes)
- Moisture (optimum range is usually between 40 and 80 percent of field capacity)
- Nutrients (concentrations should be maintained at a reasonably moderate but steady-state concentration determined experimentally)
- Electron acceptors (usually oxygen derived from air, pure oxygen, ozone, or hydrogen peroxide for aerobic studies and nitrates for anaerobic tests)
- Microorganisms (the use of introduced versus indigenous populations)
- Duration of test (sufficient to determine ability of treatment to meet removal goals)
- Inhibitory compounds and their control (dilution of media may be required)
- Impact of nonbiological removal processes (extent of volatilization, sorption, photodecomposition, leaching, as experienced by inhibited controls)

! Toxicity testing (to evaluate the risk reduction experienced during treatment)

! Bioavailability (contaminants that biodegrade easily will be utilized earliest)

In situ remedy selection treatability studies are either field plot or soil column designs. Soil column studies may also be performed ex situ, usually within a laboratory setting. Three additional ex situ experimental designs are soil pans, soil slurries, and contained soil treatment systems. Table 4 presents information on remedy selection treatability study experimental designs, including their applicability, scale, typical size, and duration.

The test system used during remedy selection testing can consist of a single large reactor or multiple small reactors. Studies which 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. When a single reactor is used, small samples may be removed at various times and compared to samples from control reactors. When using large reactors, care should be taken to ensure that the availability of supplements (i.e., oxygen and moisture) are adequate, allowing for consistent degradation rates within the reactor. Additionally, sampling must be sized so that it does not affect the operation of the overall unit. Remedy selection treatability tests should 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. Contaminant concentrations are measured in both the test reactors and the control reactors at the beginning of the study ( $T_0$ ), 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 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

**Table 4. Remedy Selection Treatability Study Characteristics**

Type of Study	Applicability	Scale	Size	Duration
Field plots	In situ bioremediation	Field-scale	1 to 1,111 yd <sup>2</sup> plot of land*	2 months to 2 years
Soil columns	In situ bioremediation	Lab- and field-scale	0.01 - 3,200 ft <sup>3</sup> 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 <sup>3</sup> to 3,900 <sup>3</sup> yds of soil	10 days to 10 months

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

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 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.

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 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 very low 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 to the extent that a statistically significant difference between the test and control means can be determined.

When designing a treatability study, the types of equipment required for the test must be considered. Standard laboratory equipment such as mixing flasks and sample collection bottles should be available for all treatability studies. A wide variety of equipment is employed during biodegradation treatability testing to contain the media under study or isolate it from the environment. During soil column studies, a metal, plastic, or glass cylinder may be used onsite or offsite as part of a laboratory study. Field plots, on the other hand, may require that in-ground barriers, such as sheets of steel driven into the ground, or above-ground barriers such as berms be used to separate testing plots from one another or from soil located outside of the testing area. Slurry reactors, which range in size from 1 fluid ounce vials to 70,000-gallon lagoons, typically utilize 0.1- to 130-gallon vessels. In contrast, contained soil treatment systems will generally require a bermed, watertight area in which the soil can be placed. The vessels required for contained soil treatability studies also vary considerably, since they may be designed to simulate composting, soil heaping, or other solid-phase biotreatment technologies. Depending on the type and scale of the system, a leachate collection system and other accessories may also be required.

## **SAMPLING AND ANALYSIS PLAN**

The Sampling and Analysis Plan (SAP) consists of two parts: the Field Sampling Plan (FSP) and the Quality Assurance Project Plan (QAPP). A SAP is required for all field activities conducted during the RI/FS. The purpose of the SAP is to ensure that samples obtained for characterization and testing are representative and that the quality of the analytical data generated is satisfactory. 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.

## **TREATABILITY DATA INTERPRETATION**

When conducting treatability studies, the test results and goals for each tier must be properly evaluated to assess the treatment potential of 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.

Interpretation of remedy selection test results should allow the RPM or OSC to determine whether the bioremediation technology used is capable of meeting cleanup standards under simulated (or actual) site conditions. The experimental design of the study should have been constructed to produce quantitative and statistically defensible estimates of the extent and rate of biodegradation. Ideally, a statistical evaluation of the difference between biodegradation rates when parameters such as nutrient addition, loading rate, and microbial composition are varied, should also be designed. Example 1 describes a remedy selection treatability test and the interpretation of the test results.

## **Estimation of Costs**

Complete and accurate cost estimates are required in order to fully recommend technologies for site remediation. Consequently, when making preliminary cost estimates for full-scale bioremediation, achievable cleanup levels, degradation rates, concentration and application frequencies of various degradation enhancing supplements (e.g., nutrients, lime, water, etc.), 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 laboratory studies. However, estimating full-scale cost from treatability study data can be difficult. Given the variability and interaction of factors such as soil temperature, pH, moisture, heterogenous contaminant concentrations, and optimal nutrient 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 that a certain set of events or values will occur based upon available empirical data. Using probabilistic simulation methods can produce time and cost estimates for a particular confidence level and a specific level of certainty (e.g., the ability to state with 90 percent certainty that the cost of the project will be within  $\pm 40$  percent of the estimate).

## **TECHNICAL ASSISTANCE**

Information from existing literature and consultation with experts are important factors in determining the need for and ensuring the usefulness of treatability studies. A reference list of sources on treatability studies is provided in the "Guide for Conducting Treatability Studies Under CERCLA" (EPA/540/R-92/071a).

It is recommended that a Technical Advisory Committee (TAC) be used. This committee includes experts who provide technical support from the scoping phase of the treatability study through data evaluation. Members of the TAC may include representatives from EPA (Regions or ORD), other Federal agencies, States, and consulting firms.

The Office of Solid Waste and Emergency Response and Office of Research and Development operate the TSP which provides assistance in the planning, performance, and review



### Example 1

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 oil and grease (O&G) removal. Based on extensive experience with O&G biodegradation, toxicity 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.

Sample	O&G				
	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
<i>Bioreactor</i>					
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
<b>Mean Value</b>	<b>41,000</b>	<b>35,000</b>	<b>23,000</b>	<b>15,000</b>	<b>14,000</b>
<i>Inhibited Control</i>					
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
<b>Mean Value</b>	<b>41,000</b>	<b>39,000</b>	<b>39,000</b>	<b>39,000</b>	<b>39,000</b>

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.

of treatability studies. For further information on treatability study support or the TSP, please contact:

#### Groundwater Fate and Transport Technical Support Center

Robert S. Kerr Environmental Research Laboratory, (RSKERL)  
Ada, OK 74820  
Contract: Don Draper  
(405) 332-8800

#### Engineering Technical Support Center (ETSC)

Risk Reduction Engineering Laboratory (RREL)  
Cincinnati, OH 45268  
Contact: Ben Blaney or Joan Colson  
(513) 569-7406 or (513) 569-7501

### FOR FURTHER INFORMATION

Sources of information on treatability studies and bioremediation are listed in the "Guide for Conducting Treatability Studies Under CERCLA" (EPA/540/R-92/071a) and the "Guide for Conducting Treatability Studies Under CERCLA: Biodegradation

Remedy Selection" (EPA/540/R-93/541A). Additionally, the Office of Emergency and Remedial Response's Hazardous Site Control for each Region should be contacted for information and assistance.

### ACKNOWLEDGMENTS

This fact sheet and the corresponding guidance document were prepared for the U.S. Environmental Protection Agency, Office of Research and Development, Risk Reduction Engineering Laboratory, Cincinnati, Ohio by Science Applications International Corporation (SAIC) under Contract No. 68-C8-0061 and Contact No. 68-C0-0048. Mr. Ed Opatken served as the EPA Technical Project Monitor. Mr. Jim Rawe served as SAIC's Work Assignment Manager. Mr. Rawe, Ms. Evelyn Meagher-Hartzell, and Ms. Sharon Krietemeyer (SAIC) were the primary technical authors. Mr. Derek Ross (ERM) and Mr. Kurt Whitford (SAIC) served as a technical experts.

Many Agency and independent reviewers have contributed their time and comments by participating in the expert review meetings or peer reviewing the guidance document.