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EPA GUIDE FOR IDENTIFYING CLEANUP ALTERNATIVES AT HAZARDOUS-WASTE SITES AND SPILLS: BIOLOGICAL TREATMENT

Prepared for
Office of Emergency and Remedial Response
U.S. Environmental Protection Agency

Prepared by
Pacific Northwest Laboratory
Operated for the U.S. Department of Energy
by Battelle Memorial Institute

Project Management by
Corvallis Environmental Research Laboratory
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Corvallis, Oregon

DISCLAIMER

This manual describes the scientifically relevant and important functional activities that concern regional, state, and local authorities during the response to hazardous substance releases It is intended to convey technical recommendations only and not to constitute agency policy The manual should not supersede specific procedures and documentation for quality assurance, chain-of-custody, and other requirements addressed by Environmental Protection Agency regulations and policy guidance documents currently in effect The research described in this report has been funded by the United States Environmental Protection Agency through interagency agreement no AD-89-F-2A115 to the United States Department of Energy. This report has been subjected to the Agency's required peer and policy review

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PREFACE

The Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA) grants the President the authority to respond to releases of hazardous chemical substances that imminently and substantially threaten public health or welfare, or the environment The Act, which establishes a $1 6-billion Superfund to finance response actions, and which charges the Environmental Protection Agency (EPA) with administering critical portions of the response program, was designed to build on the existing environmental response authority given to EPA under Section 311 of the Clean Water Act

In accordance with this mandate, the EPA is preparing a series of documents to assist state and regional officials who are responsible for instituting cleanup actions at specific hazardous-waste sites This guide deals exclusively with biological processes as tools for cleaning up a site

This guide supplies the information and sources that an official needs to decide how to proceed at a contaminated site This document also can be used by management to assist in and overview staff cleanup activities. Additionally, it is a training tool for persons who are new to the business of cleaning up hazardous-waste sites Finally, it is a resource for anyone who wishes to know more about the cleanup of hazardous-waste sites

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EXECUTIVE SUMMARY

Under the provisions of Superfund (CERCLA of 1980), the Environmental Protection Agency (EPA) has been charged with responsibility for cleaning up hazardous-waste disposal sites This guide provides direction to on-scene personnel who must assess hazardous wastes in the environment and may want to implement biological cleanup methods

Treatment for hazardous wastes are broadly grouped into physical, chemical, and biological methods This document emphasizes biological treatment, but all three methods can be integrated, as appropriate, to achieve optimal effectiveness Physical or chemical pretreatment may be necessary prior to biotreatment if a waste is toxic to microorganisms in its current form or concentration Almost all organic compounds and some inorganic compounds can be degraded microbiologically, given suitable physical and chemical conditions and sufficient time to do so

Pre-treatment site assessment is critical to provide the background data necessary to evaluate and select treatment methods Information is needed about the wastes and their concentrations, the danger of the wastes to workers, the features of the site environment, and the biotransformation capacity of microorganisms Post-treatment site monitoring is recommended to establish effectiveness of the methods applied

Biological methods can be applied to hazardous wastes either onsite or offsite Biological methods include a no-treatment option (natural biodegradation), which relies on detoxification of wastes by natural processes Biological treatment of hazardous wastes on soil is called land treatment and includes both direct application and compositing Biological methods can also be applied with special facilities developed for wastewater treatment These facilities, called bioreactors, include the activated-sludge, trickling filter, rotating disc, and aerobic and anaerobic lagoon or pond systems Hazardous wastes may also be cleaned from a site by bioaccumulation and removal techniques.

Biotreatment of hazardous waste may be enhanced by controlling environmental conditions Biotransformation of hazardous materials can be aided by dilution and by chemical and physical pre-treatment Environmental conditions in land treatment and bioreactor systems can also be enhanced to favor growth and enzymatic activities of microorganisms Enhancement of the waste environs includes adjustment of pH, temperature, nutrient levels, and aeration In some cases, specialized microorganisms can be developed and introduced to aid microbial transformation Examples of such organisms are generic microorganisms that are indigenous to the waste, the site, or the system, enriched microorganisms that are cultured to degrade a specific waste, or adapted microorganisms that are cultured for site-specific conditions

The advantages and disadvantages of various biological treatment methods are discussed in the text. Diagrams are provided to help personnel decide which treatment to select The relative effectiveness of different biotreatment procedures at locations with hazardous wastes is largely site-specific Relative effectiveness can be expected to vary from site to site because environmental parameters will vary, as will types, quantities, and conditions of hazardous-waste materials Thus, different methods may be favored at different locations.

CONTENTS

PREFACE iii

ACKNOWLEDGMENTS v

EXECUTIVE SUMMARY vii

1 0 INTRODUCTION 1 1

2 0 BIOTREATMENT OPTIONS 2 1

3 0 SELECTION OF POTENTIAL BIOTREATMENT OPTIONS 3.1

3 1 IS BIOTREATMENT VIABLE AT THE SITE? 3 1

3 2 CAN THE WASTE AT THE SITE BE TREATED BIOLOGICALLY? 3 3

3.3 WHAT QUESTIONS MUST BE ANSWERED TO EVALUATE BIOTREATMENT OPTIONS? 3 5

4 0 SITE-SPECIFIC CONSIDERATIONS AT HAZARDOUS-WASTE SITES OR SPILLS 4 1

4 1 WHAT INFORMATION SHOULD BE COLLECTED BEFORE TREATMENT? 4 1

4.2 WHAT INFORMATION SHOULD BE COLLECTED AFTER TREATMENT? 4 15

4.3 CHAPTER REFERENCES 4.24

5.0 DESCRIPTION OF BIOTREATMENT OPTIONS 5 1

5 1 NATURAL BIODEGRADATION 5 1

5 2 LAND TREATMENT 5 1

5 3 BIOREACTOR SYSTEMS 5 7

5 4 BIOACCUMULATION AND REMOVAL 5 15

5 5 ENHANCEMENT OF BIOTREATMENT PROCESSES 5 17

5.6 CHAPTER REFERENCES 5 27

6 0 BIOTRANSFORMATION CONCEPTS 6.1

6 1 ROLE OF MICROORGANISMS IN BIOTRANSFORMATION 6 1

6.2 BIOTRANSFORMATION PROCESSES 6 6

6 3 RECALCITRANT COMPOUNDS 6 14

6.4 BIOACCUMULATION 6 18

6 5 ACTIVATION 6 20

6.6 METALS—SPECIAL CASES 6 21

6 7 ENVIRONMENTAL INFLUENCES 6.23

6 8 CHAPTER REFERENCES 6.25

FIGURES

1 1 Summary of Guide Chapters as They Relate to Specific User Needs 1 1

1.2 Steps in Remedial Action Process for Responding to an Uncontrolled Hazardous-Waste Site or Spill 1 2

2 1 Concept of Onsite and Offsite Treatment Options Used for Biotransforming Hazardous Wastes 2 1

3 1 Selection of Biotreatment as a Cleanup Option 3 1

3 2 Flow Chart for Determining Whether or Not Chemicals Can Be Treated Biologically 3.4

4 1 A Flow Chart for Categorizing Priority Pollutants 4 11

4 2 Decision Matrix for Planning Post-Treatment Monitoring 4.18

5.1 Matrix Comparison of Advantages and Disadvantages of Biotreatment Options for Hazardous Wastes 5 4

5 2 Decision Process for Evaluating Land Application (Including Composting) for Biotreatment of Hazardous Wastes 5 6

5 3 Decision Process for Evaluating Activated Sludge Biotreatment 5 10

5 4 Decision Process for Evaluating Trickling Filter Biotreatment 5 11

5 5 Decision Process for Evaluating Rotating Disc Biotreatment 5 13

5 6 Decision Process for Evaluating Lagoon and/or Pond Treatment 5 14

5 7 Decision Process for Evaluating Bioaccumulation by Algae and Higher Plants (Autotrophs) 5 17

5 8 Decision Process for Determining if Enhancement is Required for Biotreatment 5.20

6 1 Hypothetical Model for Population Changes and Metabolism of a Chemcal Exposed to Mineralizing and Co-metabolizing Populations . 6.8

TABLES

4 1 EPA List of Hazardous Chemical Compounds 4.6

4 2 Known or Suspect Carcinogenic Materials 4 7

4 3 Compounds, Category Rank and Environmental Compartments of EPA Priority Pollutants 4 8

6.1 A Summary of Microorganisms Involved in Biotransformation of Chemical Compounds and Their Selective Characteristics 6.3

6 2 Information on the Biotransformation of Chemical Compounds, Including Reaction Type, Microorganisms Involved, Condition, and Transformation Products 6 9

6.3 Relative Persistence of Selected Pesticides in Soil Under Natural Conditions 6 15

6 4 Some Organic Compounds that Persist in Soils and Water for Relatively Long Periods of Time 6.17

6.5 Some Synthetic Polymers Resistant to Transformation by Microorganisms 6 17

6 6 Examples of Bioaccumulation of Synthetic Compounds by Different Microorganisms 6 18

1.0 INTRODUCTION

WHY THIS GUIDE?

This document provides guidance to help decide whether or not biological treatment is an appropriate component of the remedial action process at a hazardous-waste site or spill The guide describes biological treatment methods, conditions and factors that limit the use of biotreatment, and types of data needed to evaluate and select biotreatment methods Flow charts are included to help users determine the usefulness of each biotreatment method at specific hazardous-waste sites and spills

WHO CAN USE THIS GUIDE?

This guide is intended for use primarily by people who have limited experience in participating in or coordinating cleanup activities at uncontrolled hazardous-waste sites In addition to being a training tool, an educational resource, and a source of assistance during remedial-action analysis, this guide may be used by managers to maintain a broad overview of cleanup programs

This guide contains material designed to serve many different users (Figure 1 1) **As a decision-making tool,** the document supplies information and sources of information needed for users to make decisions about cleanup activities

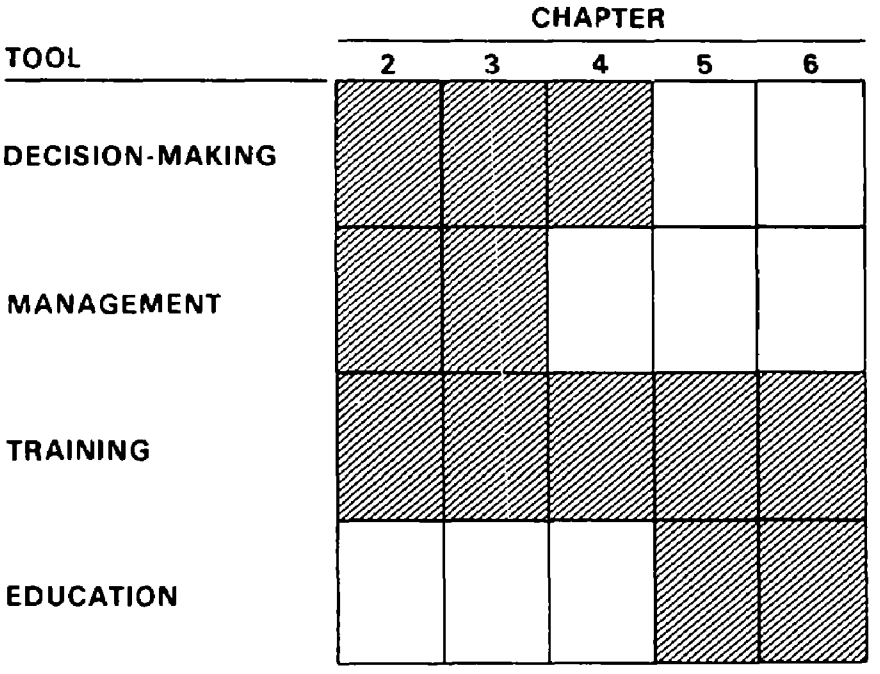

FIGURE 1.1 Summary of Guide Chapters as They Relate to Specific User Needs

As a management tool, the document contains flow charts that will help the user select cleanup alternatives It also lists advantages and disadvantages of various treatment methods to assist in and overview staff cleanup activities

As a training tool, the document reviews biotransformation techniques, discusses information needs related to selection of cleanup alternatives, and outlines assessment activities required at most cleanup sites

As an educational tool, the document reviews basic information that a user needs to understand biological cleanup methods Obviously, all the biological, chemical, and physical information necessary to understand biotransformation of hazardous waste cannot be discussed in a guide More complete information can be obtained by reading the references cited.

WHERE DOES THIS GUIDE FIT INTO THE REMEDIAL ACTION PROCESS?

This guide is intended for use during the time when chemical, physical and biological cleanup alternatives are being identified, that is, after the waste and site have been characterized and health risks have been assessed, and before the cleanup alternatives are analyzed in detail (see shaded area, Figure 1 2) The guide is not intended as a design guide nor as an encyclopedic guide for analyzing remedial-action alternatives Rather, when used, it ensures that biological methods are given proper consideration during the development of cleanup alternatives

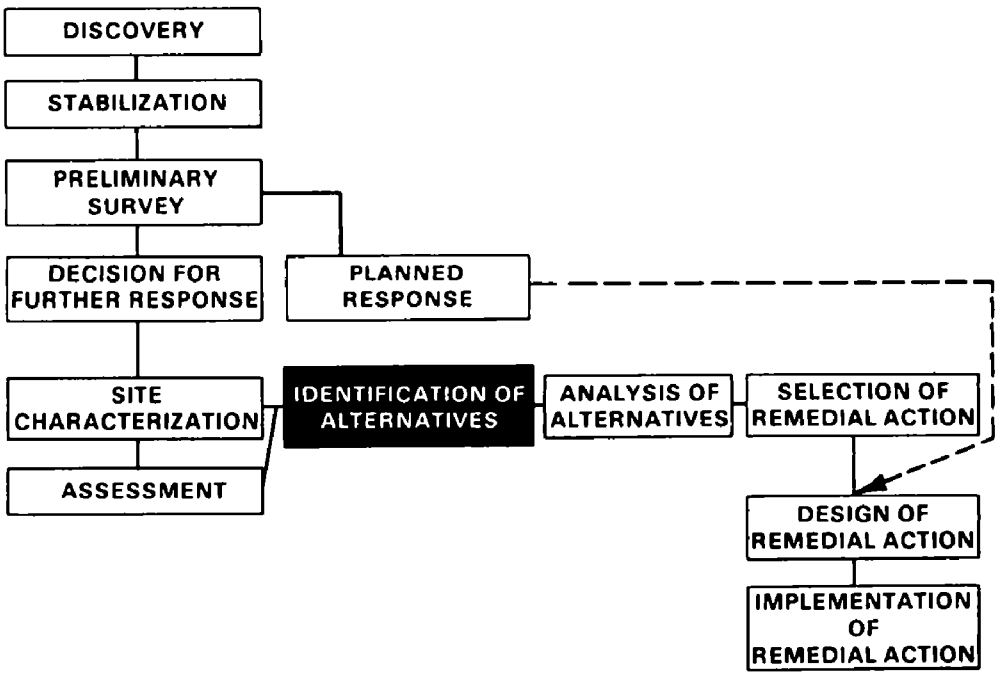

FIGURE 1.2 Steps in Remedial Action Process for Responding to an Uncontrolled Hazardous-Waste Site or Spill

WHAT IS BIOLOGICAL TREATMENT?

Biological treatment uses the activity of microorganisms to transform hazardous chemical compounds into innocuous materials. Almost all organic compounds, and some inorganic compounds, can be degraded biologically if given the proper physical and chemical conditions and sufficient time Biological processes are particularly useful for detoxifying aqueous solutions that contain dilute concentrations of hazardous materials

Microorganisms normally metabolize organic matter to produce energy by pathways involving a variety of enzyme-catalyzed reactions Simple organic compounds that occur naturally are readily incorporated into the cells of microorganisms and oxidized The larger, more complex molecules are degraded at a much slower rate Although microorganisms may be adapted and grown on many types of organic materials, some complex organic compounds are not degraded or are degraded very slowly, these compounds are called "recalcitrant" organic compounds (refractory compounds) Other organic compounds are toxic to microorganisms or inhibit their activity. Special methods may be necessary to enhance biotransformation of refractory, toxic, or inhibitory organic compounds

Inorganic materials (such as metals) and some organic compounds may be partially removed from the environment by concentration, through the mechanisms of adsorption or transport, in the microorganism during biological treatment (bioaccumulation) However, inorganic materials are rarely destroyed by biological treatment and may inhibit biodegradation at higher concentrations

The behavior of chemical substances in the environment and during biological treatment is influenced by their degradability and toxicity On the basis of these two characteristics, chemical compounds can be divided into four groups

- degradable and non-toxic
- degradable and toxic
- non-degradable and non-toxic
- non-degradable and toxic

Compounds in the first group are not objectionable over the long term Compounds in the second group, after being sufficiently diluted to prevent toxic effects, can eventually be transformed by natural biological processes Compounds of the third group may be aesthetically or otherwise objectionable Compounds of the fourth group must be prevented from entering the environment The degradable, toxic materials (second category) are the focus of this guide

2.0 BIOTREATMENT OPTIONS

Treatment of hazardous materials can be conducted onsite or offsite (Figure 2.1) Onsite treatment may be prevented by local restrictions, state regulations, population density, or other reasons The hazardous waste and contaminated soil or water must then be taken elsewhere for treatment. For both onsite and offsite treatment, the facility or degradation site must be secured to prevent unwanted human or animal activity Both onsite and offsite treatments include three options

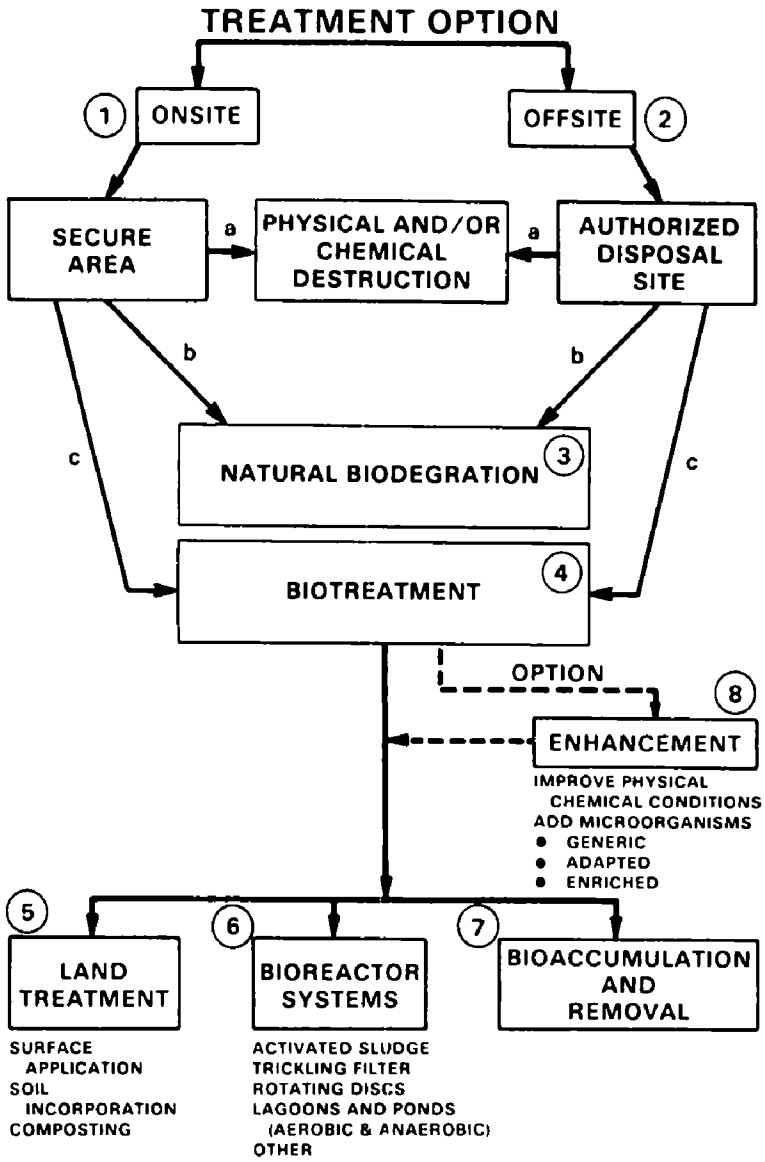

FIGURE 2.1 Concept of Onsite and Offsite Treatment Options Used for Biotransforming Hazardous Wastes

a Destroy the waste by physical or chemical methods

b Allow the waste to degrade naturally

c. Degrade the waste at a biotreatment facility (which might apply chemical and physical methods as well as biological methods)

When a waste site or spill is secured or the hazardous material is placed in an authorized disposal site, wastes may degrade naturally (3) Most hazardous organic wastes in the environment, given sufficient time, will eventually be transformed or degraded Thus, sites where hazardous materials occur will eventually return to their natural state.

The rate of waste degradation can be controlled with biotreatment methods The type of biotreatment (4) at onsite and offsite locations will depend on the feasibility of land treatment methods, the availability of special facilities such as bioreactor systems, and the opportunity for enhancing site-specific biological processes.

Land treatment (5) can be used to degrade solid, semi-solid, and liquid wastes. Application of the method requires consideration of waste loading rates, composting, and other methods such as surface tilling. Land treatment is economical but may require the commitment of large land areas for long periods of time

Bioreactor systems (6) are used primarily for liquid wastes. Bioreactors are derived from established systems that routinely treat municipal and industrial waste streams. Degradation of hazardous materials will usually take place at higher rates in bioreactor systems than in field situations because operating conditions can be controlled Bioreactor systems are usually costly to construct, operate, and maintain but are often the most effective cleanup alternative Currently, few waste-treatment systems are operating for the sole purpose of detoxifying hazardous wastes

Some wastes may be accumulated in the tissue of plants (7) The plants can be subsequently harvested and removed from the waste site or spill area Bioaccumulation is usually a final step in the cleanup process The suitability of bioaccumulation techniques depends on the toxicity of the waste, the time available to clean the site or spill, the ability to dispose of removed material, and safety and environmental factors.

Site enhancement (8) includes modifying site-specific physical and chemical conditions to improve the effectiveness of biotreatment. In some situations, degradation of wastes can be enhanced biologically by adding microorganisms Enhancement methods can be applied to land treatment, bioreactor systems, and bioaccumulation techniques

3.0 SELECTION OF POTENTIAL BIOTREATMENT OPTIONS

Biological, physical, and chemical alternatives must be considered when selecting a cleanup strategy This chapter addresses questions used to qualify biological treatment as a viable method to treat hazardous waste

3.1 IS BIOTREATMENT VIABLE AT THE SITE?

Questions for determining whether or not biotreatment is a viable cleanup alternative are outlined in Figure 3 1 Requirements for making decisions in Steps 1 through 5 of Figure 3 1 are discussed below The items listed below each question are the types of information and the sources of information needed to answer the questions. More detailed discussion of specific considerations and questions are included in subsequent chapters of this guide.

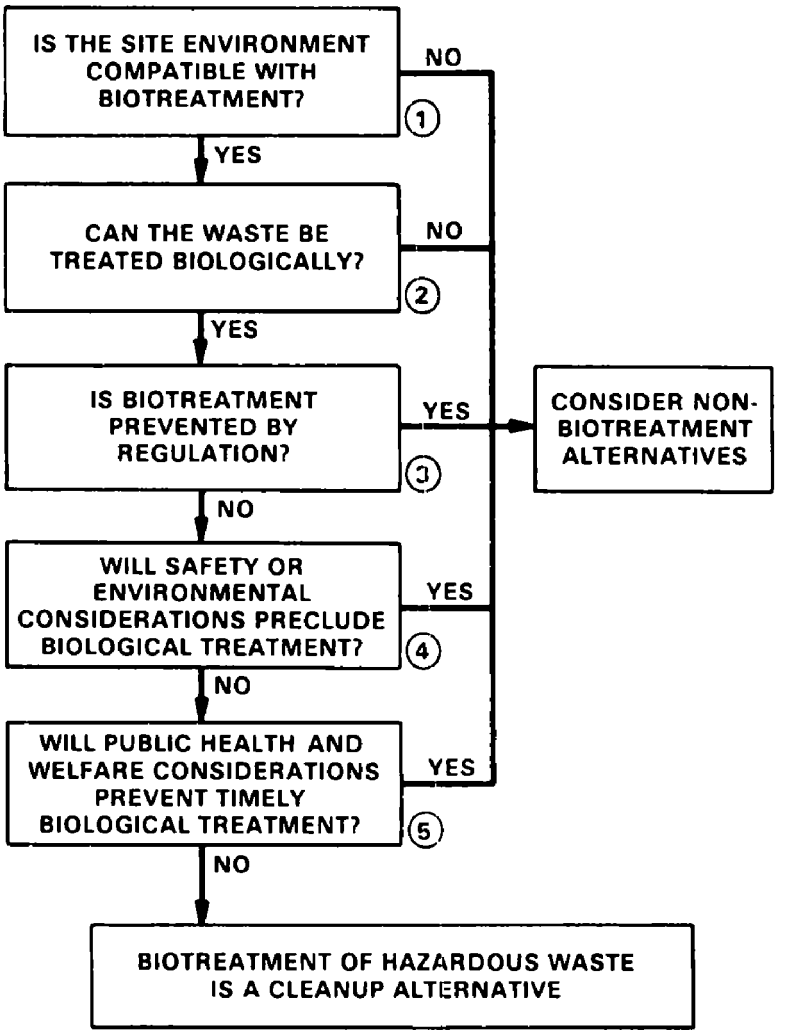

FIGURE 3.1 Selection of Biotreatment as a Cleanup Option

Is the site environment compatible with biotreatment? *(Figure 3 1, Step 1)*

- Consider the following types of information
  - climate (temperature, precipitation, wind direction)
  - biota (plants, animals, microorganisms)
  - soil (permeability, contamination)
  - topography
  - surface water (lakes, rivers, runoff)
  - ground water (location, public use).
- Consider the following sources of information.
  - public use records
  - site environmental assessment
  - environmental impact statements or management plans for other activities
  - governmental services (weather bureau, agricultural extension, soil conservation district)

Can the waste be treated biologically? *(Figure 3 1, Step 2)*

- Consider the following types of information·
  - condition of waste containers (scaled, damaged)
  - physicochemical characteristics (pH, solid, liquid)
  - toxicity
  - enhancement requirements to treat waste
  - data about degradability of related compounds
  - by-products of degradation
- Consider the following sources of information·
  - records (source of waste, condition)
  - observable physical and chemical characteristics
  - detailed chemical analysis
  - available degradation data (journal articles, technical reports, expert advice)

Is biotreatment prevented by regulation? *(Figure 3 1, Step 3)*

- Consider the following types of information:
  - regulations applicable to the type of waste
  - regulations applicable to the treatment process
  - regulations applicable to transportation requirements
- Consider the following sources of information:
  - local, state, and Federal laws, regulations, and officials

Will safety or environmental considerations preclude biological treatment? *(Figure 3 1, Step 4)*

- Consider the following types of information·
  - site characteristics
  - waste characteristics

- Consider the following sources of information:
  - local, state, and Federal laws, regulations, and officials
  - assessment of potential impacts of cleanup

Will public health and welfare considerations prevent timely biological treatment? *(Figure 3 1, Step 5)*

- Consider the following types of information
  - local population (density, proximity)
  - avenues by which population may be affected
- Consider the following sources of information:
  - risk assessment
  - site environmental assessment
  - environmental impact statements for other activities

3.2 CAN THE WASTE AT THE SITE BE TREATED BIOLOGICALLY?

Questions for determining whether or not a chemical can be biologically degraded are outlined in Figure 3 2 Requirements for making decisions in Steps 1 through 6 of Figure 3 2 are discussed below. The items listed below each question are the types of information and sources of information needed to answer the questions

Do published reports indicate the chemical has been successfully biotreated? *(Figure 3 2, Step 1)*

- Consider the following types of information
  - tables of chemical degradation rates
  - published journals
  - government publications
  - technical reports
  - acknowledged experts

Are reported rates of biodegradation sufficiently rapid for a waste site or spill? *(Figure 3 2, Step 2)*

- Consider the following types of information.
  - time allowed to complete cleanup
  - potential exposures over the cleanup period
- Consider the following sources of information:
  - published journals
  - government publications
  - technical reports
  - acknowledged experts
  - risk assessment of delayed treatment.

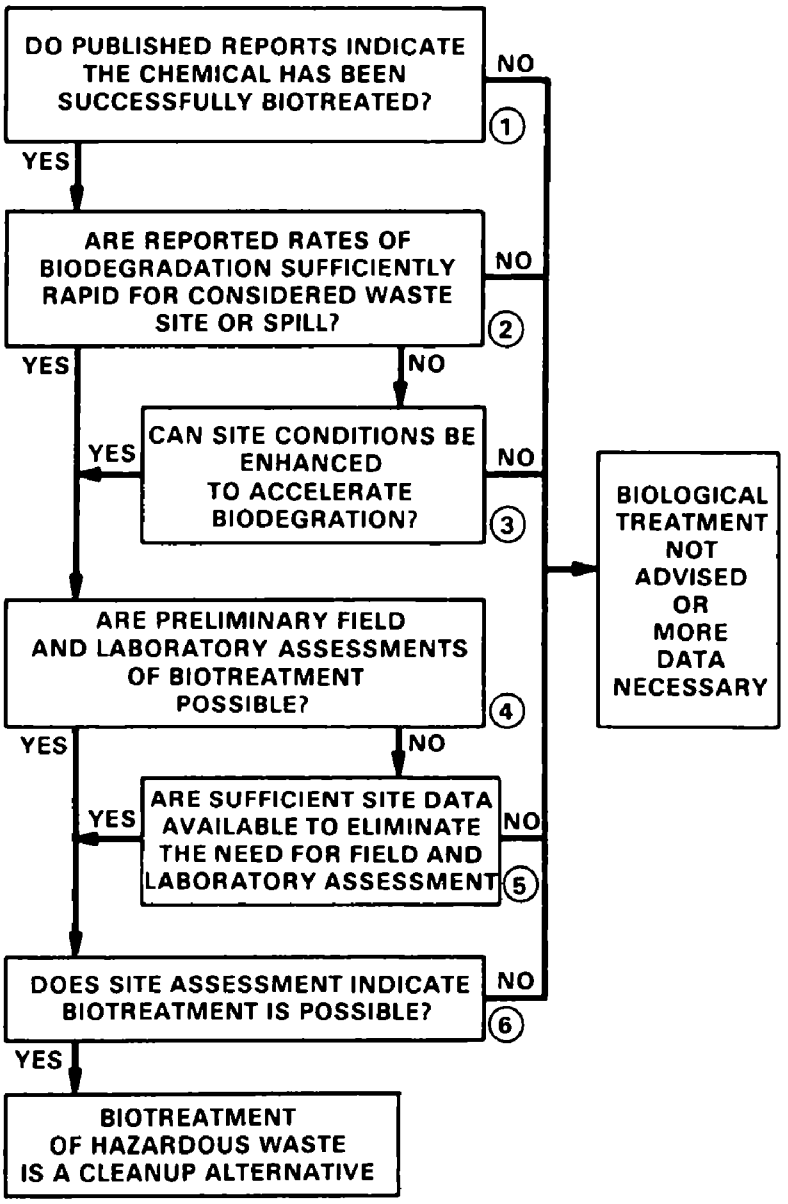

FIGURE 3.2 Flow Chart for Determining Whether or Not Chemicals Can be Treated Biologically

Can site conditions be enhanced to accelerate biodegradation? *(Figure 3 2, Step 3)*

- Consider the following types of information:
  - site characteristics [see Figure 3 1 (1)]
  - biodegradation characteristics of waste [see Figure 3 1 (2) and Figure 3 2 (2)].
- Consider the following sources of information:
  - site environmental assessment
  - environmental impact statements for other activities

Are preliminary field and laboratory assessments of biotreatment possible? *(Figure 3 2, Step 4)*

- Consider the following types of information
  - treatment schedule (how soon must waste be cleaned up?)
  - experimental data (for waste, for site)
  - assessment techniques
- Consider the following sources of information.
  - site environmental assessment
  - environmental impact statements for other activities

Are sufficient site data available to eliminate the need for field and laboratory assessment? *(Figure 3 2, Step 5)*

- Consider the following sources of information·
  - data on analogous compounds
  - previous studies at the site
  - data from similar waste sites or spills.

Does site assessment indicate biotreatment is possible? *(Figure 3 2, Step 6)*

- If assessment of the information discussed in Steps 1 through 5 results in a positive response to Question 6, biotreatment should be included as a clean-up alternative

3.3 WHAT QUESTIONS MUST BE ANSWERED TO EVALUATE BIOTREATMENT OPTIONS?

Biotreatment options are outlined in Figure 2 1. Many specific questions need to be considered when selecting a biotreatment option Some of the questions and the types of information necessary to answer these questions are listed below.

Natural Biodegradation [Figure 2.1, (3A)(3B)]

Can the waste be secured or contained?

- Consider the following types of information.
  - mobility of waste in the environment
  - volume of waste
  - safety of contained waste
  - duration of containment process
  - regulations related to containment.

Can the waste or spill site be returned to previous use in a reasonable time?

- Consider the following types of information·
  - prior use of the site
  - estimated biological and chemical half-life of the waste
  - chemical concentrations that are safe (public health and environmental)

Natural Biodegradation [Figure 3.1, (3A)(3B)] (contd)

Will natural biodegradation conditions remain constant over time?

- Consider the following types of information.
  - indigenous microorganisms
  - chemical fate in secured site
  - site characteristics

Land Treatment [Figure 2.1, (5)]

Can biotreatment be conducted at the selected site?

- Consider the following types of information.
  - population density near the site (land use, public access)
  - toxicity of wastes to humans
  - environmental conditions at the site (weather, wind direction)
  - surface- and ground-water conditions on site
  - wildlife access to site
  - domestic animal access to site.

Is sufficient area available for offsite land treatment?

- Consider the following types of information·
  - site geology
  - cost
  - transportability of waste
  - current land use
  - adjacent land use

Are the soil, hydrology, topography, and climate suitable for biotreatment?

- Consider the following types of information·
  - surface- and ground-water characteristics
  - soil depth
  - seasonal precipitation
  - site climate

Can another site be found?

- Consider the following types of information
  - transportation regulations
  - distance to new site
  - cost of transporting
  - safety considerations

Are the waste volumes and transportation costs reasonable?

- Consider the following types of information
  - waste characteristics
  - available methods of transport
  - transportation regulations
  - total quantity of waste

Land Treatment [Figure 2.1, (5)] (contd)

Is composting a viable option at land farming site?

- Consider the following types of information.
  - ability of indigenous microorganisms to transform the waste
  - energy demands
  - characteristics of compost products
  - disposal mechanism for composting products

Is land application a viable method?

- Consider the following types of information
  - loading capacity of treatment site
  - availability of treatment site
  - natural site conditions (climate, geology, soil type)

Can composting and land application be combined to treat waste?

- Consider the following types of information
  - site availability
  - area of land required
  - natural site conditions (soil type, climate, geology)

Will microbiological enhancement be required? *[Figure 2.1, (8)]*

- Consider the following types of information·
  - natural conditions and indigenous microorganisms
  - predicted degradation rates
  - microorganism activity

Are nonbiological enhancement techniques necessary or desirable? *[Figure 2 1, (8)]*

- Consider the following types of information.
  - physical conditions
  - chemical conditions
  - available nutrients

Bioreactor Systems [Figure 2.1, (6)]

Can a bioreactor system be constructed on site?

- Consider the following types of information
  - site characteristics
  - local populations
  - regulatory restrictions
  - cost

Can suspended solids be reduced to less than 1%?

- Consider the following types of information
  - total dissolved solids
  - availability of water for dilution

Bioreactor Systems [Figure 2.1,(6)] (contd)

Can pre-treatment remove solids?

- Consider the following types of information·
  - available processes
  - treatment requirements for solids.

Is sufficient land available to consider lagoon or pond systems?

- Consider the following types of information.
  - local population density
  - cost
  - wildlife restrictions (waterfowl)
  - domestic animal access to site

Will system disruption be unlikely to prevent use of an air activated sludge system?

- Consider the following types of information·
  - cost
  - treatment requirements of sludge
  - energy requirements
  - shock loading potential of suspended solids
    metal content of waste
    biocidal organic content of waste

Would the combination of trickling filter with activated sludge be a viable process?

- Consider the following types of information
  - potential system interruption (freezing, disruption of water supply)
  - microorganism sensitivity to shock loads
  - organic load of waste

Are shock loadings unlikely to prevent use of a rotating disc system?

- Consider the following types of information
  - potential system interruption (freezing, disruption of water supply)
  - system sensitivity to shock loading
  - system modification requirements
  - waste capacity.

Will microbiological enhancement be required?

- Consider the following types of information·
  - indigenous microorganisms
  - degradation rates

Bioreactor Systems [Figure 2.1,(6)] (contd)

Are nonbiological enhancement techniques necessary or desirable?

- Consider the following types of information·
  - chemical and physical conditions
  - toxicity of the wastes
  - available nutrients
  - adaptability of microorganisms to greater activity

Bioaccumulation and Removal [Figure 2.1, (7)]

Can the waste site or spill site be secured?

- Consider the following types of information
  - area of site or spill
  - public access
  - wildlife access
  - domestic animal access
  - potential for offsite biotransport

Can bioaccumulating plants grow on the waste site?

- Consider the following types of information
  - waste toxicity
  - climate
  - local flora
  - compatibility of alien flora with site and waste characteristics.

Can bioaccumulated waste be taken elsewhere?

- Consider the following types of information·
  - local regulations
  - available disposal sites
  - characteristics of the accumulated waste

Enhancement [Figure 2.1, (8)]

Because of waste or site characteristics, it may be desirable to enhance the conditions under which biodegradation occurs Enhancement involves modifying specific physical, chemical, and biological conditions to improve the effectiveness of biotreatment Enhancement is applicable to all biotreatment options identified in Figure 2 1

The following questions should be considered when selecting enhancement options

Will natural conditions and indigenous microorganisms permit timely removal of the hazardous waste through transformation, degradation, and/or absorption?

- Consider the following types of information.
  - waste degradation characteristics for site-specific conditions
  - waste concentrations
  - site recovery requirements

Enhancement [Figure 2.1,(8)] (contd)

Are physical and chemical conditions suitable for enhancement?

- Consider the following types of information
  - soil
  - climate
  - nutrient availability
  - site chemistry (aerobic, anaerobic, pH)

Will addition of nutrients and modification of physical and chemical characteristics enhance treatment?

- Consider the following types of information·
  - cost of modifications
  - characteristics of the natural microbial community

Will addition of generic organisms enhance treatment?

- Consider the following types of information
  - physical and chemical factors
  - waste degradation rates with indigenous microorganisms
  - waste concentrations

Will enriched microorganisms enhance treatment?

- Consider the following types of information
  - availability of an enriched culture
  - concentrations

Will adapted microorganisms enhance treatment?

- Consider the following types of information
  - availability of an adapted culture
  - waste concentrations

Will a combination of adapted and enriched microorganisms enhance treatment?

- Consider the following types of information·
  - availability of culture
  - waste concentrations

4.0 SITE-SPECIFIC CONSIDERATIONS AT HAZARDOUS-WASTE SITES OR SPILLS

Technical background information must be assessed before and after treatment of a hazardous-waste site or spill Pre-treatment assessment provides data necessary to begin cleanup activities and will often indicate whether a combination of physical, chemical, and biological methods is required for effective waste removal If biotreatment is selected, decisions must be made about whether to conduct cleanup of hazardous materials onsite or offsite, which biotreatment to use, and whether or not enhancement techniques are required

Post-treatment assessment helps ensure that no waste components still remain a hazard to human health or the environment Data should be collected to detect the presence of any residual hazardous materials or hazardous by-products of the biotreatment process.

The purpose of conducting pre- and post-treatment activities is to ensure human health and safety and environmental protection, and to provide data for treatment decisions Because some of the data requirements and methods used in pre- and post-treatment activities differ, this chapter is organized into two subsections

- What information should be collected before treatment?
- What information should be collected after treatment?

4.1 WHAT INFORMATION SHOULD BE COLLECTED BEFORE TREATMENT?

A hazardous-waste disposal site should be assessed before treatment methods are applied to ensure that the most appropriate cleanup method is used Pre-treatment assessment involves the following steps

1 Identify the chemicals and concentrations present

2 Determine the danger of those chemicals to human health and safety.

3 Qualify the environmental characteristics of the site.

4. Identify indigenous microorganisms.

5. Assess whether or not the waste can be treated biologically

Identify Chemicals and Concentrations Present

The first step in selecting remedial treatment is to determine the types, concentrations, and distribution of waste materials present It may be necessary to identify chemical mixtures and reaction products Hazardous materials can be identified by the following means

- records
- containers and packaging

Identify Chemicals and Concentrations Present (contd)

- observable physical and chemical characteristics of the waste
- chemical analysis

Records—Records can provide rapid and positive identification of hazardous wastes, their location, amounts, and distribution at the site. Records also indicate possible hazards that may arise during waste recovery and handling (fire, corrosion, explosion) Records usually are not available for uncontrolled or abandoned disposal sites Older records may have been lost or destroyed Records may be incomplete and may contain errors Waste manifests, which describe each shipment of waste received, may be available at controlled disposal sites

Containers and Packaging—Containers and packaging will usually help identify hazardous-waste materials, however, labels and identification marks are not fool-proof means of identification because the original contents may have been replaced with other materials Identification marks are often lost with time Wastes at disposal sites may be unconfined, leached into the soil, buried, or in ground water Excavation may be required to expose buried containers or particulate wastes

Observable Characteristics—Observable physical and chemical characteristics (odor, color, density, reaction) may be used to tentatively identify unknown materials The approach is usually limited to identifying broad classes of compounds and must not be used to identify specific hazardous wastes Observable characteristics are usually not suitable at abandoned or buried sites, or when the wastes have entered soil or water

The Oil and Hazardous Materials-Technical Assistance Data System, OHM-TADS, is maintained by the EPA and can be used to identify chemical substances based on their observable characteristics Physical properties of the unknown material (physical state, odor, color, turbidity, miscibility, reactions) are input to a computer system, which can perform a search for possible identities The OHM-TADS system contains data on approximately 850 chemicals and hazardous substances

Other sources of information can be used to identify chemicals on the basis of their physical and chemical properties These include

The EPA Field Detection and Damage Assessment Manual for Oil and Hazardous Materials Spills (EPA 1972) lists over 300 materials and identifies them by odor, color, reaction, and other properties

Physical and Chemical Properties of Hazardous-Waste Constituents (Dawson, English and Petty 1980) provides data on 250 chemicals commonly found in hazardous-waste streams

U S Coast Guard Chemical Hazards Response Information System (CHRIS) Manuals CG-446-1 and CG-446-2 (USCG 1974a, b) provide observable characteristics of approximately 900 hazardous chemicals.

Identify Chemicals and Concentrations Present (contd)

Chemical Analysis—Chemical analysis of samples may be the only way to identify hazardous-waste materials and determine the extent of site contamination Collection of samples for chemical analyses will depend largely on physical conditions at each location In some cases, soil and water samples can be obtained directly In other cases, overburden removal, excavation, well drilling, and similar efforts will be necessary to obtain representative samples Drainage or leachable liquids from sites where a variety of wastes are buried can be collected and analyzed. Plant and animal tissues can be analyzed to identify chemical wastes that have bioaccumulated Background levels of waste materials can be determined by samples collected and analyzed from uncontaminated surrounding areas Analyses of samples taken before and after site treatment will help establish the effectiveness of the treatment effort

Analytical methods can be used to determine the types and concentrations of hazardous wastes present Advanced equipment and trained manpower are usually required for analyzing hazardous wastes Standard methods of analysis for water and wastewater (APHA 1981, EPA 1976) are usually inadequate for specific identification of waste materials in the environment Hazardous wastes are typically analyzed by six instrumental techniques discussed below

Gas chromatography (GC) and high-performance liquid chromatography (HPLC) are used to analyze quantitatively the specific organic materials in wastes For the most part, they are not suitable for qualitative analysis of unknown materials in a sample unless known standards are used for comparison Gas chromatography and HPLC can be relatively expensive and time-consuming, but they can detect the presence of contaminants in environmental samples at levels in the ppb (parts per billion) range

Gas chromatography/mass spectrometry has been used primarily for qualitative analysis Improvements in equipment now permit, in some instances, use of GC/MS for quantitative analysis of pollutants in environmental samples The GC/MS technique has the ability to simultaneously identify and quantify organic compounds present in environmental samples at the ppb level This feature is particularly suitable when confirmation of contaminants in a sample is required Also, quantitative GC/MS analysis can eliminate error in quantification from the presence of an interfering contaminant The technique is expensive and time consuming, therefore, its use should be based on the data needs required from the individual sample or sample batches

Atomic absorption spectrometry (AAS) and inductively coupled, argon-plasma spectrophotmetry (ICAP) are used to quantify heavy metals in environmental samples in the ppm or ppb concentration range The AAS technique can quantify only a single compound at any given time in a sample, whereas ICAP can obtain quantitative

Identify Chemicals and Concentrations Present (contd)

information on several dozen metals from a single analysis of the sample

Chemical analysis with these analytical techniques can be used to quantify or confirm the identity of compounds in hazardous-waste materials Chemical analyses require time, funds, and a laboratory Tables summarizing EPA-approved analytical procedures for 16 classes of organic chemicals and approved analytical procedures for 80 hazardous materials are given in EPA (1982)

Determine Danger of Chemicals to Worker Health and Safety

Workers at a hazardous-waste disposal site must be aware of the dangers from chemicals present and proceed accordingly Any unidentified substance at a disposal site must be assumed to be hazardous unless firm evidence exists to the contrary

Two important characteristics of hazardous wastes are reactivity and toxicity. The reactivity of chemicals at hazardous-waste sites should be determined prior to treatment Numerous chemicals and complex compounds may have been deposited at a site where they subsequently may have mixed and reacted with each other and with natural materials in the soil to form compounds with properties different from the original components

Toxicity is a term used to define a substance's capacity to produce dysfunction, injury, malformation, or death in a living organism Toxic responses in humans are generally a result of exposure by inhalation, ingestion, or skin contact Exposures may be direct or indirect Primary concern is with organic compounds or metals that are moderately to severely toxic at normal use concentrations, or that are carcinogenic (or mutagenic and teratogenic) at low concentrations Identification of these types of compounds at hazardous-waste sites is important because many of them bioaccumulate and resist biotransformation

The reactivity and toxicity of chemicals are described in several data bases and handbooks. EPA's data base provides rapid access to information on the reactivity of over 850 chemicals; this includes identification of binary reactions, corrosiveness, recommended containers, flammability, explosiveness, and other dangerous properties. The U S Coast Guard's CHRIS Manual CG-446-2 (Arthur D Little, Inc 1972) contains information on approximately 900 chemicals, including reactivity with water and common materials (including containers), stability during transport, neutralizing agents, polymerization reactions and inhibitors, flammability, and flash points

Hatayama et al (1980) have prepared a manual on hazardous waste compatibility that includes a reference chart and a classification of common hazardous materials into 41 groups All binary reactions are identified as heat generation, toxic gas generation, flammable and nonflammable gas generation, fire and innocuous gas genera-

Determine Danger of Chemicals to Worker Health and Safety (contd)

tion, explosion, violent polymerization, solubility of toxic substances, and unknown but potentially hazardous reactions The National Fire Protection Association has published the *Manual of Hazardous Chemical Reactions* (NFPA 1975), which identifies over 3500 hazardous reactions by reactant Sax (1979) identifies chemical incompatibility of over 1000 different materials California Hazardous-Waste Regulations (Calif State Dept Health 1977) list about 800 chemicals that are considered to be hazardous or extremely hazardous, and gives the dangerous properties of each

Information on other dangerous chemical properties of materials that may be currently deposited at hazardous-waste sites is given by the sources mentioned above (USCG 1974a,b, Hatayama et al 1980, NFPA 1975, Sax 1979) Additional publications with valuable information include Plunkett (1966), Sittig (1979a, 1980), Verscheuren (1977), and Arthur D Little, Inc (1972) Requests for quick information, in event of an emergency, can be phoned to CHEMTREC (Chemical Transportation Emergency Center-CMA, 800-424-9300). This number is also listed in the chapter references

Lists of priority pollutants and hazardous materials published by EPA and the U S Coast Guard can be consulted to determine if a chemical or metal has been defined as hazardous These lists are given in The Toxic Substances Control Act, P L. 96-510, Clean Air Act, P L 91-604 as amended, and Federal Water Pollution Control Act, P L 92-500 Lewis and Tatken (1980) provide a Registry of Toxic Effects of Chemical Substances, which is updated quarterly on microfiche and as a on-line data base A list of hazardous chemical compounds prepared by the EPA is reproduced in Table 4 1 A list of known or suspect carcinogenic materials issued by The National Toxicology Program is reproduced in Table 4 2

A group of 129 chemical compounds and elements, referred to as "priority pollutants," were listed in section 307(a)(1) of the 1977 Clean Water Act (33 U.S C 466 et seq., Committee Print H R 3199) The priority pollutants were reviewed by Dryden et al (1978), and their environmental fate in water was described by Callahan et al (1979) The relative importance of each priority pollutant in environmental compartments (water, sediment, biota) was established by Chapman, Romberg and Vigers (1982), and is summarized in Table 4 3 The categories were derived by flow chart from three main characteristics persistence, accumulation, and volatility Chapman, Romberg and Vigers (1982) rated the pollutants in five categories based on the flow chart shown in Figure 4.1 Category 1 pollutants (wastes) are persistent, accumulative, and nonvolatile, and thus rate the highest environmental concern Category 2 pollutants are accumulative, persistent, and volatile Category 3 pollutants are persistent, non-accumulative, and non-volatile Category 4 pollutants are persistent, non-accumulative, and volatile Category 5 pollutants are non-persistent and rate the lowest environmental concern

TABLE 4.1 EPA List of Hazardous Chemical Compounds[a]

acenaphthene
acrolein
acrylonitrile
benzene
benzidine
chlorobenzene
1,2,4-trichlorobenzene
hexachlorobenzene
1,2-dichloroethane
1,1,1-trichloroethane
1,1-dichloroethane
1,1,2-trichloroethane
chloroethane
bis(chloromethyl) ether
bis(2-chloroethyl) ether
2-chloroethyl vinyl ether (mixed)
2-chloronaphthalene
2,4,6-trichlorophenol
p-chloro-m-cresol
2-chlorophenol
1,3-dichlorobenzene
3,3'-dichlorobenzidine
1,1-dichloroethylene
1,2-trans-dichloroethylene
2,4-dichlorophenol
1,2-dichloropropane
1,2-dichloropropylene
(1,3-dichloropropene)
2,4-dimethylphenol
2,4-dinitrotoluene
2,6-dinitrotoluene
1,2-diphenylhydrazine
ethylbenzene
fluoranthene
4-chlorophenyl phenyl ether
4-bromophenyl phenyl ether
bis(2-chloroisopropyl) ether
bis(2-chloroethoxy) methane
methyl chloride (chloromethane)
bromoform (tribromomethane)
dichlorobromomethane
trichlorofluoromethane
dichlorodifluoromethane
chlorodibromomethane
hexachlorobutadiene
hexachlorocyclopentadiene
isophorone
2-nitrophenol
4-nitrophenol
4,6-dinitro-o-cresol
N-nitrosodimethylamine
N-nitrosodiphenylamine
N-nitrosodi-n-propylamine
pentachlorophenol
bis(2-ethylhexyl)phthalate
butyl benzyl phthalate
di-n-butyl phthalate
di-n-octyl phthalate
diethyl phthalate
dimethyl phthalate
benzo(a)anthracene
(1,2-benzanthracene)
benzo(a)pyrene (3,4-benzopyrene)
3,4-benzofluoranthene
benzo(k)fluoranthane
(11,12-benzofluoranthene)
chrysene
acenaphthylene
anthracene
benzo(g,h,i)perylene
(1,12-benzoperylene)
fluorene
phenanthrene
dibenzo(a,h)anthracene
(1,2,5,6-dibenzanthracene)
indeno(1,2,3-c,d)pyrene
(2,3-o-phenylenepyrene)
pyrene
toluene
vinyl chloride (chloroethylene)
aldrin
chlordane (technical mixture and metabolites)
a-endosulfan-Alpha
b-endosulfan-Beta
endosulfan sulfate
endrin aldehyde
heptachlor
heptachlor epoxide
a-BHC-Alpha
b-BHC-Beta
g-BHC-Delta
PCB-1242 (Arochlor 1242)
PCB-1254 (Arochlor 1254)
PCB-1221 (Arochlor 1221)
PCB-1232 (Arochlor 1232)
PCB-1248 (Arochlor 1248)
PCB-1260 (Arochlor 1260)
PCB-1016 (Arochlor 1016)
2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)

(a) Source EPA, *Federal Register* 43, No 243, pp 58946-59028 (Dec 18, 1978)
Note: This list is considerably shorter than many lists of hazardous materials and should not be considered to be inclusive

TABLE 4.2 Known or Suspect Carcinogenic Materials[a]

2-acetylaminofluorene
acrylonitrile
aflatoxins
4-aminobiphenyl
amitrole
aramite
arsenic and related compounds
asbestos
auramine and its manufacture
benz(a)anthracene
benzene
benzidine
benzo(b)fluoranthene and benzo(j)fluoranthene
benzo(a)pyrene
beryllium and certain beryllium compounds
N,N-bis(2-chloroethyl)-2-naphthylamine
bis(chloromethyl) ether and its technical grade
cadmium and certain cadmium compounds
carbon tetrachloride
chlorambucil
chloroform
chromium and certain related substances
coke oven emissions
p-cresidine
cycasin
cyclophosphamide
2,4-diaminotoluene
dibenz(a,h)acridine
dibenz(a,j)acridine
dibenz(a,h)-anthracene
7H-dizenzo(c,g)carbazole
dibenzo(a,h)pyrene
dibenzo(a,i)pyrene,
1,2-dibromo-3-chloropropane
1,2-dibromoethane
dichlorobenzidine
1,2-dichloroethane
DES, 4-dimethyl aminoazobenzene
dimethylcarbamoyl chloride
dimethyl sulfate
1,4-dioxane
formaldehyde
hematite underground mining
hydrazobenzene
indeno-(1,2,3-cd)pyrene
iron dextran
isopropyl-alcohol manufacturing (strong acid process)
kepone
lead acetate and lead phosphate
lindane
melphalan
mirex
mustard gas
2-naphthylamine
nickel, certain nickel compounds and refining
N-nitrosodi-N-butyl-amine
N-nitrosodiethanolamine
N-nitrosodiethylamine
N-nitrosodimethylamine
N-nitrosodi-N-propylamine
N-nitroso-N-ethylurea
N-nitroso-N-methylurea
N-nitroso-methylvinylamine
N-nitrosomorpholine
N-nitrosonornicotine
N-nitrosopiperidine
N-nitrosopyrrolidine
N-nitrosoarcosine
oxymetholone
phenacetin
phenazopyridine hydrochloride
phenytoin
PCBs
procarbazine and its hydrochloride
b-propiolactone
reserpine
saccharin
safrole
soots, tars and mineral oils
streptozotocin
TCDD
thorium dioxide
o-toludine hydrochloride
toxaphene
tris(1-aziridinyl)phosphine sulfide
tris(2,3-dibromopropyl) phosphate
vinyl chloride

(a) Source National Toxicology Program, 2nd Annual Report on Carcinogens, April 30, 1982

TABLE 4.3 Compounds, Category Rank (see Figure 4 1), and Environmental Compartments of EPA Priority Pollutants (Chapman, Romberg and Vigers 1982)

Compound	Category Rank	Environmental Compartment		
		Water	Sediment	Biota
Metals and inorganics				
Antimony	3	X		
Arsenic	1		X	X
Asbestos	3	X		
Beryllium	1		X	X
Cadmium	1		X	X
Chromium	1		X	X
Copper	1		X	X
Cyanides	5	X		
Lead	1		X	X
Mercury	1		X	X
Nickel	1		X	X
Selenium	1		X	X
Silver	1		X	X
Thallium	1		X	X
Zinc	1		X	X
Pesticides				
Acrolein	2		X	X
Aldrin	2	X		X
Chlordane	2		X	X
DDD	1		X	X
DDE	1		X	X
DDT	1		X	X
Dieldrin	1		X	X
Endosulfan and endosulfan sulfate	3		X	
Endrin and endrin aldehyde	1	X	X	X
Heptachlor	1		X	X
Heptachlor epoxide	1	X	X	X
Hexachlorocyclohexane (α, β, δ isomers)	3	X	X	
γ-Hexachlorocyclohexane (lindane)	3	X	X	
Isophorone	3	X		
TCDD	1		X	X
Toxaphene	1		X	X
PCBs and related compounds				
Polychlorinated biphenyls (6 PCB arochlors)	1		X	X
2-Chloronaphthalene	1		X	X
Halogenated aliphatics				
Chloromethane (methyl chloride)	4	X		
Dichloromethane (methylene chloride)4	X			
Trichloromethane (chloroform)	4	X		
Chloroethane (ethyl chloride)	4	X		
Tetrachloromethane (carbon tetrachloride)	4	X		
1,1-Dichloroethane (ethylidine chloride	4	X		
1,2-Dichloroethane (ethylene dichloride)	4	X		

TABLE 4.3 (Contd)

Compound	Category Rank	Environmental Compartment		
		Water	Sediment	Biota
Halogenated aliphatics (contd.)				
1,1,1-Trichloroethane methyl chloroform)	4	X		
1,1,2-Trichloroethane	4	X		
1,1,2,2-Tetrachloroethane	4	X		
Hexachloroethane	4	X		
Chloroethene (vinyl chloride)	4	X		
1,1-Dichloroethene (vinylidine chloride)	4	X		
1,2-Trans-dichloroethene	4	X		
Trichloroethene	4	X		
Tetrachloroethene (perchloroethylene)	4	X		
1,2-Dichloropropane	4	X		
1,3-Dichloropropene	4	X		
Hexachlorobutadiene	1		X	X
Hexachlorocyclopentadiene	1		X	X
Bromomethane (methyl bromide)	4	X		
Bromodichloromethane	3	X	X	
Dibromochloromethane	3	X	X	
Tribromomethane (bromoform)	3	X	X	
[a]Dichlorodifluoromethane	4	X	X	
[a]Trichlorofluoromethane	4	X	X	
Ethers				
[a]Bis(chloromethyl) ether	3	X		
Bis(2-chloroethyl) ether	3	X		
Bis(2-chloroisopropyl) ether	3	X		
2-Chloroethyl vinyl ether	4	X	X	
4-Chlorophenyl phenyl ether	1		X	X
4-Bromophenyl phenyl ether	1		X	X
Bix(2-chloroethoxy) methane	3	X		
Monocyclic aromatics				
Benzene	4		X	
Chlorobenzene	2		X	X
1,2-Dichlorobenzene (o-dichlorobenzene)	2		X	X
1,3-Dichlorobenzene (m-dichlorobenzene)	2		X	X
1,4-Dichlorobenzene (p-dichlorobenzene)	2		X	X
1,2,4-Trichlorobenzene	2		X	X
Hexachlorobenzene	1		X	X
Ethylbenzene	4		X	
Nitrobenzene	3		X	
Toluene	4		X	
2,4-Dinitrotoluene	3		X	
2,6-Dinitrotoluene	3		X	
Phenols and cresols				
Phenol	3	X		
2-Chlorophenol	3	X		
2,4-Dichlorophenol	5	X		

TABLE 4.3 (Contd)

Compound	Category Rank	Environmental Compartment		
		Water	Sediment	Biota
2,4,6-Trichlorophenol	3		X	
Pentachlorophenol	1		X	X
2-Nitrophenol	3		X	
4-Nitrophenol	3		X	
2,4-Dinitrophenol	3		X	
2,4-Dimethylphenol	1		X	X
p-Chloro-m-cresol	3		X	
4,6-Dinitro-p-cresol	3		X	
Phthalate esters				
Dimethyl phthalate	1		X	X
Diethyl phthalate	1		X	X
Di-n-butyl phthalate	1		X	X
Di-n-octyl phthalate	1		X	X
Bis(2-ethylhexyl) phthalate	1		X	X
Butyl benzyl phthalate	1		X	X
Polycyclic aromatics				
Acenaphthene	1		X	X
Acenaphthylene	1		X	X
Anthracene	1		X	X
Benzo(a)anthracene	1		X	X
Benzo(b)fluoranthene	1		X	X
Benzo(k)fluoranthene	1		X	X
Benzo(ghi)perylene	1		X	X
Benzo(a)pyrene	1		X	X
Chrysene	1		X	X
Dibenzo(a,h)anthracene	1		X	X
Fluoranthene	1		X	X
Fluorene	1		X	X
Indeno(1,2,3-cd)pyrene	1		X	X
Naphthalene	1		X	X
Phenanthrene	1		X	X
Pyrene	1		X	X
Nitrosamines and miscellaneous compounds				
Dimethyl nitrosamine	3	X		
Diphenyl nitrosamine	1		X	X
Di-n-propyl nitrosamine	1	X	X	X
Benzidine	3		X	
3,3'-Dichlorobenzidine	1		X	X
1,2-Diphenylhydrazine (hydrazobenzene)	1		X	X
Acrylonitrile	4	X	X	

(a) These compounds have been removed from the EPA priority pollutant list

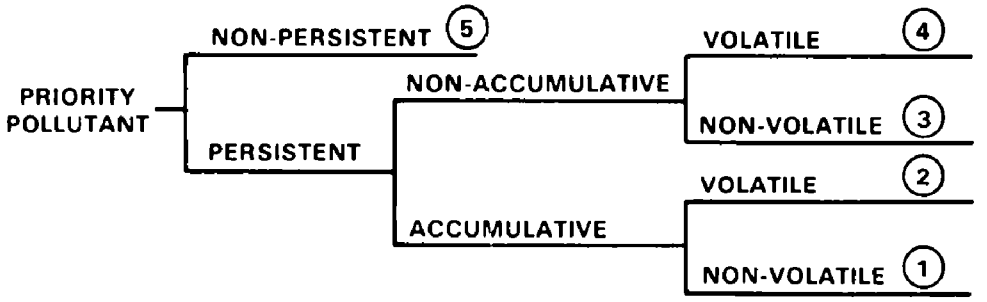

FIGURE 4.1 A Flow Chart for Categorizing Priority Pollutants (modified from Chapman, Romberg and Vigers 1982)

Identify the Environmental Characteristics of the Site

Physical and chemical features of a hazardous-waste disposal site must be determined to evaluate the feasibility of biotreatment methods Waste characteristics and worker health and safety have already been discussed Other factors to consider in evaluating the general site environment include

- topography and size
- hydrology
- climate
- soil composition
- human activities
- biota

Topography and Size—The topography and size of a site, as well as the types and quantities of toxic wastes present, will influence decisions about the feasibility of various treatment methods Clean-up activities can be conducted more efficiently on a relatively flat site than on a sloping site Steep sites may lead to equipment operational problems, soil erosion, and excessive surface runoff If the terrain is hilly or undulating, low areas may tend to collect surface runoff and provide suitable locations for waste lagoons Sites crossed by ravines and gullies are subject to substantial surface runoff, if wastes are present in such locations, surface runoff should be confined and controlled A certain amount of land around a waste disposal site is desirable for a buffer strip, access roads, soil stockpiles, construction of special biotreatment facilities such as bioreactor systems, and related activities

Hydrology—Generally, any form of treatment (biological, chemical or physical) must avoid contamination of surface and ground water If a bioreactor system is used, it must be operated as an isolated facility and designed and constructed to prevent seepage, leachates or overflow (Fields and Lindsey 1975) Surface-water flows must be controlled and confined, and other measures must be taken to avoid contamination of navigable streams, lakes, reservoirs, and domestic or public water supplies

Identify the Environmental Characteristics of the Site (contd)

Depth to water table will influence contamination of ground water beneath waste disposal sites Deep water tables beneath an impermeable overburden are not apt to be contaminated If toxic wastes enter the water table, special isolation and removal work may be needed The direction and rate of ground-water flow will influence offsite spread of contaminated water Special underground barriers and water-collection systems may be required

Climate—Seasonal temperature and rainfall (or total precipitation) are the primary climatic factors to consider Air temperature and moisture will influence the rate of microbial activity in bioreactor systems exposed to the atmosphere Temperature and moisture will also influence biotransformation rates in the soil, and the uptake and bioaccumulation of hazardous materials by photosynthetic organisms (bacteria, algae, and higher plants) Within limits for most microorganisms in wastewater treatment facilities, the higher the temperature and the greater the moisture, the greater the rate of biotransformation activity Low temperature and dry climates limit biological treatment Cold climates are unfavorable for biotransformation of hazardous wastes, whereas warm or temperate climates are favorable. Evaporation rates will affect the movement of low-solubility contaminants to the atmosphere (Mackay and Leinonen 1975) Wind forces and direction will influence the spread of noise, dust, and odors during onsite cleanup activities These factors will also affect possible offsite spread of contaminated materials and exposure of onsite workers

Soil Composition—Physical and chemical characteristics of the soil will influence biotransformation processes in several ways Soil nutrients (e g , organic carbon, nitrogen, phosphorous, potassium, iron and magnesium) are important to the activity of many photosynthetic organisms such as phototrophic bacteria, algae and larger plants, and to the activity of microorganisms using organic compounds as energy sources

Low pH usually inhibits the growth of most microorganisms in soil and water, increases the solubility of metals, and affects the ion exchange and absorption properties of the colloidal fraction of soils (Fields and Lindsey 1975, Epstein and Chaney 1978) Clays are more effective absorbers of heavy metals at higher pH levels The cation exchange capacity of soils depends to a large extent on clay content, but it increases in direct proportion to pH-dependent charged particles such as hydrous metal oxides and organic matter (Sittig 1979b)

Organic carbon may be a function of normal organic content of the soil, or the chemicals and other materials present at a waste disposal site Transformation activities of many microorganisms are fueled directly or indirectly by organic carbon, other conditions being favorable If concentrations of an organic compound are low, the microorganisms may not obtain enough energy to be self-

Identify the Environmental Characteristics of the Site (contd)

sustaining, some microorganisms require less chemical energy than others, and the phototrophs require sunlight (Chapter 6)

Soil Permeability—Soil permeability depends on soil texture and structure (Sittig 1979b). Fine-grained, poorly-structured soils have the lowest permeabilities If a soil is already saturated with water, its permeability will be low Impermeable soils will have more surface runoff from precipitation than other soils Hazardous waste is most likely to leach from permeable soils Permeability will also influence the depth of underground penetration of liquid wastes, and the action of aerobic and anaerobic microbial processes

Human Activites—Population density near a waste disposal site will influence onsite activities. Generally, disturbance of people must be minimized Noise, odors, dust, and emission of toxic or irritating gases must be kept minimal If population levels are high in surrounding areas, biotreatment of hazardous wastes at another location may be desirable Access to a disposal or cleanup site by the general public should be discouraged or prevented Special problems may be encountered when dealing with sites near hospitals, schools, jails, retirement homes, parks, fishing areas, libraries, colleges, training centers, and other facilities in heavy public use

Abandoned hazardous-waste sites may now be used for other purposes Current use of the site may conflict with cleanup activities Such a conflict normally can be resolved because protection of people and the environment from toxic waste has priority over most other land uses If cleanup is effective, the land can be used for other purposes Biotreatment processes, because they are relatively slow, may require a longer commitment of the site for cleanup activity than is required for chemical and physical treatments

In some forms of biotreatment, particularly land treatment, there are no residues that require further disposal In comparison, most chemical and physical treatment processes leave some residue for disposal, which requires commitment of land for this purpose

Biota—Biotransformation activities at hazardous-waste disposal sites could lead to contamination of nearby vegetation and local and migratory animals Risk of contamination is increased because biotreatment usually takes longer than chemical and physical treatments, and biotreatment is often conducted under open situations that require exposure of contaminated soil and liquids to air and precipitation

Waste bioreactor systems that are exposed to the elements (activated sludge units or lagoons and ponds) should be surrounded by a fence to block access of large animals Access of birds and small animals also should be prevented when possible Burrowing animals, including insects and small mammals, may become contaminated through contact with waste materials in soils at land treatment sites Many of these small animals may travel offsite

Identify the Environmental Characteristics of the Site (contd)

Where soil cultivation is practiced, grasses and other vegetation may accumulate hazardous materials Thus, grazing by domestic animals should be prohibited and use by game animals should be minimized Harvesting of plants and animals from a hazardous-waste treatment site should be prohibited until post-treatment monitoring provides assurance of safety

Sampling and chemical analyses of plants and animals at a hazardous-waste site before and after treatment will demonstrate the effectiveness of the method applied Some chemicals and metals bioaccumulate and are persistent and may exist for years in higher organisms

Identify Indigenous Microorganisms

Indigenous microorganisms are those that occur naturally at a particular site The organisms may be capable of biotransforming the waste materials by direct metabolic activity, indirect co-metabolic activity, photosynthetic activity, or by some other method if they are able to grow

Indigenous microorganisms should usually be evaluated as a complex microbial community rather than as individual species This is particularly true if the hazardous waste is represented by complex mixtures rather than by physically separate toxic materials A single species of organism, however, can sometimes be isolated directly from one type of material, the organism can then be adapted to use and transform the specific material

Indigenous microorganisms have several characteristics that enable them to change toxic materials to innocuous materials They tolerate the toxic materials on which they occur, many have the ability to attack, use, and degrade the toxic materials on which they live, they are adapted to the physical and chemical conditions of the site where they are found, they can be used to inoculate bioreactor systems without extensive culture; and they can be cultured as adapted and enriched populations for inoculation of composting or bioreactor systems

Assess Whether Waste Can Be Treated Biologically

Biotransformation data from the literature can be examined to answer the following questions about pre-treatment site assessment

- Is a chemical compound known to be biotransformable and, if so, under what conditions?
- What are the relative rates and processes of biotransformation?
- What environmental features inhibit biotransformation?
- What concentrations of a waste inhibit biotransformation?
- What microorganisms or groups of organisms are most useful for biotransforming a particular compound?

Assess Whether Waste Can Be Treated Biologically (contd)

An extensive amount of literature is available that describes the biotreatment of chemical compounds Considerable information on such studies is available in this guide and its references (for example, see Table 6 2)

Progress has recently been made in defining biotransformation pathways of a variety of synthetic compounds, pesticides, and hazardous-waste materials in laboratory cultures of single microorganism species Studies of single species transformations in artificial media are relatively simple, because the test compound is frequently present in proper concentrations, the microorganisms consist of one species, and none of the complexing materials and surface characteristic of soils, sediment, sewage, and surface water are present In contrast, studies of environmental fate are more difficult

Laboratory studies with pure cultures and test solutions provide a wealth of background information on the biotransformation pathways of compounds that are of environmental concern, such as pesticides and aliphatic and aromatic compounds. Laboratory studies can be considered an exploratory phase of biotransformation processes They do not necessarily portray what happens in outdoor ecological systems represented at hazardous-waste disposal or accidental-release sites

Available biotreatment options usually employ mixed populations of indigenous microorganisms rather than single-species populations Assessment of biotreatability should be based on analyses that monitor or detect any disappearance of the chemical under consideration Assessment should identify compounds formed by the biotransformation process

4.2 WHAT INFORMATION SHOULD BE COLLECTED AFTER TREATMENT?

Post-treatment monitoring, in many cases, will merge with treatment monitoring Post-treatment monitoring, in a strict sense, refers to locations where biotreatment is applied to return a site to its former, natural condition However, land farming practices and operation of bioreactor systems may involve long-term operations during which more wastes are added as activities are continued Thus, monitoring is also necessary during biotreatment to ensure that the effort is succeeding and that environmental components are not being damaged

Data from post-treatment monitoring may also be compared with data from pre-treatment monitoring to determine the effectiveness of treatment effort Such comparisons aid in determining the cost-effectiveness of treatment activities

Post-treatment site assessment involves the following activities

1. Gather technical background information.
2. Identify environmental components for monitoring
3. Develop a sampling program
4. Determine when monitoring should be terminated

Gather Technical Background Information

Much of the technical background information obtained in pre-treatment site assessment or during biotreatment activities will be of value in post-treatment monitoring Post-treatment monitoring will concern not only chemical compounds and waste mixtures identified prior to biotreatment, but possible intermediate products and by-products resulting from biotreatment Since biotreatment involves the use of microorganisms, the following questions should be examined

- Were the expected degradation reactions carried to completion and the hazardous materials rendered harmless?
- Has sufficient time passed for the hazardous materials to be biodegraded?
- Were undesirable intermediate products or by-products produced?
- Do any environmental components (soil, water, sediment, air, plants, animals) remain contaminated?
- What residual levels of the remaining hazardous materials are acceptable to people and the environment?
- Did significant offsite movement of hazardous materials take place?

Post-treatment monitoring consists largely of a planned sampling and chemical characterization program Sampling designs are largely site-specific For example, sampling the effluent from a bioreactor system is relatively simple to do, whereas sampling various environmental components (air, soil, water, plants, wildlife) at an abandoned waste disposal site is complex. (See Section 4 1 for a discussion of chemical analysis)

Sampling schemes may consist only of periodic samples from a single source for chemical analyses (e g , to monitor effluent from a bioreactor system) Complex sampling designs to monitor several environmental components over a period of time (e g , at waste disposal sites) require the services of a statistician, preferably one with a background in ecological monitoring Because analytical procedures for low chemical concentrations have special requirements and may be costly, a chemist should also help establish a practical sampling program

Identify Environmental Components for Monitoring

In most cases of post-treatment monitoring, it is not necessary to sample nearby human populations to determine the effectiveness of biological cleanup Human health problems will usually be examined before and during cleanup activities, if the hazardous waste situation warrants Concern for human health will always be paramount (EPA 1982)

The main environmental components to be considered for post-treatment monitoring are

- air
- soil
- water
- sediment
- plants
- domestic animals
- wildlife

Each environmental component can be related to one or more areas of concern Post-treatment monitoring to evaluate the effectiveness of biotreatment and other cleanup activities must also examine potential pathways that link the environmental component to areas of concern A pathway becomes important only if the cleanup effort was ineffective or partially effective, and an environmental component remains contaminated with hazardous materials

Monitoring is usually a complex and expensive task Only those components of the waste site and surrounding environs that are most important to the assessment should be monitored Figure 4 2 is a decision matrix by which the relative importance of environmental components and areas of concern can be evaluated For instance, if the contaminants appear in the soil only, the other environmental components need not be included in monitoring If the land in question will revert to residential use after cleanup, then the areas of concern would include 1) human densities and distribution, 2) public resources and institutions, and 3) uses of land Monitoring should be designed to assure that contaminant levels be low enough to protect the areas of concern Evaluation must be site-specific because the characteristics considered vary widely from site to site, and sampling programs must be designed accordingly

Eight major areas of concern given in Figure 4 2 must be considered

(1) Human density and distribution
  Are population densities large or small?
  Are people scattered or grouped?
  Do many people live or work near the treatment site?
  Are population centers downwind?

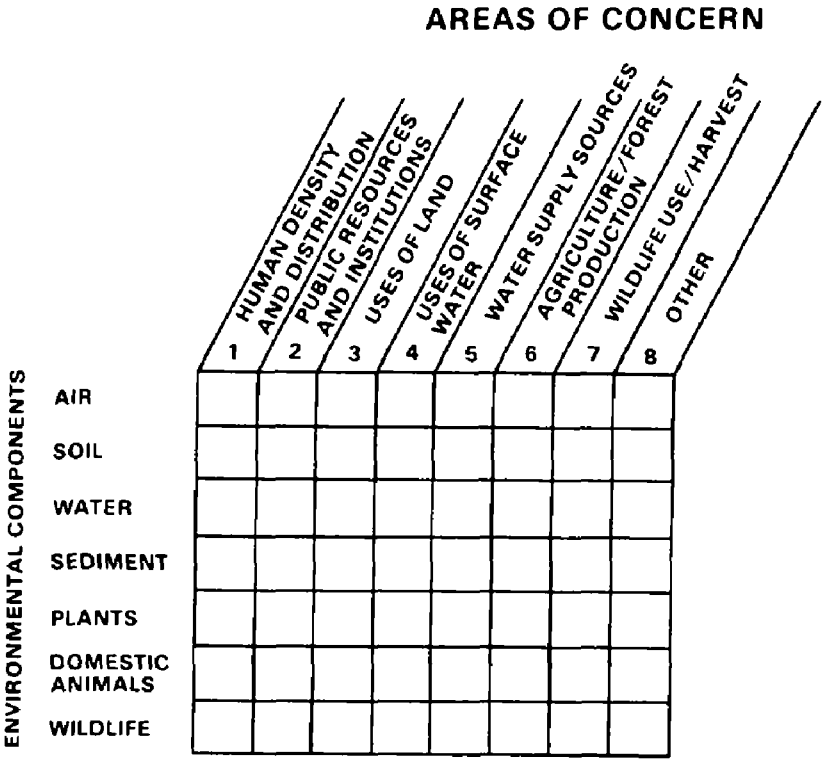

FIGURE 4.2 Decision Matrix for Planning Post-Treatment Monitoring Environmental components should be sampled in relation to areas of concern

Identify Environmental Components for Monitoring (contd)

(2) Public resources and institutions.
What public resources and institutions are located near the treatment site? (parks, hospitals, libraries, retirement homes, market centers)

(3) Uses of land
What are the dominant uses of the land surrounding a treatment site? (recreational, commercial, agricultural, livestock)

(4) Uses of surface water
What uses are made of surface waters near the treatment site? (fishing, swimming, boating, irrigation, livestock)

(5) Water supply sources
What are the sources of water for domestic, municipal and industrial users near a treatment site? (aquifers, wells, reservoirs, rivers)

(6) Agriculture and forest production
What agricultural and forestry products are grown near a treatment site? (crops, forage, animals, lumber, trees)

Identify Environmental Components for Monitoring (contd)

(7) Wildlife use and harvest
What wildlife species occur near the site?
What forms are harvested as game animals?
What forms are migratory?

(8) Other
What other critical areas might be adversely affected by incomplete or ineffective biotreatment?

Air—Air quality may deteriorate during biodegradation of hazardous materials if obnoxious gases are produced or chemical compounds evaporate Once biotreatment is complete, these concerns are normally resolved However, effective biotreatment often takes more time than physical or chemical treatment, and gases may be emitted over a period of years. Where biotreatment is a continued operation (land cultivation, composting, activated sludge system, lagoons and ponds), some emission of gases may be unavoidable Monitoring of air quality during and after biotreatment is warranted by concerns that involve humans (see Figure 4 2, Areas of Concern 1 and 2) Emissions must be controlled if large human densities, public institutions, or public facilities are located downwind from the treatment site While the gaseous emissions may not be hazardous in themselves, their presence may cause public displeasure

Selected references on air monitoring are grouped in Section 4.3

Soil—Post-treatment monitoring of soil is necessary to document low or harmless concentrations of former wastes before land can be reclaimed and returned to normal uses Some hazardous materials such as metals are complexed in soil Future declines in soil pH from acid precipitation may change harmless levels of hazardous materials in soil, particularly certain metals, to levels capable of environmental damage (EPA 1980, Haines 1981)

Soils may retain some toxic substances and toxic by-products after biotreatment has been applied Retention is most likely to occur at abandoned waste disposal sites and land treatment sites, where the original hazardous materials were mixed with soil by composting, layering, or tilling over large areas at various depths In such situations, isolated sections may contain wastes that escape effective biotreatment

Soil samples are collected most efficiently at the surface where biotreatment is usually most effective If surface samples show that no hazardous materials remain or that residuals are at harmless levels, surface runoff is not likely to be contaminated, crops may be grown, and other human uses can be permitted Subsurface areas may resist biotreatment more than surface areas, especially if moisture and aeration is lacking Therefore, some subsurface or underground locations may remain contaminated longer than others Untreated or ineffectively treated locations below the surface may

Identify Environmental Components for Monitoring (contd)

eventually affect ground-water quality Future construction activity may also expose subsurface layers that remain contaminated Deep locations in soil are difficult to sample effectively, but may be identified through ground-water samples or by well-drilling and core-sampling techniques

These considerations indicate that soil could contain some areas with residual hazardous materials or by-products after biotreatment is considered to be complete Such residuals could be harmful to people in the future The sampling design for soils should be designed to be adequate to detect the presence of such residuals. If significant amounts of residuals are found, other methods of treatment or long-term site isolation may be warranted

Selected references on soil monitoring are grouped in Section 4 3

Water—Water is relatively easy to sample and it provides a reliable way to detect the presence of residual hazardous wastes or by-products Water samples can be collected from surface runoff, leachate, ground water from land treatment sites or from liquid effluents from bioreactor systems. Sampling wells can be drilled at land treatment sites to determine if ground water is contaminated with hazardous materials or their by-products

Ground water at abandoned waste disposal sites and accidental spill sites is frequently contaminated Withdrawal of contaminated ground water, followed by biotreatment and reinjection to the ground, is one method useful for site cleanup Periodic chemical analysis of ground-water samples will show the relative effectiveness and progress of biotreatment efforts

Water represents a direct pathway for potential effects on human health Areas of concern (Figure 4 2) include "uses of surface water" and "water supply sources " Post-treatment monitoring must include onsite and offsite sampling of these sources to provide assurance of continued safety to people and animals Sources to monitor include aquifers, wells, springs, lakes, reservoirs, and streams Domestic, municipal and industrial drinking water sources must be examined for the presence of carcinogenic compounds

Information on public and private water supplies can be obtained from such sources as public health departments, water supply companies, well drillers, and residents of the area Previous analyses performed on water supplies may be identified and used as baseline data for post-treatment assessment Knowledge of the drainage basin will aid in selecting sampling locations in reservoirs, lakes, and streams

Selected references on surface- and ground-water monitoring are grouped in Section 4 3

Sediment—Sediment is a deposit of fine particulate matter by wind or water Sediment can be deposited on land, in or near running

Identify Environmental Components for Monitoring (contd)

water, or beneath standing water Hazardous materials such as metals can be complexed in sediment, as they are in soils. Deposits of sediment in water may function as ecological "traps" for hazardous materials

Selected references on soil and water monitoring are grouped in Section 4 3 and include references for sediment monitoring

Plants—Plants will grow at field locations used for biotreatment when waste concentrations in surface layers of the soil are not phytotoxic (toxic to plants) Some organic compounds and metals at low concentrations are bioaccumulated by terrestrial and aquatic plants Former land-treatment and sludge-disposal sites may be used to grow crops However, if trace amounts of toxic materials remain in the soil, they may bioaccumulate and enter the food chain when plants are consumed by people and animals (Chaney et al in press, Chaney 1982)

When studying plants, the main areas of concern are with agriculture and forest products Private gardens, orchards and small farmlots must be included in the sampling design if the area is populated

Plant samples are easy to collect But concentrations of waste materials or treatment by-products in plant tissues do not correlate readily with concentrations in the environment (soil, water or air) This is because several variables (including waste concentration and chemical form, soil properties, and soil processes) influence the kinetics of uptake reactions, particularly for metals (Cataldo and Wildung 1978)

Therefore, the aim of post-treatment monitoring of plants should be to detect concentrations of hazardous substances and treatment by-products in leafy tissues that might be eaten by humans and animals Analyses of plant tissues to quantify residual levels of waste materials in soil and water are of limited value

Domestic Animals—Domestic animals using land treatment sites or accidental release sites after biotreatment will be exposed to residual hazardous wastes or by-products that remain in the soil surface, in plants, or in surface waters Exposure is usually by ingestion or inhalation Post-treatment monitoring will be required to detect possible bioaccumulation and possible harmful effects on animal reproduction and health Many toxic materials that bioaccumulate and go undetected in domestic animals are readily transferred to humans

Wildlife—Wildlife using land treatment sites and accidental release sites after biotreatment will also be exposed to hazardous wastes or by-products that remain The difference is in degree use of land and water by domestic animals can usually be controlled, whereas use by wild animals usually cannot Many forms of wildlife are migratory and will use a site only temporarily Others reside at a

Identify Environmental Components for Monitoring (contd)

site throughout the year Some migratory species such as birds will use sites seasonally for breeding and rearing young

Small mammals and insects will dig burrows in the soil, birds will consume invertebrates in the soil, fish will live in water, and carnivores will consume prey Food-chain pathways for wildlife are more varied and complex than those for domestic animals Game animals and game fish harvested for human consumption might not be subject to marketing restraints, whereas agricultural products usually are.

The aim of post-treatment monitoring of wildlife, however, is the same as that for domestic animals, that is, to determine concentrations of hazardous substances and treatment by-products in body tissues that adversely affect their reproduction and health processes, and concentrations that might be transferred to humans

State and local environmental groups will have information on wildlife use The U S Fish and Wildlife Service and state departments of fish and game will have information on the biology, ecology, and harvest of wildlife Collection of wildlife samples will require permits or assistance from the Federal government (migratory species), state departments of fish and game (resident species, game or non-game animals), or both

Selected references on biological sampling (plant, domestic animals and wildlife) are grouped in Section 4 3

Develop a Sampling Program

Post-treatment monitoring teams must assume that all results from sampling of environmental components might be used in future legal proceedings Therefore, sampling programs must be developed on a sound scientific basis and adhere to quality control procedures. Numerous manuals exist which review methods and designs to be used for obtaining statistically valid data from the environment In general, the following items should be considered when selecting a sampling design (EPA 1972)·

- Is the method suitable for collecting statistically sound (time and space) samples?
- Does the method provide for test and control samples?
- Is the method technically defensible, generally accepted, and cited in standard texts?
- Can an adequate number samples be collected and analyzed with the time and staff available?
- Will the method give quick preliminary results?
- Does the method sample the environmental components of concern, with emphasis on foodchain pathways to humans?

Develop a Sampling Program (contd)

High standards of performance, care, analysis, and documentation are as important as a standardized method of sampling to ensure the quality of data Consistently uniform procedures are important if the data will be statistically evaluated For this reason, a statistician and analytical chemist must both be involved in establishing the sampling design before post-treatment monitoring begins

Selected references on sampling requirements, including statistical considerations, are grouped in Section 4 3

Determine When Monitoring Should Be Terminated

Post-treatment monitoring begins when biotreatment activities are believed to have significantly reduced hazardous-waste materials, or to have rendered them innocuous to humans and the environs In many situations, monitoring will be conducted during biotreatment to check on progress, to determine the effect of environmental and microbiological enhancement efforts, and to adjust the biotreatment process (operational monitoring)

Eventually, post-treatment monitoring must end Termination occurs when the evidence collected and analyzed indicates that the site no longer represents a hazard to humans and the surrounding environs Where biotreatment is a continuing process, as in most bioreactor systems, monitoring is an ongoing phase of the operation that ends only when the operation terminates

A specific time to terminate post-treatment monitoring cannot be established in advance A termination date will depend on the success of the biotreatment process Success of biotreatment will depend on numerous biotic and abiotic factors that vary over time and from site to site If operational monitoring indicates limited success, chemical and physical methods of waste detoxification, waste removal, or waste isolation must be adopted

Post-treatment monitoring can be terminated when

- The wastes are removed or eliminated from the site
- The wastes are reduced to acceptable levels, and no toxic by-products are produced
- The wastes are contained and possible future releases represent minimal environmental risk
- Sufficient post-treatment monitoring data are obtained to establish that the site or waste materials are no longer hazardous

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5.0 DESCRIPTION OF BIOTREATMENT OPTIONS

As discussed in Chapter 2, biotreatment options vary with environmental conditions, local restrictions, state regulations, population density, and waste characteristics This chapter describes some of the available treatment methods, provides flow charts for selecting specific methods, and compares the advantages and disadvantages of each option

Four treatment options are diagrammed in Figure 2 1 and discussed in the following text

- natural biodegradation
- land treatment
- bioreactor systems
- bioaccumulation and removal

Also illustrated in Figure 2 1 and discussed in the following text are enhancement techniques that can be applied to biotreatment options

5.1 NATURAL BIODEGRADATION

Most hazardous wastes in the environment, given sufficient time, will eventually be transformed or degraded by a combination of chemical, physical and biological processes In some cases, it may take decades or centuries for the wastes to degrade The hazardous nature of many wastes, their environmental persistence and mobility, and their high volumes make rapid conversion to innocuous compounds necessary One way to increase the rate of degradation is to control the physical, chemical, and biological processes through biotreatment methods

Chemical degradation can result from oxidation-reduction, hydrolysis, depolymerization, or isomerization Physical degradation can result from sunlight and radiation Microbial degradation is influenced by the microbial population and by soil conditions that affect their activity These conditions include pH, moisture, temperature, exposure to air and sunlight, and presence of organic matter The chemical nature of a waste and its structure can also influence degradation

5.2. LAND TREATMENT

Solid or liquid wastes can be biologically treated by applying the wastes to the land surface, by composting, or by combining both methods The concept of land treatment described here does not include landfills, overland flow pretreatment, deep well injection, or soil reactor units Brown and Deuel (1980) and Overcash and Pal (1979) describe the application of land treatment methods to hazardous wastes These studies also discuss land treatment and selection of potential land treatment sites

Land treatment involves mixing or dispersing the wastes in soil in relatively thin layers This has the effect of increasing the availability of the waste to

microorganisms in the soil It assumes that some microorganisms will survive the initial shock of being exposed to the wastes, will develop suitable enzyme systems, and will biodegrade the compounds present In its most simple form, land treatment uses microorganisms indigenous to the soil Under some conditions, however, it may be necessary to add induced or adapted microbial cultures

Land application rates can be determined for most industrial wastes The decision to proceed or not with land treatment is generally based on cost, not technical feasibility Soil properties and waste composition determine the application rate If land area is not sufficient to handle all of the waste at a predetermined maximum rate of application, alternative disposal methods will be necessary

To determine if a site is acceptable for land treatment, characteristics of the surface water, ground water, climate, geology, topography, and soil must be known Land treatment is an "open system" that potentially can lead to onsite and offsite contamination of ground water, surface water, and air Therefore, design, operation, and adequate monitoring are important factors for land treatment Regulations for land treatment of hazardous wastes have been published by the U S Environmental Protection Agency (EPA 1980)

Land Treatment Systems and Limitations

The first step in designing a land treatment system is to determine the capacity of the site to assimilate waste constituents Overcash and Pal (1979) describe procedures to determine the ratio of waste generation (kg/yr) to land capacity [(kg/ha)/yr)] This ratio is the area required for effective land treatment disposal

The form of waste may be liquid, slurry, sludge, or solid The difference in application of waste forms is primarily in the equipment used to spread the material on the land Pre-treatment may be needed to reduce the concentration of the land limiting constituent (LLC) The LLC is the waste component that first exceeds an acceptable concentration in the soil (Overcash and Pal 1979) Pre-treatment may allow a greater application rate or the use of less land area A land application system should be designed to assimilate 100 percent of the LLC applied Wastes can usually be pumped and handled as liquids if they contain less than 8 percent solids and if particle sizes are smaller than 2 5 cm in diameter

Common land application methods include sprinkler irrigation, overland flow, tank truck, and truck spreaders Most hazardous wastes should be mixed with the soil by some tillage procedure to ensure that the material remains where it is placed

An aerobic system is usually effective for biotreating most waste compounds If loading rates are too high or if applications are too frequent, anaerobic conditions will develop and slow the rate and extent of biotransformation The same factors that influence the decomposition of sewage sludge and crop residues in soils also influence the decomposition rate of hazardous wastes Therefore,

Land Treatment Systems and Limitations (contd)

agricultural publications discussing disposal of sewage sludge on land will explain the principles controlling the decomposition of hazardous materials in soils

There may be times during which application of hazardous or toxic wastes to land is not possible For example, periods of high rainfall will saturate the soil and periods of extended freezing or snow cover will prevent mixing of waste and soil In these cases, interim storage of the waste may be necessary in earth ponds, concrete tanks, or in piles of relatively dry waste solids Waste integrity can be maintained with covers, liners, and other barriers that prevent moisture saturation, leaching, or surface runoff

The advantages and disadvantages of land treatment of hazardous wastes are listed below For comparison with other methods discussed in this chapter, see Figure 5 1

Advantages:

- The treatment costs less than most physical or chemical processes [Costs associated with some land treatment activities are discussed by EPA (1981)]
- The method is relatively simple to apply
- The method is effective on wastes with relatively high metal content

Disadvantages:

- Large areas of land may be required
- Degradation takes a long time and may never be complete for many wastes
- Highly concentrated wastes may kill indigenous microorganisms and sterilize the soil
- Toxic volatile compounds may be produced
- Toxic intermediate compounds, in rare instances, may form
- Heavy metals will remain in the soil

Composting

Techniques for composting hazardous waste are still in early development Wilson et al (1982a,b) describe composting as a pretreatment method of detoxifying wastes prior to land application Composting is the microbial conversion of organic waste materials in the presence of air and moisture into a product with the general appearance and other characteristics of fertile soil The waste is conditioned for composting by bulking materials (woodchips, leaves, refuse) to render it permeable to air As the aerobic microorganisms oxidize the sludge, they release heat and the temperature of the waste rises

Composting (contd)

COMPARISON CRITERIA		BIOTREATMENT OPTION: NATURAL BIODEGRADATION	LAND TREATMENT	COMPOSTING	BIOREACTORS	AIR-ACTIVATED SLUDGE	TRICKLING FILTER	ROTATING DISC	LAGOONS AND PONDS	BIOACCUMULATION AND REMOVAL
COST	MONEY	●	●	●	○	○	◑	○	●	●
	LAND	◑	○	○	●	●	●	●	○	◑
	WATER	◑	◑	○	◑	◑	◑	◑	◑	◑
	TIME (SETUP)	●	●	●	○	◑	◑	◑	◑	●
	TIME (DEGRADATION)	○	○	○	●	◑	●	◑	◑	◑
	ENERGY	●	●	●	◑	○	◑	◑	●	●
	ENHANCEMENT	●	◑	◑	◑	◑	NA	○	NA	◑
FLEXIBILITY	WASTE CONCENTRATION	●	◑	◑	◑	◑	NA	●	○	○
	WASTE FORM (LIQUID/SOLID)	●	●	●	○	●	○	○	○	◑
	LOAD RATE	NA	○	◑	○	NA	●	●	◑	NA
	ORGANICS	◑	●	●	◑	●	●	●	◑	○
	METALS	◑	●	●	◑	NA	NA	●	◑	●
BY-PRODUCTS	VOLATILES	◑	○	○	◑	○	◑	◑	○	●
	ODORS	◑	◑	◑	◑	◑	○	○	○	●
	SLUDGE	NA	NA	●	◑	○	○	NA	NA	●
	SOLIDS	NA	○	○	○	○	●	NA	NA	●
	LIQUIDS	NA	NA	NA	●	●	○	◑	○	●
	INTERMEDIATES	◑	○	◑	◑	◑	○	NA	NA	○
INTERRUPTIONS	FREEZING	NA	NA	NA	○	○	○	○	○	○
	DRYING	NA	◑	◑	○	○	○	○	○	○
	SHOCK LOADING	NA	NA	NA	○	○	●	○	○	NA
	CLOGGING	NA	NA	NA	○	◑	○	●	NA	NA
	RECOVERY TIME	NA	NA	NA	◑	◑	○	○	NA	NA

● ADVANTAGE ◑ ADVANTAGE/DISADVANTAGE DEPENDENT ON SITE SPECIFIC FACTORS ○ DISADVANTAGE NA NOT APPLICABLE

FIGURE 5.1 Matrix Comparison of Advantages and Disadvantages of Biotreatment Options for Hazardous Wastes

Composting (contd)

The most fundamental composting process involves placing the waste or soil in windrow piles Aeration to enhance degradation by aerobic microorganisms is achieved by periodically spreading or turning the pile If pH levels are kept neutral to alkaline, metals not absorbed on the soil or organic components of the waste pile will usually be present as precipitate This process is discussed by Berkowitz, Funkhouser and Stevens (1978)

Several types of composting systems are available One innovative system with promise for hazardous wastes is the Beltsville Aerated Pile Composting Method (Wilson et al 1980), which has been used primarily for composting sewage sludge. A mixture of waste and bulking material is placed over a network of perforated pipes connected to a blower that draws air down through the mass Exhaust gases are scrubbed through a pile of finished compost to remove odor before release to the atmosphere A blanket of compost over the composting sludge prevents the escape of obnoxious odors and insulates the pile to increase its temperature

The advantages and disadvantages of composting of hazardous wastes are listed below For comparison with other methods discussed in this chapter, see Figure 5 1

Advantages:

- The system has high tolerance to microbially toxic chemicals (i e , metals)
- Energy demands are low (Fuel costs for earth-moving equipment are the most expensive requirement)
- No sludge or brine disposal is required
- Most types of organic compounds are degraded microbiologically

Disadvantages:

- Long retention or degradation periods are required
- Acclimation is needed for aerobic microorganisms
- Sufficient moisture must be present

Land treatment of hazardous waste, including composting, is most effective for sites where

- Toxic wastes are present in relatively low concentrations
- The site does not have to be cleaned up or detoxified rapidly
- Soil moisture and temperature levels are favorable

Slow degradation is a problem only if the compounds are extremely hazardous, if the site cannot be secured, or if there are

Composting (contd)

land-use conflicts Good management practices can often accelerate the degradation process to some extent Some waste materials are highly toxic to microorganisms, or are present in sufficiently high concentrations to sterilize the soil Control of application rates and sound treatment practices would minimize this problem

If concentrated wastes from an abandoned site or a spill area can be removed, they can be stored and applied to an approved land treatment site at diluted or non-toxic concentrations Again, good management practices and proper loading rates will resolve many of these problems Application of water by sprinkler systems or hoses will increase the water/waste ratio in soil or composts and enhance degradation at many sites However, such dilution should be avoided at sites where contamination of surface or ground water is possible, or where seepage and runoff cannot be controlled The course of action depends, to a large extent, on the site and types of wastes present.

The decision process used to determine if land treatment methods are practical for detoxification of hazardous wastes is shown in Figure 5 2

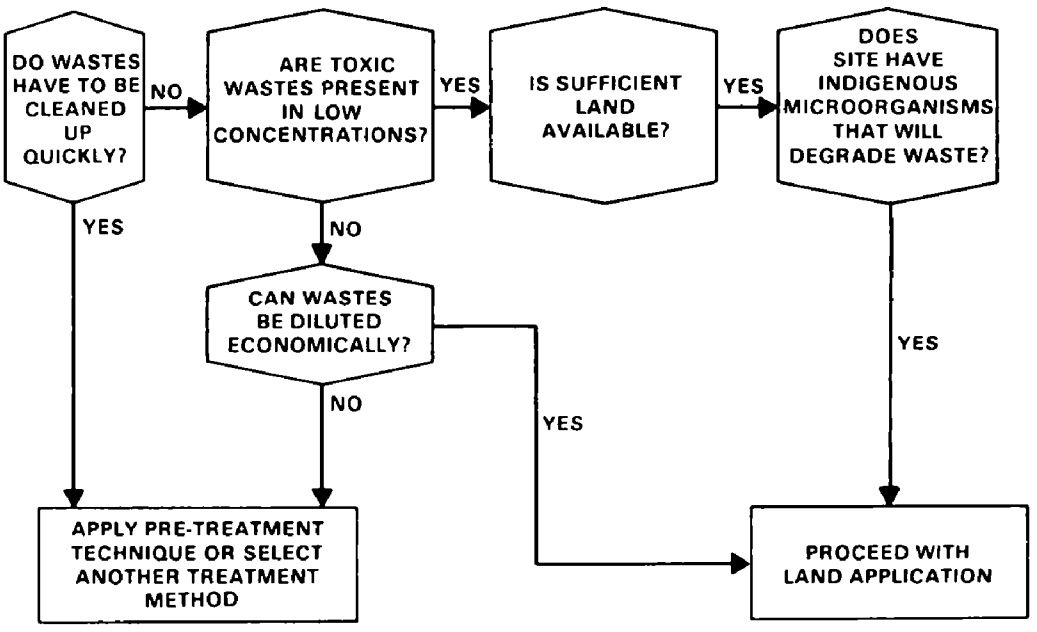

FIGURE 5.2 Decision Process for Evaluating Land Application (Including Composting) for Biotreatment of Hazardous Wastes

5.3 BIOREACTOR SYSTEMS

Biological treatment of municipal and industrial wastes is relatively common Microorganisms, however, have not been used extensively to transform or degrade hazardous materials in bioreactor systems Microorganisms have degraded many synthetic compounds under controlled laboratory conditions, and their potential for similar activity in large-scale, engineered systems is good

The advantages and disadvantages of bioreactor treatment of hazardous wastes are listed below For comparison with other methods discussed in this chapter, see Figure 5 1

Advantages:

- Degradation proceeds more rapidly than under field situations
- Degradation may proceed more rapidly than under land treatment activities
- Physical and chemical conditions are readily modified

Disadvantages:

- High construction, maintenance, and operational costs
- System disruptions must be prevented
- Sludges accumulate and must be removed
- Use is restricted to primarily liquid wastes

Application of biotreatment to hazardous waste liquids and leachates in bioreactors is considered by EPA (1982), Shuckrow, Pajak and Oshaka (1981), and Berkowitz, Funkhouser and Stevens (1978) A study by SCS Engineers (1979a) describes successful and unsuccessful biodegradation in wastewater treatment processes, gives estimates of the time required to decompose different wastes, and makes engineering and economic comparisons

Bioreactor systems to treat toxic or hazardous wastes must be designed to

- enhance oxygen transfer to microorganisms for aerobic systems
- enhance contact between wastes and microorganisms
- reduce toxic or inhibitory effects of the wastes
- prevent abrupt "shock" loadings

The most common bioreactor systems are air-activated sludge, trickling filter, rotating disc, aerobic ponds and lagoons, and anaerobic ponds and lagoons A general description, advantages, disadvantages, applications, and flow charts for each system follow.

Air-Activated Sludge The air-activated sludge process involves pumping air into sludge reactor tanks containing the waste The process relies on microorganisms being continuously circulated while in contact with organic materials and oxygen The operator can modify aeration

Air-Activated Sludge (contd)

and mixing by changing the amount of air blown into the reactor tanks Secondary clarifiers are used to separate activated sludge solids from the mixed liquor, and to produce concentrated solids for the return flow necessary to sustain the microbial population Eventually, the sludge must be removed and deposited elsewhere for the system to function.

Metals at low concentrations, non-toxic to microorganisms, are concentrated and separated by the treatment Most insoluble metals should be removed chemically by sedimentation before treatment. Metals can have an adverse effect on metabolism of microorganisms

Preliminary analysis of the waste helps determine if additional nutrients are necessary. If the waste is deficient in either nitrogen or phosphorus, the necessary nutrient is added Lime is almost always added to maintain an alkaline pH and to precipitate metals

Problems with toxic compounds are low because the aeration unit mixes and dilutes toxic components Following treatment, however, the resulting sludge may be hazardous because of sorption and concentration of toxic compounds such as metals In addition, the mixing action of the aeration unit may release volatile compounds to the atmosphere Loss of volatile compounds can aid biotreatment but may also degrade air quality and affect personnel safety (Berkowitz, Funkhouser and Stevens 1978)

Pre-treatment and post-treatment methods may be required to meet discharge objectives (Shuckrow, Pajak and Touhill 1980) The methods include precipitation of metals, carbon sorption, ultrafiltration, chemical oxidation, wet air oxidation, ion exchange, and electrochemical treatment

The advantages and disadvantages of air-activated sludge treatment of hazardous wastes are listed below For comparison with other methods discussed in this chapter, see Figure 5 1

Advantages:

- The method is acceptable for industrial wastewater treatment
- The method is adaptable to different types of waste streams
- The method is reliable
- The method can handle higher organic loads than many biotreatment processes

Disadvantages:

- The method is relatively expensive
- Toxic gases could be released during aeration

Air-Activated Sludge (contd)

- Sludge that can be high in metals and organic compounds is produced
- The method is sensitive to shock loads of suspended solids, metals, and biocidal organic compounds
- Energy requirements are relatively high

The activated sludge process is sensitive to disruption from suspended solids, oil, and grease The Environmental Protection Agency (EPA 1982) recommends that suspended solids be less than 1 percent, with oil and grease less than 75 mg/l (preferably less than 50 mg/l), for effective treatment The waste should also be free of heavy metals at levels that inhibit microbial activity The waste must also be free of any toxic organic compounds at levels high enough to kill microorganisms or to prevent their growth If this occurs, the wastes will not decompose or will do so slowly Finally, the system must be cost-effective compared to alternative methods The decision process used to determine if activated sludge treatment may be applied to hazardous wastes is shown in Figure 5 3

Trickling Filter

Trickling filters have biological films grown on a fixed bed of crushed rock or some other support medium Liquid waste is applied to the top and passes over the slime layers, which consist of a complex mixture of microorganisms Organic wastes are biotransformed by the microbial population, and biotransformation products are returned to the liquid waste stream

Trickling filters can accept shock loads better than the activated sludge process Various support media and depths of media are used for different hydraulic loads The process requires that the filter bed be aerobic As a result, the wastewater to be treated should contain less than 1 percent solids, that is, it should be almost entirely liquid Higher solid concentrations cause the support media to plug and the system to become anaerobic

The trickling filter does not produce as high a quality of effluent as the activated sludge process However, the system may be less troublesome to operate and is less likely to strip volatile compounds (Berkowitz, Funkhouser and Stevens 1978)

The advantages and disadvantages of trickling filter treatment of hazardous wastes are listed below For comparison with other methods discussed in this chapter, see Figure 5 1

Advantages:

- The method has short hydraulic residence times
- The method has low sensitivity to shock loads
- Suspended or colloidal matter can be removed

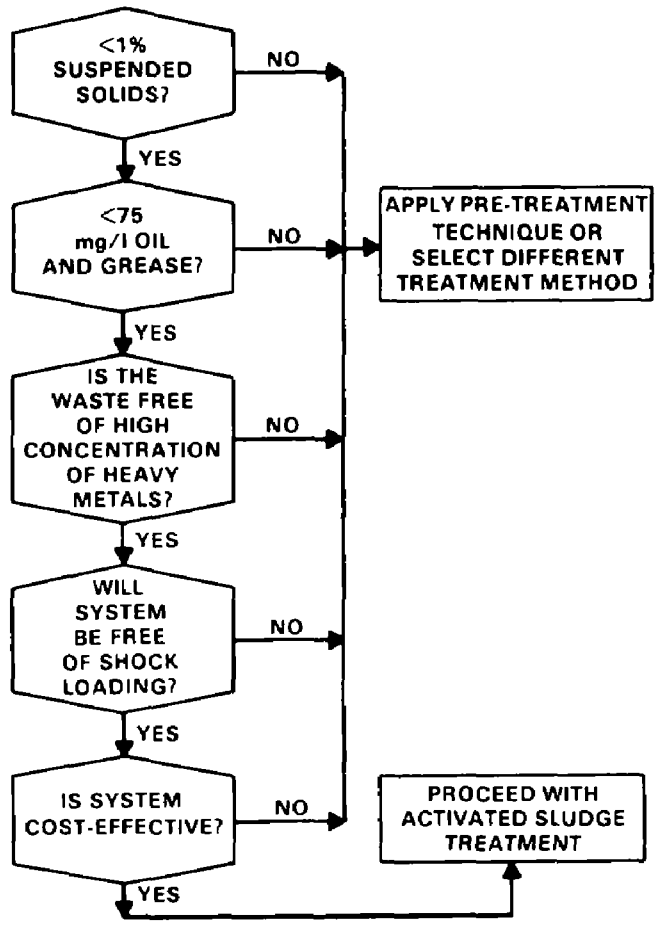

FIGURE 5.3 Decision Process for Evaluating Activated Sludge Biotreatment

Trickling Filter (contd)

- The method can be used as a roughing filter to moderate organic loads

Disadvantages:

- The method is applicable only to liquid waste streams
- Treatment capability is restricted in a one-pass-through operation
- Odors may be a problem
- Flexibility and product control are relatively limited
- Microorganisms are slow to recover if disrupted
- After shutdown, the system recovers slower than the activated sludge treatment
- Freezing temperatures may prevent operation

Trickling Filter (contd) Because the trickling filter process involves continuous trickling of water containing wastes over a support medium, the system should not be disrupted Disruption may permit the medium to dry out, thus destroying the microbial population Freezing temperatures may also disrupt the system by solidifying the liquid waste and preventing it from passing through the filter bed Pretreatment of wastes may be necessary if odors are a problem Trickling filters are usually used with other treatment methods because the short retention time does not generally produce an effluent low in toxic components The decision process used to determine if trickling filter treatment may be applied to biotransform hazardous liquid wastes is shown in Figure 5 4

Rotating Disc The rotating disc process is similar to the trickling filter process in that both use a fixed-film growth of microorganisms to degrade wastes A series of perforated disks are mounted on a horizontal shaft and then placed in a tank having a contoured bottom The discs are immersed approximately 40 percent The liquid trickles from the void spaces and the biomass is aerated (Metry 1980) when the disks are rotated in the tank.

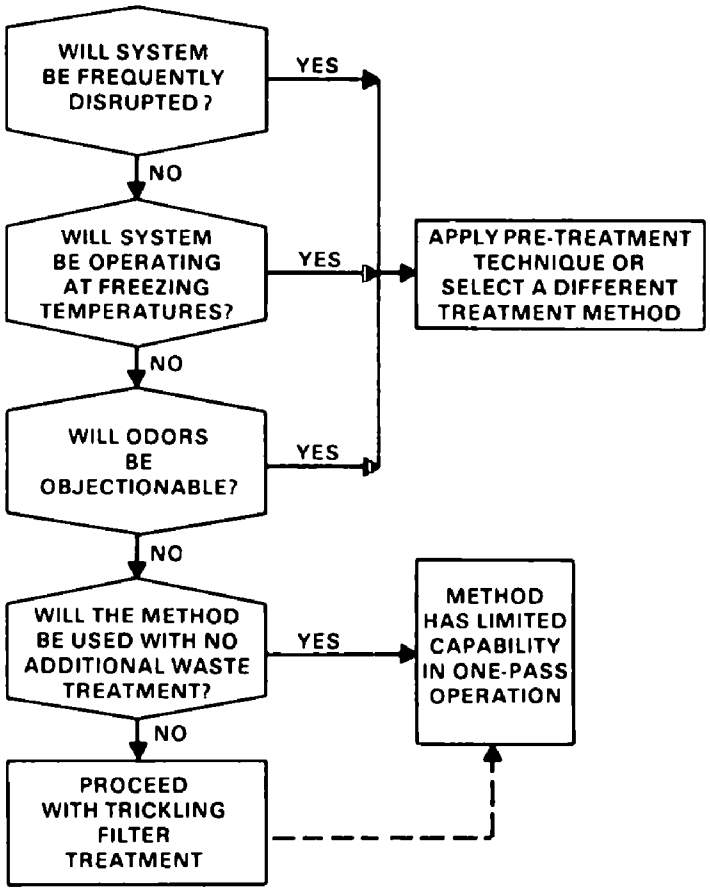

FIGURE 5.4 Decision Process for Evaluating Trickling Filter Biotreatment

Rotating Disc (contd)

The advantages and disadvantages of rotating disc treatment of hazardous wastes are listed below For comparison with other methods discussed in this chapter, see Figure 5 1

Advantages:

- Operation is relatively flexible (Biomass, waste water and aeration rate can be controlled by the rotation of the discs)
- Treatment capacity is relatively high (Retention time can be controlled by varying tank size)
- The system rarely clogs (Shearing forces continuously and uniformly strip excess growth)
- The method can be modified to meet varied waste loadings.

Disadvantages:

- The method is applicable only to liquid waste streams
- More aeration may be needed for high organic loads
- Odors may be a problem
- Microorganisms are slow to recover if disrupted
- Organism activity recovers more slowly after a shock load than in the activated sludge treatment

Rotating disks have been used for wastewater treatment in the United States only since 1969, but their use is increasing

As is the case with trickling filters, the discs may dry and damage the microbial population if the system is disrupted for any length of time. Also, the method cannot operate when ice forms Additional aeration may be required for waste streams with high organic loads, because aeration is provided only by disc rotation.

The decision process used to determine if rotating disc treatment may be applied to hazardous wastes is shown in Figure 5 5

Aerobic and Anaerobic Lagoons and Ponds

Lagoons and ponds used as bioreactor systems are large basins that rely on long waste retention times and both natural and forced aeration for waste decomposition In the aerated basin, the wastes are artificially aerated with diffused air or mechanical aerators Basin treatments differ from activated sludge processes in that they do not recycle biomass Waste lagoons and ponds are more sensitive to high concentrations of inorganic materials and suspended solids than are other biotreatment methods If there is no mixing, suspended solids will settle, creating an excessive load and a sludge blanket on the bottom

The advantages and disadvantages of lagoon treatment of hazardous wastes are listed below For comparison with other methods discussed in this chapter, see Figure 5 1

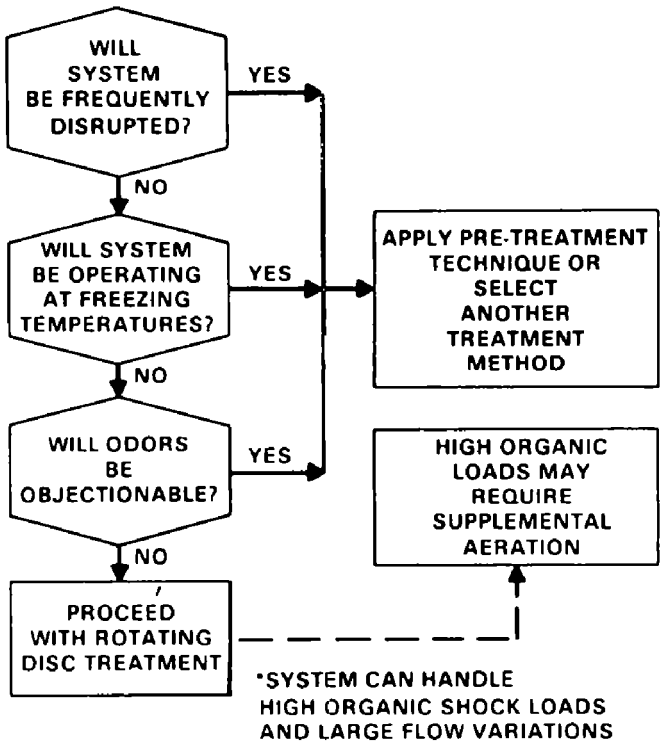

FIGURE 5.5 Decision Process for Evaluating Rotating Disc Biotreatment

Aerobic and Anaerobic Lagoons and Ponds (contd)

Advantages:

- Operating costs are low
- The treatment is an effective final-phase method for waste cleanup
- Energy requirements are low

Disadvantages:

- Only low-strength wastes can be treated
- Concentrations of suspended solids and metals must be low
- Requires large areas of land
- Freezing temperatures limit operations
- Flexibility is limited
- Volatile gases may be emitted
- Odors may be a problem

In general, lagoons and ponds can treat only low-strength wastes Therefore, they should be used only after other treatments have been applied

Aerobic and Anaerobic Lagoons and Ponds (contd)

Aerated basins may result in greater evaporation of waste compounds, which might impair air quality The large size of most basins, however, provides dilution and buffering of fluctuations in waste loads Anaerobic lagoons and ponds are sensitive to waste composition because anaerobic organisms are sensitive to environmental changes The methane-forming bacteria are highly sensitive to pH levels, operating best in a narrow range of pH of 6 8 to 7 5 (Berkowitz, Funkhouser and Stevens 1978) For both aerobic and anaerobic basins, buildup of sludge may require periodic shutdown for removal This residue, which may, in itself, be hazardous, must be disposed of elsewhere at an environmentally favorable site

The decision process to determine if lagoon and pond systems may be used to biotransform hazardous wastes is shown in Figure 5 6

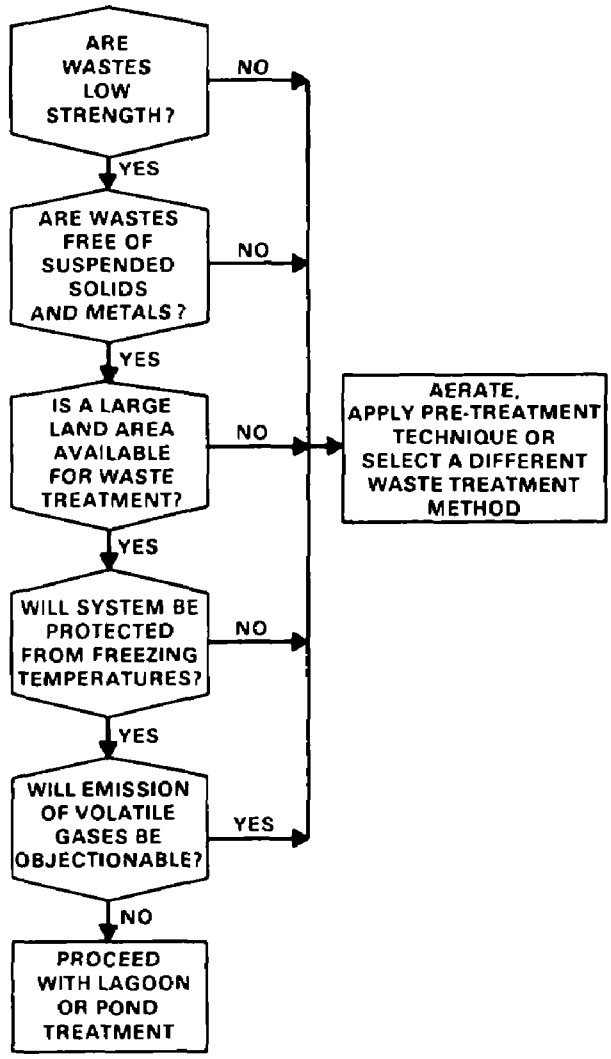

FIGURE 5.6 Decision Process for Evaluating Lagoon and/or Pond Treatment

5.4 BIOACCUMULATION AND REMOVAL

Bioaccumulation occurs because algae and some higher plants can increase the concentration of certain chemical substances in their cells to a level higher than the levels in the adjacent environs Sorption of certain pesticides by aquatic microorganisms and algae is also known to occur (Paris et al 1975)

Mass culture of the photosynthetic organisms (autotrophs) on hazardous wastes, followed by harvest and disposal, is a possible biological cleanup technique

The ability of algae to bioaccumulate waste materials is related to their high adsorptive/absorptive nature, which is a function of their high surface-to-volume ratio The potential use of algae and higher plants to bioaccumulate should be considered when confronting certain waste disposal situations Generally, only some metals and some organic materials can be bioaccumulated, but bioaccumulation often plays a natural role in the environmental transfer of chemical components (Jenkins 1975)

Little information is available on bioaccumulation as a method of removing hazardous wastes from the environment Most interest has been in the use of algae to absorb excess nutrients (mainly nitrogen and phospherus) in stabilizing ponds prior to release of treated water to streams or lakes (Fitzgerald and Rohlich 1964, Sufferin, Fitzgerald and Szluha 1981) One limitation of bioaccumulation is that only certain concentrations can be accumulated (especially of the heavy metals) before autotrophs are inhibited or killed A number of interacting factors influence the accumulation of heavy metals by plants (Cataldo and Wildung 1978)

Tolerance of algae and higher plants to toxic metals and organic compounds varies widely with species and environmental conditions Some chemicals will inhibit bioaccumulation of other chemicals (Cushing and Rose 1970, Soeder et al 1975, Hart 1977) Algae and plants can be killed directly by waste constituents, by waste degradation products and by-products, and by indirect effects of the wastes on nutrient transfer (Chaney 1981b)

In theory, algae can be grown in bioreactor systems containing liquid wastes Higher plants can be grown on land treatment sites, on the overburden of abandoned hazardous-waste disposal sites, or on the site of accidental waste release In all cases, the concentrations of hazardous wastes must be sufficiently low so that autotrophs will not be destroyed or their growth inhibited Because of this requirement, bioaccumulation is usually a final step in the cleanup process

The advantages and disadvantages of bioaccumulation treatment of hazardous wastes are listed below For comparison with other methods discussed in this chapter, see Figure 5 1

Advantages:

- The method is relatively inexpensive if physical and chemical conditions are suitable for autotrophic growth

- The method can be used as final cleanup of an abandoned waste site, spill site, or land treatment site
- The method can be used for pre- and post-treatment monitoring
- Algae and higher plants that bioaccumulate may be used for food, fertilizer, and other uses if toxic levels do not preclude their use

Disadvantages:

- Waste concentrations cannot exceed levels that are lethal to autotrophs, thus, the method is restricted to low-concentration wastes
- Use throughout the year is restricted to certain locations, because temperature and sunlight will affect growth rates
- Depending upon bioaccumulation levels, disposal of harvested biomass may be a problem

In general, bioaccumulation offers few advantages over conventional waste treatments However, the tendency of algae and some larger plants to accumulate hazardous materials may be of some value if combined with chemical and physical treatment and with microorganism activities Some bioaccumulation will be unavoidable at sites containing hazardous materials For example, plants will grow on contaminated soil and composted materials, leachates will drain into sumps and algae will grow in the liquid mix, and algae will grow in waste-treatment lagoons and ponds Where natural bioaccumulation occurs, human and wildlife access must be limited to control movement of waste offsite

Bioaccumulation by algae and some higher plants for hazardous-waste recovery and removal cannot be used at all sites If used in field situations, the method is limited to areas with suitable thermal and sunlight regimes present throughout the year (i e , the sun belt) The method is restricted to those hazardous wastes that are accumulated by algae and larger plants (some metals and organic materials), and are not toxic or inhibitory to autotrophic growth.

Determining Whether or not Bioaccumulation and Removal is an Option

The decision process to determine if bioaccumulation and removal is a biotreatment option is shown in Figure 5.7. The following information must be obtained to answer the questions in Figure 5 7

- wastes involved
- concentration of wastes in water or soil
- toxicity of wastes to algae and higher plants
  - at ambient concentrations
  - at accumulated concentrations
- environmental characteristics
  - physical - light, temperature, flow, etc
  - chemical - nutrient availability, pH, etc
  - biological - will mass culture be possible, will grazing animals limit crop?

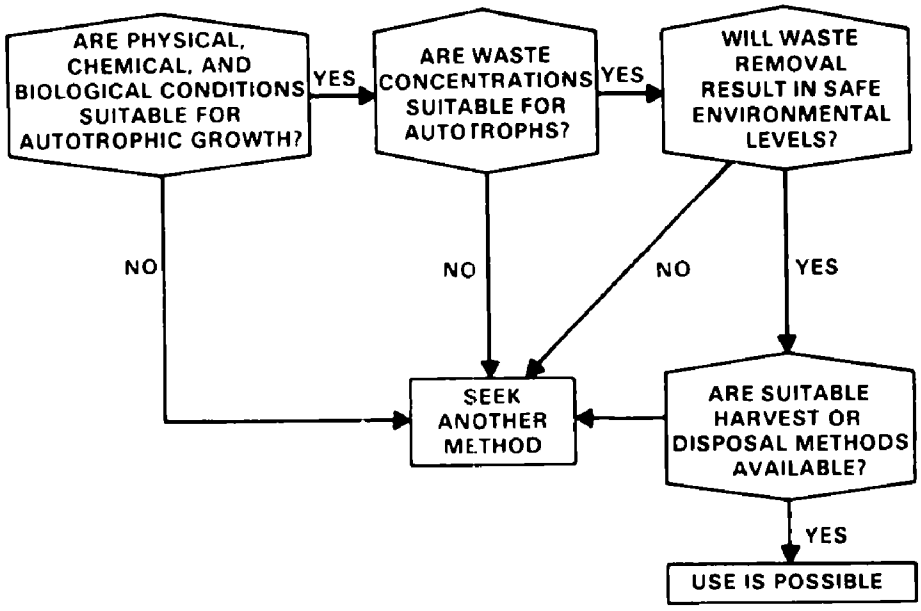

FIGURE 5.7 Decision Process for Evaluating Bioaccumulation by Algae and Higher Plants (Autotrophs)

Determining Whether or not Bioaccumulation and Removal is an Option (contd)

- percent of waste removal necessary
- eventual disposal
  ease of harvesting
  disposition of biomass - Is the problem just moved from one place to another?
- accessibility of the site to humans and animals

5.5 ENHANCEMENT OF BIOTREATMENT PROCESSES

The objective of pre-treating hazardous wastes is to render them suitable for biotransformation The wastes can be pre-treated by physical and/or chemical methods to reduce their toxic or inhibitory effects on microorganisms, or to alter their chemical composition to a form suitable for further microbial transformation

Hazardous wastes may destroy or inhibit the activity of microorganisms The main hazardous components of complex toxic waste mixtures, which may occur at disposal sites, are

- heavy metal cations (Sb, As, Cd, Cr, Hg, Pb, Zn, Ni, Cu, V, P, Be, Se, Mn, Ti, Sn, Ba, etc)
- heavy metal anions (chromates, chromites, arsenates, arsenites, etc)
- non-metallic toxic anions (cyanides, sulfides, thiocyanates, etc)

- organics (hydrocarbons, organic acids, organic peroxides, esters, alcohols, aldehydes, phenols, chlorocarbons, amines, analines, pyridines, organic sulfur compounds, organic phosphorous compounds, alkaloides, sterols, etc)

The most common form of pre-treatment is mixing with water In field situations where hazardous materials are dispersed in the soil, mixing occurs from precipitation or ground-water infusion, or can be accomplished with overhead sprinkler or underground injection systems Onsite application of water, however, must avoid contamination of surface-water and ground-watersupplies, and prevent the spread of toxic materials beyond site boundaries Hence, depending on ambient conditions, confinement systems may be required

Neutralization and precipitation are two other common practices used to reduce the toxicity of hazardous wastes Since heavy metals remain in solution over a certain acidic pH range, neutralization (pH adjustment) and precipitation often occur together

Chemical and physical processes other than dilution, neutralization and precipitation may be necessary to reduce toxicity of waste materials prior to land treatment or introduction to bioreactor systems Treatment processes common to municipal and industrial waste streams are discussed by Landreth and Rogers (1974), SCS Engineers (1979a), Arthur D Little, Inc (1977), Berkowitz, Funkhouser and Stevens (1978), NATO (1981), and Shuckrow, Pajak and Osheka (1981) In addition, chelating agents can be used to aid removal or detoxification of heavy metals (Jenkins et al 1981, Muramoto 1981)

Potentially useful pre-treatment steps applicable to liquid wastes include

- chemical coagulation - Lime or alum can be added to form precipitates that scavenge toxic substances such as heavy metals
- carbon sorption - Packed activated carbon can be added to the wastes to reduce toxicant levels, particularly organics
- ultrafiltration or reverse osmosis - Large molecular compounds, which typically include the toxic or refractory components can be removed, while leaving the biodegradable components
- steam stripping - Numerous, low molecular weight organic compounds can be removed, but most of these are also biodegradable
- aeration, sedimentation, and filtration - Heavy metals can be precipitated to facilitate removal by sedimentation or filtration.
- chemical oxidation - Certain materials can be detoxified, but most oxidizable materials are readily biodegradable

Since the purpose of biotreatment is to transform materials from hazardous to innocuous forms, post-treatment is generally not considered However, when products arising from bioreactor units fail to reach acceptable environmental standards, post-treatment will be required The need for post-treatment will be determined through monitoring the treated waste materials (see Chapter 4)

Considerations for Enhancing of Biological Processes

Enhancement should be considered when the biotransformation rate is otherwise too slow to be practical Microbial degradation of hazardous wastes can sometimes be enhanced in field situations and bioreactor systems by adding exogenous microorganisms (Atlas 1977, Daughton and Hsieh 1977, Barles, Daughton and Hsieh 1979, Kobayashi and Rittmann 1982)

Biotransformation processes can be slowed when degradation by microorganisms is limited by their low numbers or when a waste inhibits microorganism activity The rate at which microorganisms degrade hazardous materials depends on their inherent ability and on the physical and chemical properties of the environs that affect their growth (viability) Rates of microbiological transformation may be affected by physical and chemical factors such as the lack of nutrients or extremes in pH, Eh, temperature, moisture content, and salinity

Microbial activity in field situations or in a bioreactor system can be increased by pre-treating the wastes, altering the physical and chemical environs, and introducing cultured or engineered microorganisms The waste environment is generally altered by adding appropriate nutrients, chemically adjusting the pH, or adjusting the Eh through flooding or cultivation Needs are identified by site-specific chemical analysis The natural microbial community is generally augmented by introducing enriched or adapted microorganisms to degrade a specific waste This process is sometimes called "biological seeding," and it involves applying select cultures of microorganisms to special wastes (SCS Engineers 1979a)

The initial biotransformation of a hazardous waste will usually involve indigenous microorganisms that occur at the site Whether or not these organisms can completely degrade the waste to innocuous compounds will determine if other methods, including microbial enhancement, should be used

Factors to consider when evaluating the usefulness of enhancement in biotreatment options are grouped in Figure 5 8 The types and sources of information necessary to answer the questions in Figure 5 8 are listed throughout the following text

Are natural conditions and indigenous microorganisms suitable to transform, degrade, and/or adsorb the hazardous wastes? *[see Figure 5 8, (1)]*

- Consider the following types and sources of information
  - waste concentrations
  - site characteristics (surface and ground water, soil, climate, topography)
  - site use characteristics
  - local population characteristics

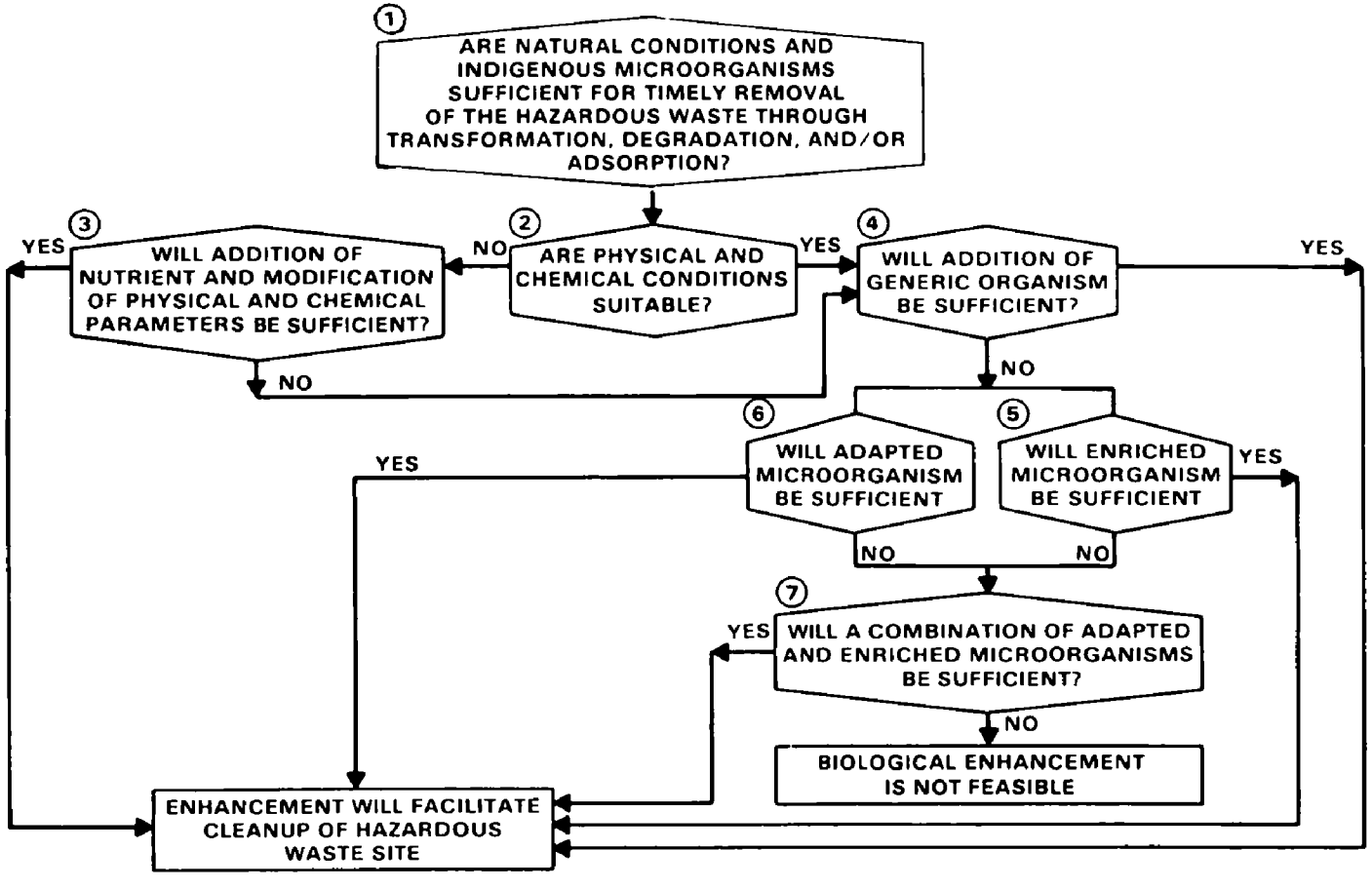

FIGURE 5.8 Decision Process for Determining if Enhancement is Required for Biotreatment

Considerations for Enhancing of Biological Processes (contd)

- Consider the following sources of information
  - published reports (journal articles, technical reports, government reports, expert advice)
  - environmental impact assessments

Enhancement of Site-Specific Conditions

Natural conditions at field locations and in bioreactor systems can be altered to optimize growth and survival of microorganisms that biotransform waste Optimization requires characterization of the physical and chemical properties of the soil, water, geosubstrate, ground water or bioreactor system, identification of the factors that limit transformation rate, and selection of ways to alter factors that limit microbial growth

Several physical and chemical conditions can influence the density and composition of a microbial community

- moisture
- aeration
- temperature
- organic matter
- pH
- inorganic nutrients

Moisture—The moisture level influences biotransformation rates in soils, sediments, and solid-waste treatment systems (composting) It is not important in liquid media Most aerobic soil bacteria usually occur when the moisture level is between 50 and 75 percent of the soil's retention capacity The moisture level may vary in sediments and solid-waste treatment systems with rainfall and dehydration Anaerobic systems are generally controlled by keeping the medium saturated with water while limiting aeration Optimal moisture levels can be maintained by sprinkler and drainage systems

Aeration—Aeration is necesary for aerobic biotransformation processes Air should be forcibly injected into solid or semi-solid substrates, as in aerobic composting Contaminated surface soils and sediment can be aerated by regular cultivation Liquid waste streams are easily aerated by mixing, spilling, air injection, and other methods

Temperature—Temperature influences microbial activity at all hazardous waste sites and in all biotreatment processes The optimal temperature for most bacteria ranges from 25° to 35°C, but bacteria can multiply at 15° to 45°C (Alexander 1977) Temperature also affects the biotransformation rate For every 10°C increase in temperature, the rate should increase about twofold Temperature control is difficult under field conditions Microbial activity in some situations (e g , composting) generates heat To estimate the degradation rate, or the time to site recovery, the

Enhancement of Site-Specific Conditions (contd)

influence of temperature on microorganism activity must be considered Various procedures are used to maintain warm temperatures in bioreactor systems

Organic Matter—The number (biomass) of soil microorganisms can, in many cases, be increased by adding organic matter such as manure, crop residues, forest residues, and some industrial organic wastes Commercial fertilizers can also be used (Jobson et al 1975) An increase in biomass, however, may depend on other nutrients Adding organic matter or nutrients is useful when more microorganisms will increase the biotransformation rate In some cases, adding organic matter may slow biotransformation activity and, therefore, inhibit the removal of hazardous components The value of organic matter or nutrients can often be determined from simple laboratory studies, where the degradation is examined under controlled conditions

pH—The growth of microorganisms is usually greatest within a pH range of 6 to 8 Several methods are available to adjust the pH The pH of soils, for example, is generally lowered by adding sulfur or raised by adding lime (Tisdale and Nelson 1966) Most state agricultural experiment stations and soil testing laboratories have quantified the lime requirements of major soil types in the areas they serve For land areas contaminated by hazardous materials, methods used to adjust pH for crops can be used to adjust pH for indigenous microorganisms

Inorganic Nutrients—In many areas, microbiological transformation rates are slowed because one inorganic nutrient is absent or is present in low quantities The nutrient is usually nitrogen or phosphorus Microbial activity can be increased by adding the appropriate inorganic nutrient

Physical and chemical factors should be considered when determining if enhancement is required for biotreatment The types of information necessary to answer Questions (2) and (3) in Figure 5 8 are listed below

Are physical and chemical conditions suitable *[Figure 5 8 (2)]?*

- Consider the following types of information·
  - moisture content
  - oxygen content (aerobic conditions require sufficient aeration, anaerobic conditions require little oxygen)
  - temperature
  - organic carbon
  - pH
  - inorganic nutrients

Enhancement of Site-Specific Conditions (contd)

Will addition of nutrients and modification of physical and chemical parameters improve microbial activity *[Figure 5 8 (3)]?*

- Consider the following types of information
  - cost
  - degradation and transformation rates after modification

Addition of Generic Microorganisms

Generic microorganisms are microbes that occur naturally and are indigenous to the waste, the site, or the biotreatment system

Generic microorganisms are not intentionally adapted or enriched for a specific waste material Sources of generic microorganisms include surface soils, sediments, and sewage sludge In general, the method involves amending the site or the biotransformation process with indigenous microorganisms

Generic microorganisms can be used in land treatment or various bioreactor systems In either application, generic microorganisms are usually added when an increase in microbial biomass will increase the degradation rate significantly The procedure is best when indigenous microorganisms already present can degrade the hazardous material Generic microorganisms are generally used to begin biotreatment processes

Will addition of generic organisms be sufficient to degrade or transform the wastes *[Figure 5 8 (4)]?*

- Consider the following types of information
  - physical and chemical characteristics
  - concentrations of the hazardous wastes
  - site recovery potential
  - site characteristics (surface and ground water, soil, climate, topography)
  - toxic by-products potential
  - human populations
  - hazardous materials transportability
- Consider the following source of information
  - published reports (journal articles, technical reports, government reports, expert advice)

Culture and Introduction of Enriched Microorganisms

Enriched microorganisms are microbes cultured for transforming a specific hazardous waste

Biotransformation rates can be inhibited because the microorganisms that degrade a compound are only a small part of the total microbial community, and because natural pathways leading to degradation are limited

Increasing the ratio of effective microorganisms to total available microorganisms can be accomplished by introducing cultures enriched to transform the waste

Culture and Introduction of Enriched Microorganisms (contd)

A microbial community (in soil, sediment, sewage sludge, or elsewhere) can be supplemented by adding microorganisms developed to use a specific waste as the sole source of carbon for metabolic growth Both batch and continuous culture techniques are suitable to grow enriched microorganisms

In batch enrichment, microorganisms are grown in a closed system such as test tubes or bottles where the concentrations of nutrient and metabolic products change during incubation Enrichment is usually initiated by transferring a sample from a complex microbial community to a culture medium that contains the waste as the sole source of carbon Microorganisms unable to use the waste for metabolic energy will disappear Repeated transfer of the culture to fresh enrichment media after a period of growth will, in many cases, lead to a stable microbial population capable of, and specifically enriched for, degrading the target waste

In continuous enrichment, microorganisms are grown in a chemostat (Senior, Bull and Slater 1976), which continuously supplies the nutrient that limits growth and removes metabolic products By using the target waste as the nutrient that supplies carbon, a stable microbial community may develop that will biotransform the target waste

Several techniques can be used to increase biotransformation rates of previously enriched cultures, to develop enriched cultures for resistant organic compounds, or to apply in situations where normal enrichment procedures are unproductive Attempts to increase the activity of enriched microorganisms in cultures have been made by mutating an enriched culture and repeating the enrichment process The strains that demonstrate an increased biotransformation rate are then identified (Wong, Leong and Dunn 1978)

When direct enrichment is difficult, pre-enrichment with related organic substrates may aid the process An enrichment culture for the specific organic substrate can then be prepared (Brilon et al. 1981)

A more elaborate procedure is plasmid-assisted molecular breeding This is a recently developed technique (Kellogg, Chatterjee and Chakrabarty 1981, Chatterjee, Kilbane and Chakrabarty 1982) used to evolve a strain of bacteria that rapidly degrades 2, 4, 5-trichlorophenoxyacetic acid (2,4,5-T) The technique is a modification of the continuous culture (chemostat) enrichment procedure The initial bacterial inoculum (soil, sediment, sewage sludge) is amended with specific bacterial strains

The new strains contain plasmid-encoded pathways that may evoke degradation of the target organic substrate Enrichment

Culture and Introduction of Enriched Microorganisms (contd)

begins with a low concentration of substrate and a high concentration of a plasmid substrate Over the culture period, the concentration of the plasmid substrate is lowered and the concentration of the target substrate is raised. If successful, a strain of the microorganism or a mixed microbial population capable of degrading the target waste will be produced Enriched cultures are not readily available, and must be developed under laboratory conditions

Initial efforts have been made by genetic engineering to produce microorganisms that degrade specific organic mixtures such as oils (Atlas 1977) These efforts to date have not produced microorganisms that can degrade organic compounds when mixed microbial populations (normally associated with natural systems or biotreatment processes) are present However, genetic engineering, which includes the biochemistry of gene splicing, microbial genetics, and gene amplification, shows future promise

Enrichment and adaptation are not mutually exclusive In many instances, both processes occur simultaneously For example, when the substrate is changed, both adaptation and enrichment of the microorganisms take place

Enriched cultures can be used as the initial inoculum for starting various bioreactor systems In some cases, they can be used to enhance biotransformation processes at field locations where hazardous materials are present Enriched cultures are generally used when the hazardous material is not degraded within suitable time limits by generic (indigenous) microorganisms.

Will addition of enriched microorganisms be sufficient to degrade the waste *[Figure 5 8 (5)]?*

- Consider the following types of information
  - physical and chemical characteristics
  - hazardous-waste concentrations
  - commercial availability of enriched culture
  - potential for site recovery
  - time necessary to prepare enriched culture
  - site characteristics (surface and ground water, soil, climate, topography)
  - toxic by-products
  - local human populations
  - government regulations for use of enriched microbiological cultures
  - safety considerations (worker health and safety, environmental)
  - transportability of hazardous material

Culture and Introduction of Adapted Microorganisms

Adapted microorganisms are microbes cultured for site-specific physical and chemical conditions

The activity of indigenous or enriched microorganisms at a hazardous-waste site can be limited by nutrients, extremes in the environment (e g , pH, Eh, temperature, salinity), or toxic components (e g , trace metals, organic compounds, and degradation products) Any one of these factors may depress microbial activity If limiting conditions occur, microbiological growth can be enhanced by either altering physical and chemical conditions (see Enhancement of Site-Specific Conditions, p 5 21) or by introducing organisms specifically adapted to site-specific conditions Adapted microorganisms are generally used when the wastes are toxic to microorganisms or physical and chemical enhancement of the site is not possible

A microbial population can be adapted by incrementally adjusting the environs over time to the chemical and physical conditions desired The initial microorgaisms are generally obtained from soils, sediments, sewage sludge, and/or enrichment cultures Like enrichment, adaptation is usually conducted in either batch or continuous culture (chemostat) incubation systems

Batch adaptation may require gradual transfer of microorganisms from an unadapted culture to a culture representing the desired physical and chemical conditions When adaptation is difficult, samples can be transferred through a series of batch cultures in increments that approach final conditions In the latter situation, each batch culture is allowed to reach a predetermined growth level before a sample is transferred to the next incremental level Complete adaptation occurs when the microorganisms actively grow on a medium simulating physical and chemical conditions of the waste site

Continuous culture (chemostat) systems have also been used for adaptation By gradually altering the initial growth medium, conditions can be changed to the desired parameters A culture is adapted if it actively grows when the target conditions are attained Adapted cultures are not readily available and must be developed under laboratory conditions

Adaptation and enrichment within a culture occur primarily by two processes Both processes select microorganisms that can survive and increase as cultural conditions are changed These processes also select for spontaneous mutations that occur in a cultured population In some cases, the mutants can grow under the target conditions In this regard, adaptation may be enhanced by inducing mutations among cultured microorganisms (Wong, Leong and Dunn 1978) Mutations are induced conveniently by the batch adaptation system

Culture and Introduction of Adapted Microorganisms (contd)

Adaptation and enrichment are not mutually exclusive In many instances, both processes occur simultaneously. For example, when the substrate is changed, both adaptation and enrichment of the microorganisms take place.

Adapted microorganisms can be used as the initial inoculum for starting various bioreactor systems In some cases, they can be used to enhance indigenous biotransformation processes at abandoned hazardous-waste sites or accidental-release sites The use of adapted cultures is generally considered when the hazardous material is not degraded within suitable time limits by generic (indigenous) microorganisms

Will addition of adapted microorganisms be sufficient to degrade the wastes *[Figure 5 8 (6)]?*

- Consider the following types of information
  - physical and chemical site characteristics
  - hazardous-waste concentrations
  - availability of adopted culture
  - potential for site recovery
  - time to prepare culture
  - site characteristics (surface and ground water, soil, climate, topography)
  - toxic by-products
  - local human populations
  - government regulations
  - safety of adapted culture (worker health and safety, environmental)

Culture and Introduction of Adapted and Enriched Microorganisms

Will a combination of adapted and enriched microorganisms be sufficient to degrade the waste *[Figure 5 8 (7)]?*

The information to answer this question is similar to that given for adapted microorganisms [Figure 5 8 (6)] and enriched microorganisms [Figure 5 8 (5)]

5.6 CHAPTER REFERENCES

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6.0 BIOTRANSFORMATION CONCEPTS

This chapter reviews information on mechanisms by which microorganisms degrade or transform hazardous materials It also describes the functions and limitations of microorganisms for biotreatment, Users of this guide with limited backgrounds in microbiology may benefit from this overview of the concepts

A combination of natural physical, chemical, and biological processes usually interact to transform hazardous materials in the environment (Rogers and Landreth 1975, Alexander 1981, Kobayashi and Rittmann 1982) Of these, the most important appear to be biological In some instances, physical and chemical methods can be used to aid or enhance biological transformations (Fields and Lindsey 1975, MacGregor and Metry 1977, Epstein and Chaney 1978, SCS Engineers 1979b, Wyss, Willard and Adams 1980, Shuckrow, Pajak and Osheka 1981, North Atlantic Treaty Organization 1981)

Biological transformation is caused by the enzymatic activity of indigenous microorganisms or, in some cases, by microorganisms specifically cultured or genetically engineered Current evidence suggests that indigenous microbial populations, particularly heterotrophic bacteria and fungi, are the chief agents for changing chemical molecules in waters and soils

6.1 ROLE OF MICROORGANISMS IN BIOTRANSFORMATION

Microbial activity can reduce or eliminate hazardous materials by

- aerobically or anaerobically degrading them into innocuous forms
- aerobically degrading them to carbon dioxide and water (mineralization)
- anaerobically decomposing them to carbon dioxide and methane.

Some chemical compounds, however, are not removed efficiently by existing biological treatment techniques, either because they are metabolized very slowly or because they are resistant to microbial degradation under prevailing conditions in the environs (Kobayashi and Rittmann 1982)

Microorganisms are ubiquitous They induce biotransformation of natural and synthetic compounds at rates that vary with environmental conditions and compound structure Microorganisms that transform specific compounds can be isolated, and many can be cultured, enriched, or adapted in the laboratory Microorganisms may also be genetically engineered to transform certain compounds The basis of biotransformation is the enzyme pathways of these microorganisms, which can lead to appreciable changes in the molecular structure of most organic and some inorganic compounds Nonenzymatic reactions also occur, but they generally hold a lesser role in breaking down synthetic compounds

Several groups of microorganisms, listed below in order of importance, biotransform hazardous materials

- heterotrophic bacteria
- fungi
- anaerobic bacteria
- actinomycetes
- phototrophic microorganisms
- oligotrophic bacteria

Each group has special characteristics that are useful for detoxifying hazardous-waste materials These characteristics are summarized in Table 6 1

Heterotrophic Bacteria Decomposition of synthetic organic compounds by heterotrophic bacteria is the most commonly studied biotransformation process Characteristically, bacterial species are not identified individually but are listed only by source of the inoculum (e g , sewage or soil) In most cases a mixed population of species is involved The most common heterotrophic bacteria isolated from areas contaminated by different organic compounds are represented by the genera *Pseudomonas, Achromobacter, Arthrobacter, Micrococcus, Vibrio, Acinetobacter, Brevibacterium, Corynebacterium,* and *Flavobacterium* *Corynebacteria* spp are believed to be major agents for breakdown of hydrocarbons and heterocyclic compounds in aqueous environments *Pseudomonas* spp are ubiquitous and able to biotransform many different synthetic compounds For example, they are adaptable to biotransform different chlorobenzene isomers through plasmid transfer of genetic material

Fungi Fungi have considerable ability to degrade or transform hydrocarbons of complex structure or long chain length Bacteria and yeasts, on the other hand, show decreasing abilities to degrade alkanes with increasing chain length Organisms in two orders of fungi—Mucorales (such as *Cunninghamella*) and Moniliales (*Fusarium, Aspergillus,* and *Penicillium*)—show high biotransformation potential For example, *Fusarium oxysporum* has the ability to completely degrade DDT, no other microorganism with this ability has been detected Because filamentous fungi and yeasts have nonspecific enzyme systems for aromatic compounds, they are believed to be more capable of degrading polychlorinated biphenols (PCBs) than are bacteria However, fungus metabolism often results in incomplete breakdown of a chemical structure In this case, subsequent association with bacteria is necessary to achieve mineralization

Anaerobic Bacteria Anaerobic transformation of organic matter to carbon dioxide and methane involves groups of strictly anaerobic bacteria Little is known about species composition and the changes that occur during biotransformation However, evidence suggests that at least four interacting groups of bacteria are involved·

TABLE 6.1 A Summary of Microorganisms Involved in Biotransformation of Chemical Compounds and Their Selective Characteristics[a]

Microorganism	Selective Characteristics[b]	Significance
Fungi		
Yeast	pH < 5, ae-mae, high O_2 tension, pH < 5, moisture about 50%	Attacks and partially degrades complex compounds not readily metabolized by other organisms Wide range of nonspecific enzymes
Mold		
Algae	ae-mae, light 600-700 nm, low carbon flux	Self-sustaining population, light is primary energy source, partially degrades certain complex compounds, photochemical reactions, oxygenates effluent, supports growth of other microbes, no aeration needed, effective in bioaccumulation of hydrophobic substances
Cyanobacteria (formerly called blue-green algae)	ae-mae, an, light 600-700 nm, low carbon flux	See algae
Bacteria		
Heterotrophs (aerobic)	ae, proper organic substrate, growth factors as required, Eh 0 45 to 0 2 V	For many compounds, degradation is more complete and faster than under anaerobic conditions High sludge production
Anaerobic (fastidious)	an, Eh <-0 2 to -0 4 V	Conditions for abiotic or biological reductive dechlorination, certain detoxification reactions not possible under aerobic conditions, no aeration, little sludge produced
Facultative anaerobes	mae-an, Eh <-0 2 V	No aeration necessary, reductive dechlorination possible
Photosynthetic bacteria		
Purple sulfur	an (light), mae (dark), Eh 0 to -0 2 V, $S^{=}$ 2 to 8 mM, 0 4-1 mM, light 800-890 nm at 1000-2000 lux, high intensities near limit, low C flux	Self-sustaining population able to use light energy, conditions right for reductive dechlorination, no aeration
Purple nonsulfur	an, Eh 0 to -0 2 V, light 800-890 nm, low carbon flux	See purple sulfur bacteria, also nonspecific enzymes
Actinomycetes	ae, moisture 80-87%, temp 23-28°C, urea as nitrogen source	Universal scavengers with range of complex organic substrates often not used by other microbes
Oligotrophs (may be from almost any group above)	ae, carbon flux of <1 mg/L/d, favorable attachment sites	Removal of organic contaminants in trace concentrations, many inducible enzymes for multiple substrates

(a) From Kobayashi and Rittmann (1982)
(b) Possible characteristics for selection, not growth range
ae = aerobic, mae = microaerophilic (<0 2 atm oxygen), an = anaerobic

Anaerobic Bacteria (contd)

- hydrolytic bacteria, which break down major biomass components such as saccharides, proteins, and lipids
- H_2-producing, acetogenic bacteria, which break down products such as fatty acids and neutral end-products produced by hydrolytic bacteria
- homoacetogenic bacteria, which break down multicarbon compounds to acetic acid
- methanogenic bacteria, which use hydrogen and carbon dioxide for growth

Anaerobic bacteria require oxygen-free conditions and oxidation-reduction potentials of less than -0 2 V These organisms occur in anaerobic sediments, in sewage sludge, and in stomachs (rumen) of cattle The role of strictly anaerobic organisms in biotransformation is indicated by several detoxification reactions that occur in the rumen of cattle These reactions include reductive dechlorination (or dehalogenation), a possible limiting factor in degradation of certain compounds, nitrosamine degradation, a removal mechanism for a suspect carcinogen, reduction of epoxide groups in various compounds to olefins, reduction of nitro groups, as found in nitrophenol, and breakdown of aromatic structures

In addition to strict anaerobes, bacteria capable of living in anoxic but not necessarily reducing environs, and bacteria that are both aerobic and anaerobic, are important to biotransformation processes These organisms are abundant in soil and sewage, and occur at sites where reductive chlorination occurs

Actinomycetes

Actinomycetes often occur in association with unusual chemical compounds They can attack numerous complex organic materials such as phenols, pyridines, glycerides, steroids, chlorinated and nonchlorinated aromatic compounds; and even lignocellulose, which very few microorganisms attack

The most common aquatic actinomycete is *Nocardia* spp , which typically feed on lipids in activated sludge Actinomycetes can grow under low nutrient levels (e g., in distilled water), tolerate a wide temperature range, resist dessication, and exist under a wide range of pH

Organic decomposition by actinomycetes generally results in metabolites that can be mineralized in the presence of other microorganisms Hence, mixed culture systems are necessary when actinomycetes are used The number of nitrogenous compounds that can be used by actinomycetes is limited, due to low cell synthesis, most of the nitrogen in the substrate is liberated as ammonia Low cell synthesis also tends to keep the populations of actinomycetes low under natural conditions Actinomycetes may be especially useful in treatment of contaminated soil where composting would be practical

Phototrophic Organisms

Algae, cyanobacteria (blue-green algae), and photosynthetic bacteria are phototrophic microorganisms Since they obtain energy from sunlight and carbon by carbon dioxide fixation, they are able to exist on substrates low in nutrients In addition, some cyanobacteria and photosynthetic bacteria are able to fix nitrogen and survive in soils where low nitrogen levels would not otherwise support bacterial growth

In general, phototrophic organisms promote partial rather than complete degradation, hence, interactions with other microorganisms are important The prolific growth of phototrophs will promote the growth of heterotrophic organisms, which feed on the metabolic products formed by the phototrophs Algae can readily biotransform various synthetic compounds if proper densities of bacteria are maintained (O'Kelley and Deason 1976, Matsumura and Esaac 1979)

In addition to their capacity to break down organic materials, phototrophic organisms are potentially useful because they can bioaccumulate hydrophobic compounds Bioaccumulation is a feature shared by many different micro- and macroorganisms Bioaccumulation by phototrophs is increased because they can sustain relatively large populations without needing organic matter in concentrations high enough to serve as carbon and electron donors In addition, some algae (especially *Chlorella* and certain diatoms) can be manipulated physiologically to develop large lipid stores in which hydrophobic compounds are sequestered

Cyanobacteria in general, and the green algae *Chlorella* in particular, are tolerant of pollution and low concentrations of dissolved oxygen *Dunaliella* can tolerate a wide range of salinity and can be used where salt concentrations vary Some phototrophs, particularly some species of cyanobacteria, appear to be easier to manipulate genetically than bacteria

Photosynthetic bacteria can metabolize numerous substances, including simple sugars, alcohols, volatile fatty acids, tricarboxylic acid (TCA) cycle intermediates, benzoates, and 1,3,5-trihydroxybenzene These bacteria produce a range of inducible enzymes, and they are widely used in wastewater processes that treat a variety of organic compounds

Chromatiacae tolerate pollution and occur in waste-treatment lagoons *Rhodopseudomonas capsulata,* a purple nonsulfur bacteria, can degrade nitrosamines (tumor-inducing agents) to innocuous compounds Some purple nonsulfur organisms are able to grow anaerobically as phototrophs, but also can live aerobically in the dark as heterotrophs This characteristic is typical of photosynthetic bacteria

Phototrophic microorganisms, because they are easy to grow selectively, can grow under anaerobic conditions if light is available.

They normally occur, however, in only small numbers in wastewater treatment systems

Oligotrophic Bacteria Oligotrophic bacteria are microorganisms that can live under conditions of low carbon flux (less than one milligram of carbon per liter per day) These organisms do not represent a taxonomic group, but exist in almost any group of bacteria other than chemotrophs Oligotrophs are obtained only from low nutrient environments, oligotrophs can be readily adapted to high nutrient levels, but readaptation to low nutrient levels is difficult

Oligotrophs generally have a high surface-to-volume ratio and high affinities for substrate They appear to prefer attachment to a free-living existence and, thus, are common in biofilms An important feature of oligotrophs is that they often appear to have multiple inducible enzymes, can shift metabolic pathways, and can take up and use mixed substrates One species of *Clostridium* can grow on at least 20 different substrates

Oligotrophs can potentially be used to remove trace concentrations of organic contaminants from water, treat effluent from wastewater treatment processes, or treat leachate from hazardous-waste disposal sites Unlike bacteria, which cannot grow and live under low-nutrient conditions, certain oligotrophs such as species of Actinomycetes (*Nocardia*), Coryneforms, and Mycobacteria can control their metabolism to survive as long as 30 days when no nutrients are available

6.2 BIOTRANSFORMATION PROCESSES

Microorganisms are noted for two biochemical processes involving energy transfer (metabolism)

- assimilation (anabolism) - where organic compounds are synthesized from CO_2, water, and other components; this process is endothemic and primarily reductive
- dissimilation (catabolism) - where organic compounds are decomposed to CO_2 and water, or partially decomposed to lower molecular weights (e g , alcohols to aldehydes or acids), this process is exothermic, and involves oxidation or reduction

The organisms that assimilate are autotrophic, while those that dissimilate are heterotrophic Autotrophic organisms may assimilate photosynthetically with sunlight for energy, or chemosynthetically with chemical reactions for energy, whereby inorganic elements are oxidized Heterotrophic organisms obtain energy from the decomposition of organic materials

Most degradation or transformation of organic compounds in the environment is done by heterotrophic microorganisms Heterotrophs may be either aerobes, obligate anaerobes, or facultative anaerobes Aerobes require molecular oxygen for respiration and synthesis, obligate anaerobes require the

complete absence of oxygen, facultative anaerobes can grow either in the presence or absence of oxygen Aerobic synthesis is faster than anaerobic synthesis and has a higher growth yield of cells that produces more inert materials (e g , sludge)

Dissimulation of organic compounds by heterotrophic microorganims can occur by

- mineralization
- co-metabolism

Mineralization pathways characterize the microbial breakdown of several classes of synthetic compounds (Alexander 1981) As organic material is converted to inorganic material, the responsible microorganisms use some of the substrate carbon and release enough energy to synthesize cell constituents for growth Thus, mineralization is typically a growth process for microorganisms Detoxification of a chemical is a common outcome of mineralization, but sometimes the mineralized product (e g , nitrate or sulfide) is also of environmental concern

Co-metabolism (co-oxidation) occurs when microorganisms are able to transform a chemical compound without using it as a source of nutrients (Horvath 1972, Alexander 1981, Morrill, Mahilum and Mohuiddin 1982, Kobayashi and Rittmann 1982) In other words, the microbial population is growing on one substrate while performing a biotransformation on another

A number of important differences exist between mineralization (direct transformation) and co-metabolism (indirect transformation) of a chemical compound

Mineralization	Co-metabolism
Microorganisms increase in numbers and biomass	No increase in numbers and biomass
Microorganisms transform waste material directly	Waste material is transformed indirectly
Waste transformation is rapid, rate increases with time	Transformation is slow, rate does not increase with time
Waste breaks down completely	Waste breakdown is incomplete to structurally similar compounds

Some of these relationships are shown in Figure 6 1

Mineralization and co-metabolism represent extremes The complete biotransformation of most, if not all, hazardous organic wastes fall somewhere between these extremes

Current knowledge of the chemistry of biotransformation and breakdown of synthetic compounds by microorganisms is still limited Few biochemical precedents exist for many of the compounds of environmental concern Investigation of metabolic pathways are often conducted in microbial cultures because the studies are easier and the data are less equivocal (Alexander 1981)

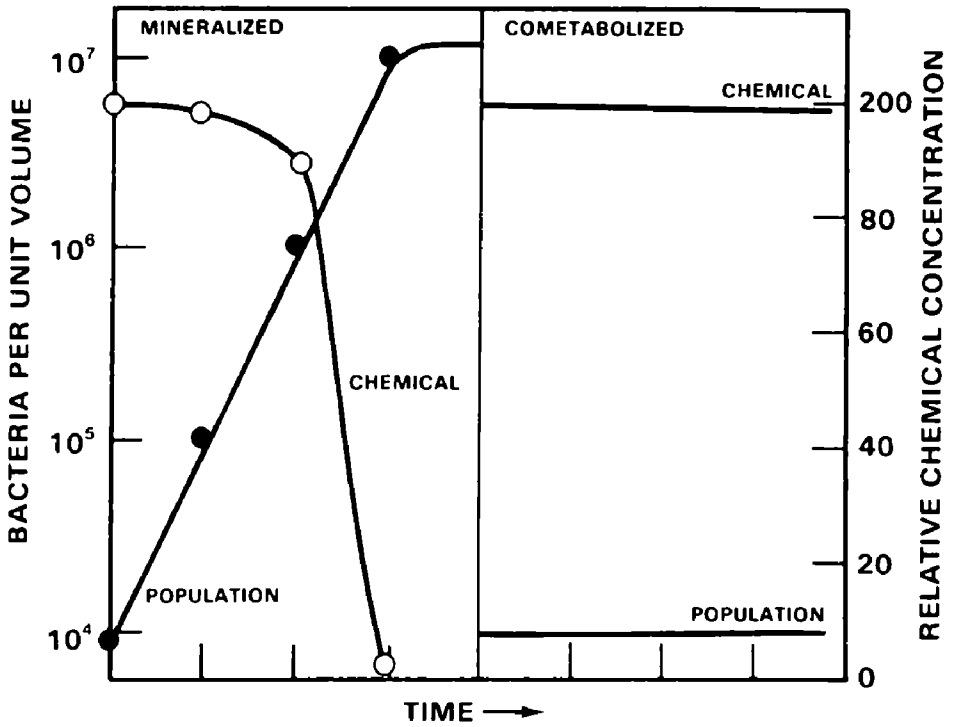

FIGURE 6.1 Hypothetical Model for Population Changes and Metabolism of a Chemical Exposed to Mineralizing and Co-metabolizing Populations (from Alexander 1981)

Laboratory studies, however indicative, cannot always be used to predict detoxification of hazardous materials in field situations

Information on chemical reactions resulting from the activities of microorganisms on an array of chemical compounds is given in Table 6 2

The biotransformation or biodegration of a compound may involve various types of reactions The reactions represent sequences established in model ecosystems or, in some instances, soil or water The chemical may be modified, as indicated by the reaction type, or the reaction may apply to a late step in the transformation or degradation process In some cases, the reaction may not be microbial because some steps may be partially or wholly nonenzymatic Furthermore, the types of product listed are found only outside of the cell and at concentrations that are readily detectable

Not all chemical transformations that are effected by microorganisms in soil and water are completely degradative A number of compounds are modified to yield methyl, simple acyl, nitro, and nitroso derivatives and other intermediate products

TABLE 6.2 Information on the Biotransformation of Chemical Compounds, Including Reaction Type, Microorganisms Involved, Condition (Aerobic or Anaerobic), and Transformation Products

Compound	Microorganisms and Media	Products	References (see Sect 6 8)
		AEROBIC	
Dehalogenation			
R-CH_2Cl–RCH_2OH			
Chlordane	Soil *Nocardiopsis* sp	Pesticide/chlordane, chlorohydrin, etc	(24)
4 Chloroaniline	Yeast, *Rhodosporidium* sp	4-amino phenol	(45)
2 4-dichlorophenoxyacetic acid	*Pseudomonas* sp	2-chlorophenoxyacetic acid	(68)
4-chloro-2 methyl phenoxyacetic acid	*Pseudomonas* sp	Chlorine lost after ring cleavage	(75, 76)
2 3 6 trichlorobenzoate	*Brevibacterium* sp	3 5-d chlorocatechol	(103, 105)
2 4 5-trichlorophenoxyacetic acid	*Brevibacterium* sp	Dichlorocatechol	(102)
Lindane (1 2 3 4 5 6 hexachloro cyclohexane)	Soil, *Escherichia coli*	Di,tri tetra pentachlorobenzene (cyclohexanes)	(72, 130)
2 4-dichlorophenol	*Rhizoctonia praticola*	2-chloro-1 4-benzoquinone	(134)
Halogenated alkanes and fatty acids	*Pseudomonas* sp	Not identified	(144)
Kepone	*Pseudomonas aeruginosa* mixed culture	Monohydrokepone, dihydrokepone	(145)
Fluorobenzoic acids	*Pseudomonas* sp	The fluorine is lost after ring cleavage	(159)
DDT	Water *Dunaliella* sp *Azmenellum* sp	$RCCl_3$ – RCOOH	(149)
N serve	Soil	$RCCl_3$ – RCOOH	(152)
DDT	*Aerobacter aerogenes*	$RCCl_3$ – RCOOH	(184)
Deamination			
Benzylamine	*Pseudomonas putida*	Benzaldehyde	(62)
Decarboxylation			
4 5-dihydroxyisophthalate	Soil bacterium	Protocatechuate	(64)
4 hydroxyisophthalate	*Pseudomonas putida*	Protocatechuate	(63)
o-phthalate	*Pseudomonas* sp *Alcaligenes* sp	Protocatechuate	(65)
2 3 6-trichlorobenzoate	*Brevibacterium* sp	2,3,5-trichorophenol	(103, 105)
3 5-cyclohexadiene-1,2 diol-1 carboxylic acid	*Alcaligenes eutrophus*	Catechol	(153)
Dichlorofop-methyl (herb cide)	Soil	$RCH(CH_3)$ COOH – RCH_2CH_3	(167)
DDT	*Pseudomonas putida*	Ar_2 CH COOH – Ar_2CH_2	(169)
3-(3' 5'-dichlorophenyl)-5,5 dimethyloxazolidine 2,4 dione (DDOD fungicide)	Soil	Ar NR COOH – Ar N (R)H	(171)
Methyl oxidation			
Xylene	*Pseudomonas* sp	Toluic acid	(57)
Cresol	*Pseudomonas putida*	Hydroxybenzoates	(100)
p-xylene	*Nocordia corollina*	p-toluic acid	(113)
Linalool	*Pseudomas* sp	Oxygenation at C-10 methy	(135)
Alkanes alkenes	Fungi	Carboxylic acids, alcohols	(129)
Pristane	*Corynebacterium* sp	Pristanic acid	(141)
Alkanes	*Pullularia pullulons*	Carboxylic acids	(133)
Acetone	*Mycobacterium vaccae*	Pyruvic acid	(176)
Hydroxylation and Ketone Formation			
Toluene xylenes	*Pseudomonas* sp	Benzoate, toluates	(190)
Alkanes	*Pseudomonas oleovorans*	Aliphatic alcohols, aldehydes, and acids	(1, 2)
Benzoic acid	*Trichosporon cutaneum* *Rhodopseudomonas* sp	4-hydroxybenzoic acid	(6, 53)
Chlorophenols	*Arthrobacter* sp	Catechol	(20)

TABLE 6.2 (Contd)

Compound	Microorganisms and Media	Products	References (see Sect 6 8)
Hydroxylation and Ketone Formation (contd)			
Naphthaline, 1 and 2-naphthalene sulfonic acid	*Pseudomonas* sp *Cunninghamella elegans* *cyanobacterium microalgae*	1, and 2-naphthol, naphthoquinone	(25, 34, 35, 36, 37, 58, 83, 114)
Biphenyl	*Pseudomonas putida*	2,3 dihydroxybiphenyl	(26)
Naphthalene	Several fungal sp	1,2 naphthalene oxide	(36)
Naphthalene	*Oscillatoria* sp (Cyano bacterium)	*Cis*-1,2-dihydroxy-1,2-dihydronaphthalene	(33)
Alkanes, cyclic alkanes, aromatics	*Methylococcus capsulatus*	Alcohols	(42)
3- and 4 hydroxphenyl acetic acid	*Escherichia coli*	3,4-dihydroxyphenol acetic acid	(43)
Naphthalene	*Pseudomonas putida*	1,2-dihydroxy-1,2-dihydronaphthalene	(47)
3-hydroxybenzoate	*Bacillus* sp	2,5-dihydroxybenzoate	(48)
2-hydroxybenzoate	*Pseudomonas testosteroni*	2,3-dihydroxybenzoate	(55)
Yrezon (5-amino-4-chloro-2-phenyl-3(2H)pyridazinone)	Bacteria	5 amino-4-chloro-2-(2,3-*cis*-dihydroxycyclo hexa-4,6-diene-l-yl)-3(2H)-pyridazinone	(59)
5-hydroxyisophthalic acid	Soil bacterium	4,5-dihydroxyisophthalate	(64)
4-chlorophenoxyacetate	*Pseudomonas* sp	4-chloro-2-hydroxyphenolacetate	(67)
2 4-dichlorophenoxyacetate	*Pseudomonas* sp	2,4 dichloro-6-hydroxyphenoxy-acetic acid	(68)
o-phthalic acid	*Pseudomonas* sp *Alcaligenes* sp	Protocatechuate	(63)
Diphenylamine (DPA)	Sludge	4-hydroxydiphenylamine	(74)
4-chloro-2-methylphenoxyacetate acid	*Pseudomonas* sp	5-Chloro-3-methylcatachol, 5-chloro-o-cresol	(75, 76)
p-xylene	*Pseydomonas putida*	3,6-dimethylpyrocatechol	(86)
Carbofuron	Soil	3-hydroxycarbofurans, 3-ketocarbofurans	(90, 194)
p-cresol	*Pseudomonas putida*	p-hydroxybenzaldehyde	(99)
2 3 6 trichlorobenzoate	*Brevibacterium*	2,4,3-trichloro-4-hydroxybenzoate	(105)
Chlorobenzoate	Mixed culture (sludge)	Chlorocatechol	(106)
2-chlorobenzoate	*Arthrobacter* sp	4-chlorocatechol	(107)
Fluorobenzoates	Mixed culture	Fluorocatechol	(108)
Benzoate anthranilate	*Pseudomonas aeruginosa*, *Micrococcus ureael*, *Pseudomonas fluorescens*	Catechols	(110)
p-xylene	*Nocardia corallina*	3,6-dimethylpyrocatechol	(113)
3-chlorobenzoic acid	*Pseudomonas* sp	3-hydroxybenzoic, 2 5 dihydroxy-benzoic acids	(115)
Chlorophenols	*Pseudomonas* sp	Chlorocatechols	(123)
Benzoic acid	*Alcaligenes eutrophus*	3,5-cyclohexadiene-1,2-diol-1-carboxylic acid	(155, 156)
Hexazinone	Soil	3,(4-hydroxycyclohexyl)-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione	(154)
1,3-diphenylbutane	*Bacillus* sp, *Pseudomonas* sp	2-phenyl-4-hydroxyphenylbutane	(164)
Cyclohexane carboxylic acid	*Alcaligenes* sp	p-hydroxybenzoate	(175)
Naphthalene	Soil bacterium (gram neg)	1 2-dihydro-1,2-dihydroxynaphthalene	(183, 186)
7 12-dimethylbenz(o)anthracene	*Pseudomonas aeruginosa*	methyl-hydroxylated metabolites	(191)
Benzoic acid	*Pseudomonas arvilla*	1,2-dihydro-1,2-dihydroxybenzoic acid	(193)
1-naphthal	Water	4-hydroxy-1-tetralone	(22)
Denmert fungicide	Soil	$R(R')(R'')\ CCH_3 - R(R')(R'')CCH_2OH$	(184)
B-oxidation			
Cannabinoids	*Nocardia salmonicolor*	Reduction of aliphatic side chains	(2)
Phenylalkanoic acid	*Micrococcus cerificans*	Phenylacrylic or phenylproprionic acid	(61)
Dicarboxylic acid	*Pseudomonas fluorescens*	Acetate and CO_2	(98)
Pristane	*Corynebacterium* sp	α-methylglutaric acid	(141)
Cyclohexane butyric acid	*Arthrobacter* sp	Cyclohexaneacetic acid	(146)
Eugenol	*Corynebacterium* sp	Protocatechuic acid	(172)
ω-(2,4-dichlorophenoxy)-alkanoic acid	Soil	$Ar\ O(CH_2)_n\ CH_2\ CH_2\text{-}COOH - Ar\ O(CH_2)_n\ COOH$	(91)

TABLE 6.2 (Contd)

Compound	Microorganisms and Media	Products	References (see Sect 6 8)
Epoxide formation			
RCH + CHR → R-CH-CH-R'			
1 alkene	*Pseudomonas aleovorans*	1,2 epoxyalkanes	(1, 2, 132)
9 10 dihydoxy-9 10-dihydro-benzo(a)pyrene	*Cunninghamella elegans*	Benzo(a)pyrene, 9,10-diol 7,8-epoxide	(27, 28, 29, 30
Aromatic hydrocarbons	Fungi bacteria, yeast	Aromatic oxides	(31, 32)
Linalool	*Pseudomonas*	7,8-dihydro-7,8-epoxy linalool	(135)
1-octene 1 7 octadiene	*Pseudomonas oleovorans*	1,2 epoxioctane, 7,8-epoxy-1-octane	(160, 174)
Oxidation			
Benzene	*Pseudomonas* sp	e-benzene-dihydrodiol	(8, 83, 84, 85)
Cyclohexane-1 2 diol	*Acinetobacter* sp	2-hydroxycyclohexane-1-one	(56)
Alkylbenzenes	*Micrococcus cirificans*	Phenylalkynoic acid	(61)
1 1-diphenylethane	Mixed culture	Atrapic acid	(71)
Methylation			
Methyl arsenate	Soil sewage	$(CH_3)Hg^+$ or $(CH_3)_2$ Hg CH_3 As(O)OH_2 → $(CH_3)_2$ AsOH or $(CH_3)_3$ As	(46)
Pentachlorophenol	*Trichoderma virgatum*	ArOH → Ar OCH_3, pentachloroanisole	(49)
2 3 4 6-tetra and penta chlorophenol	*Aspergillus sydow Scopulari-opis brevicaulis Penecillum* sp , *Enterobacter aerogenes* & soil bacteria	ArOH → Ar OCH_3	(50)
Mercuric ions	*Enterobacter aerogenes* soil	H_2^{2+} → CH_3 Hg^+ → $(CH_3)_2$ Hg	(93)
Ether Formation			
Diphenylmethane	*Pseudomonas putida*	RCH_2R → (R(R) C-O-C R	(170)
Acetylation			
2 6-dichloro-4-nitroaniline	Soil *Pseudomonas cepacia*	4-nitro-2,6-dichloroacetanilide	(4, 21, 3)
4-chlorobenzene	Yeast Rhodosporidium	4-chloro-2-hydroxyacetanilide	(45)
2 4-dichloroaniline	Soil	3,4-dichloroacetanilide	(181)
4-chloroaniline	*Fusarium oxysporum*	4-chloroacetanilide	(119)
N-nitrosation			
Trimethylamine	*Escherichia coli* *Streptococcus*	Dimethylnitrosamine	(9, 10)
Dimerization/polymerization			
Dichloroanaline	Soil	3,3',4,4'-tetrachloroazobenzene	(14, 15)
2 chlorophenol	*Rhizoctonia praticola*	Oligomeric products	(165)
2,4-dichlorophenol	*Rhizoctonia praticola*	dimeric quinones	(134)
2 and 4 chlorophenol	*Rhizoctonia praticola*	Polymers	(165, 166)
2,4-dichlorophenol	*Rhizoctonia praticola*	Polymers	(134)
3 4-dichloroaniline	Soil	3,3',4,4'-tetrachloroazobenzene	(181, 120)
2 4 6 trinitrotoluene	*Pseudomonas sp*	2,2',6,6'-tetranitro-4-azoxytoluene	(188)
2,6-dichloro-4-nitroaniline	Soil	Unidentified dimer	(4)
4-chloroaniline	*Fusarium oxysporum*	2Ar NH_2 → Ar-N=N-Ar	(119)
Dinmert	Soil	R-S-R' + RSH → R-S-S-R	(143)
Amino Oxidation			
4-chloroaniline	*Fusarium oxysporum*	R-NH_2 → R-NO_2 4-chloronitrobenzene	(119)
Diflubenziuon	H_2O		(112)
Hexazinone	Soil	3-(4-hydroxy cyclohexy)-6-(methylamino)-1-methyl-1,3,5-triazene-2,4(1H,3H)dione	(154)
Benzylisothiocyanate	*Enterobacter cloacae*	Benzylamine	(173)
Nitrilotriacetic acid	*Pseudomonas* sp	Iminodiacetate ek	(180)
Yrpazon	Water	5-amino-4-chloro-3(2H)-pyridazinone	(195)
Trimethylamine	*Mortierella parrispora* soil	Dimethylamine	(9, 10)
Thiron	*Rhizobium*	(CH_3)-N-C-SH → $(CH_3)_2$NH	(142)
Aldachlor	*Chaetomium globosum*	CH_3 R-(R') N R' → RNH_2	(179)

TABLE 6.2 (Contd)

Compound	Microorganisms and Media	Products	References (see Sect 6 8)
Amino Oxidation (contd)			
Biphenyl	*Beijerinckia* sp	2,3-dihydroxybiphenyl	(78)
Benzo(a)pyrene	*Beijerinckia* sp	Dihydrodiols	(79)
Benzo(a)anthracene	*Beijerinckia* sp	Dihydrodiols	(79)
Ethylbenzene	*Pseudomonas putida*	2,3-dihydroxy-l-ethylbenzene	(80)
Halogenated benzenes	*Pseudomonas putida*	3-halogenated catechols	(81)
Toluene	*Psuedomonas putida*	2,3-dihydroxy-1-methylcyclohexa-4,6-diene	(82, 124)
Resorcinol	*Azotobacter vinelandii*	Pyrogallol	(89)
Resorcinol	*Pseudomonas putida*	Hydroxyquinol	(39)
Cresols	*Pseudomonas putida*	Methylcatechols	(100)
Dibenzo-p-dioxin	*Pseudomonas* sp , *Beijerinckia* sp	1,2-dihydroxydibenzo-p-dioxin	(121, 122)
Benzoate, methylbenzote	*Pseudomonas arvilla*	Catechol, methyl catechol	(138)
Orcinol (3 5-dihydroxytoluene)	*Pseudomonas putida*	2,3,5 trihydroxytoluene	(38)
Sulfur Oxidation			
Aldicarb	Soil	Aldecarb sulfoxide, aldecarb sulfone	(7, 38)
Temik	Soil	Temik sulfoxide, Temik sulfone	(44)
Parathion	Mixed culture, water		(137, 196)
Denmert	Soil		(143)
Reduction of double bond			
Aldrin	Water, algal culture	-CCl = CCl - — -CHCl — CHCl—	(149)
DDT	*Aerobacter aerogenes*	Ar_2 - C=CHCl — Ar_2 -CH-CH_2Cl	
Hydration of Double Bond			
1-diphenylethylene	Mixed culture	2,2 diphenylethanol	(71)
Reduction of Nitro-groups			
4-chloronitrobenzene	*Rhodosporidium* sp	4-chloroaniline	(45)
N-nitropendimethalin	*Streptomyces* sp	Mixture of amino substituted products	(128)
2 4-dinitrotoluene	*Mucrosporium* sp	2-amino-4-nitrotoluene, 4-amino-2-nitrotoluene	(139)
2 4 6-trinitrotoluene	Groundwater, *Pseudomonas* sp	Amino-dinitrotoluene	(150, 188)
2,6-dichloro-4-nitroaniline	*Escherichia coli, Pseudomonas cepacia*	2,6-dichloro-4-aminoaniline	(3)
Fenitrothion	Water	Amino fenitrothion	
Parathion	*Mixed culture*	Amino parathion	
Glycerol trinitrate	Sludge	Glycerol dinitrate and mononitrate	(185)
Oxime Formation			
Temik (insecticides)	Soil	RCHNOR' — RCH = N — OH	(44)
Nitrile/amide metabolism			
Acetonitrile	*Nocardia rhodochrous*	Acetic acid, NH_3	(60)
Propionitrile	*Nocardia rhodochrous*	Propionic acid, NH_3	(60)
Bromoxynil	*Flexibacterium* sp	2,4-dibromo-3-hydroxy-benzoate	(168)
Benzonitrile	*Nocardia* sp	Benzoate, NH_3	(94)
Ester hydrolysis			
Phthalate esters	*Nocardia* sp , *Arthobacter* sp , *Pseudomonas* sp	Phthalic acid	(66)
Phthalate esters	*Nocardia ereuthropolis*	Phthalic acid	(126)
Malathion	Water	Malathion β-mono acid	(147, 148)
Butoxyethyl ester of 2,4-dichlorophenoxyacetic acid	Water	2,4-dichlorophenoxyacetic acid	(147, 148)
Phthalate esters	Water	Phthalic acid	(158)
Dichlorofap-methyl	Soil	R-CH_2-CH_2-$COOCH_3$ — R-CH_2-CH_2-CO_2H	(167)

TABLE 6.2 (Contd)

Compound	Microorganisms and Media	Products	References (see Sect 6 8)
Ether Cleavage			
Diethyl ether	*Methylococcus capsulatus*	Ethanol	(142)
4-chlorophenoxyacetic acid	*Pseydomonas* sp	4-chlorocatechol	(67)
2-4-dichlorophenoxyacetic acid	*Pseudomonas* sp *Arthrobacter* sp	2,4-dichlorophenol	(68, 178, 177)
2 4-dichlorophenoxyacetic acid	*Alcaligenes paracloxus*	2,4-dichlorophenol	(70)
2-methyl-4 chlorophenoxyacetic acid	*Pseudomonas* sp	5-chloro-o-cresol, glyoxylate	(73)
Polyehtylene glycols	*Psuedomonas aeruginosa*	mono or di-ethylene glycols	(92)
2 4,5-trichlorophenoxyacetic acid	*Brevibacterium* sp	2 4-dichlorophenol	(102)
Polyehtylene glycols	*Pseudomonas alcaligenes*	mono- or di-ethylene glycols	(109)
2 4-dichlorophenoxyacetic acid	*Arthrobacteria* sp	2,4-dichlorophenol	(127)
Fenitrothion	Water	R-O-CH_3-R-OH	(136)
C-N-Cleavage			
Ethylenediaminetetraacetic acid	Soil	Ethylene diamine	(18)
Pyrazon	Bacteria	5-amino-4-chloro-3(2H)-pyridazinone	(59)
Amide hydrolysis			
Propanil	Soil	3 4-dichloroaniline	(41, 14, 15 13)
Carbomate cleavage			
Chloropham	Water	3-chloroaniline	(148, 187)
Benomyl	Soil soil fungi	2-amino-benzimidazole	(16)
Phenylcarbomate pesticides	Soil	RNHC(O)R' → RNH_2	(118)
Cleavage of R = NOC(O) R			
Aldicarb	Soil	RCH = NOC(O) R → RCH = NOH	(7, 116)
C-S Cleavage			
Denmart	Soil	R—C—S-R' →R—C—S-R'(R")	(143)
C-Hg			
Phenylmercuric acetate	Soil	R Hg R' → RH and/or Hg	(93)
C-O-P phosphate ester cleavage			
Tris (2,3-dibromopropyl) phosphate (TRIS)	Activated sewage sludge	bis (2,3-d bromopropyl) phosphate	(5)
Tri-p-cresylphosphate (TCP)	Sludge	p-hydroxybenzoic acid	(125)
Parathion	*Bacillus* sp , *Pseudomonas* sp	p-aminophenol	(162, 163)
Malathion	*Trichoderma viride Pseudomonas* sp	$(AlkO)_2$ P(S)R → AlkO(OH) P(S) R and/or $(HO)_2$ P(S)R	(131)
Parathion	Mixed culture, soil	ArOP(S) RR' → ArOH and/or HO P(S)RR'	(137, 161)
Diazinon	Soil	ArOP(S)RR → Ar OH and/or HOP(S)RR'	(162)
C-O-S Sulfate ester cleavage			
Sesone	Soil, *Bacillus* sp	RCH_2 $OS(O_2)$ OH → RCH_2 OH and/or HO $S(O_2)OH$	(182)
S-S Cleavage			
Thiram	*Rhizobium* sp	RSSR→-2RSH	(142)
Ring fission			
Catechols	*Pseudomonas* sp	Mono-and di-carboxylic acids	(17, 51, 52, 53, 54)
Chlorocatechols	*Arthrobacter* sp	Dicarboxyl c acids	(19)
Methylcatechol	*Arthrobacter* sp	Dicarboxylic acids	(101)
Chlorocatechol	*Arthrobacter* sp	2,4 dichloromuconic acid	(101)
3,5-dichlorocatechol	*Arthrobacter* sp	2,4, dichloromuconic acid	(178)
2,3 5-trihydroxytoluene	*Pseudomonas putida*	2,4,6-trioxoheptanoate	(38)

TABLE 6.2 (Contd)

Compound	Microorganisms and Media	Products	References (see Sect 6 8)
		ANAEROBIC	
Dehalogenation			
DDT	*Hydrogemonas* sp *Arthrobacter* sp	DDD, DDMS, DBP	(151)
Reduction of double bond			
Phenol	Soil	n-caproic acid	(11)
Benzoate	Methanogenic bacteria	Methane-3	(69)
Ferulic acid	Methanogenic bacteria	Phenylpropionicacid-4	(97)
DDT	*Hydrogemonas* sp *Arthobacter* sp		(151)
Aldrin	Water, algal culture	—CCl = CCl — → -CHCl—CHCl—	(149)
Reduction of nitro-group			
2,6-dichloro-4-nitroaniline	Flooded soil	2,6-dichloro-p-phenylinediamine	(4)
Parathion	Flooded soil	Amino parathion	(88, 117, 40)
Hexahydro-1,3,5-trinitro 1,3,5 triazine	Activated Sludge	Hydrazine, methanol	(140)
Ring fission			
Phenol	Soil	n-caproic acid	(11)
Catechol, phenol	Methanogenic culture	Methane	(96)
N-acylation			
Chloronated anilines	*Paracoccus* sp	Chlorinated acetoanilides	(23)
2 4-dinitrotoluene	*Mucosporium* sp	4-acetamide-2-nitrotoluene	(139)
C-N Cleavage			
Choline	*Vibro cholinicus*	Trimethylamine, EtOH + acetic acid	(95)
Trifluralin	Soil	RN $(Alk)_2$ → R'NH Alk and/or RNH_2	(87)
Glyphosate	Soil, water	$RNH_2 CH_2 R'$ → RNH_2	(157)
Reduction of nitro-groups (continued)			
Parathion	Flooded soil	Amino parathion	(162)
2,4,6-trinitrophenol	*Pseudomonas acruginosa*	2-amino-4,6-dinitrophenol	(192)
Parathion	Flooded soil, *Flavobacterium* sp	Amino parathion	(40)
Dimerization/polymerization			
Trifluralin	Soil	2 $ArNH_2$ → ArN = N-AR	(87)
Dehalogenation (continued)			
Trifluvalin	Soil	R-CF_3 → RCOOH	(87)
Pentachlorophenol	Soil	$ArCl_5$ → $ArCl_4OH$	(111)
DDT	Soil	$RCCl_3$ → RCOOH	(152)

6.3. RECALCITRANT COMPOUNDS

Many synthetic organic molecules are transformed very slowly or not at all These molecules persist for years and become potential environmental hazards Organic materials that persist in natural ecosystems because microorganisms lack the ability to degrade or transform them are known as recalcitrant (persistent) compounds (Alexander 1973, 1981, Horvath 1972)

Recalcitrant compounds in soil and water include plastics, other synthetic polymers, chlorinated aromatic compounds of various types, pesticides, and other industrial chemicals A recalcitrant compound is not necessarily hazardous, but some are toxic and others are aesthetically undesirable Some recalcitrant compounds are not toxic at concentrations found in soil and water, but are subject to bioaccumulation because of their persistence

Many chemical substitutes, when attached to the parent organic compound, may cause it to be recalcitrant Among these substituents are amine, methoxy, sulfonate, and nitro groups, halogens, substitutions in the meta position of benzene rings, ether linkages, and branched carbon chains (Campbell 1977) In addition, larger molecules are generally less degradable than smaller ones Tables 6 3, 6 4, and 6 5 list several synthetic materials that resist biodegradation Some environmental concerns regarding recalcitrant compounds are that they may

- persist for years
- accumulate over time
- prove aesthetically objectionable
- be transported for some distances
- prove to be hazardous
- be bioaccumulated or biomagnified
- be difficult to remove physically, chemically, or biologically

The persistence of, and lack of rapid microbial attack on, a chemical may be due to chemical or physical causes Some molecules may completely resist attack by microorganisms (e g , synthetic polymers such as polyethylene and polyvinyl chloride) and persist for years or decades Some resistant compounds may be acted upon enzymatically, but the reaction is co-metabolic and, hence, slow Some chemicals are easily transformed in one habitat and long-lived in another, depending on environmental factors such as the presence or absence of oxygen Some easily-transformed compounds may be rendered resistant when complexed with resistant organic materials in soils

TABLE 6.3 Relative Persistence of Selected Pesticides in Soil Under Natural Conditions[a]

Pesticide	Persistence	
	Edwards (1973)	Alexander (1973)
Chlorinated hydrocarbon insecticides		
Chlordane	5 yr	>15 yr
DDT	4 yr	>15 yr
BHC	3 yr	—
Dieldrin	3 yr	—
Heptachlor	2 yr	>14 yr
Aldrin	2 yr	>15 yr
Endrin	—	>14 yr
Lindane	—	>15 yr
Toxaphene	—	>14 yr

TABLE 6.3 (Contd)

Pesticide	Persistence	
	Edwards (1973)	Alexander (1973)
Urea, triazines, and picloram herbicides		
Propazine	18 mo	2-3 yr
Picloram	18 mo	>5 yr
Simazine	12 mo	—
Atrazine	10 mo	—
Fenuron	8 mo	—
Diuron	8 mo	>15 mo
Linuron	4 mo	—
Prometryne	3 mo	—
Monuron	—	3 yr
Phenoxy, toluidine, and nitrile herbicides		
Trifluralin	6 mo	>40 wk
2,4,5-T	5 mo	>190 days
Dichlobenil	4 mo	—
MCPA	3 mo	—
2,4-D	1 mo	—
Phosphate insecticides		
Diazinon	12 wk	—
Disulfoton	4 wk	—
Phorate	2 wk	—
Malathion	1 wk	—
Parathion	1 wk	>16 yr
Benzoic acid and amide herbicides		
2,3,6-TBA	12 mo	2 yr
Bensulfide	10 mo	—
Diphenamide	8 mo	—
Amiben	3 mo	—
CDAA	2 mo	—
Dicamba	2 mo	4 yr
Carbamate and aliphatic acid herbicides		
TCA	12 wk	—
Dalapon	8 wk	—
CIPC	8 wk	—
CDEC	6 wk	—
IPC	4 wk	—
EPTC	4 wk	—
Barban	2 wk	—
Other pesticides		
2- (2,4-DP)	—	30 days
Fenac	—	>18 mo
Fluometuron	—	195 days
Pentachlorophenol	—	>5 yr

(a) Adapted from Edwards (1975) and Alexander (1973), differences in listed persistence times for identical compounds may reflect differences in test or environmental conditions, such as pH

TABLE 6.4 Some Organic Compounds that Persist in Soils and Water for Relatively Long Periods of Time[a]

Acetylethanolamine	2,2-Dimethyl-1,3-propanediol
Acetylmorpholine	2,2-Dimethylsuccinic acid
Aminobenzenesulfonic acids	Dinitrobenzenes
tert-Amyl alcohol	Dioxane
Anthraquinonesulfonic acid	bis-2-Ethoxyethyl ether
tert-Butyl alcohol	Ethylenediaminetetraacetic acid
Chloroanilines	Methoxyanilines
Chloronitrobenzenes	Morpholine
Chlorophenols *meta*-substituted	Nitroanilines
Diaminobenzenes	Nitroanisoles
2,6-Dibromohexanoic acid	Nitrobenzenesulfonic acids
Dichloroacetic acid	Nitrotoluenes
2,3-Dichloropropionic acid	Pentaerythritol
Diethylaniline	*tert*-Pentanol
Diethylene glycol	Phenoxyalkanecarboxylic acids, α-substituted
Diethyl ether	2-Phenylbutyric acid
3,3-Diethylglutaric acid	Poly(ethylene glycol) 400
3,3-Dimethyl-1-butanol	Tetraethylene glycol
2,2-Dimethylglutaric acid	Toluenesulfonic acid
1,1-Dimethylhexanol	Triethanolamine
Dimethylmalonic acid	Triethylene glycol
2,2-Dimethyloctanoic acid	Trimethylacetic acid
1,1-Dimethyl-1-pentanol	
2,2-Dimethyl-1-pentanol	

(a) Adapted from Alexander (1973)

TABLE 6.5 Some Synthetic Polymers Resistant to Transformation by Microorganisms[a]

Acetate rayon (Estron)	Poly (methyl methacrylate)
Acrylonitrile-vinyl chloride copolymer (Dynel)	Polymonochlorotrifluoro-ethylene
Carboxymethylcellulose (high degree of substitution)	Polystyrene
Cellulose acetate (fully acetylated)	Polytetrafluorethylene (Teflon)
Cellulose acetate-butyrate	Polyurethane (polyether linked)
Cellulose acetate-propionate	Poly (vinyl alcohol)
Ethylcellulose	Poly (vinyl butyral)
Ethylene glycol terephthalate (Dacron)	Poly (vinyl chloride)
Nylon	Poly (vinyl chloride)-acetate
Phenol-formaldehyde	Poly (vinylidene chloride)
Polyacrylonitrile (Orlon)	Resorcinol-formaldehyde
Polydichlorostyrene	Silicone resins
Polyethylene (high-molecular-weight)	Vinylidene chloride-vinyl chloride copolymer (Saran)
Polyisobutylene (high-molecular-weight)	Zein formaldehyde (Vicara)

(a) Adapted from Alexander (1973)

6.4 BIOACCUMULATION

Hazardous materials in the environment are often bioaccumulated by living organisms Two terms, bioconcentration and biomagnification, are used to describe this phenomena The words are often used interchangeably, but have different meanings

- Bioconcentration—the uptake and retention of a chemical from the environment by an organism or tissue to the extent that the organism eventually acquires levels of the chemical in its body that exceed levels in the environment (e g , transfer from environment to organism)
- Biomagnification—the uptake and retention of a chemical by an organism or tissue from ingestion of other organisms containing the chemical so that concentrations are magnified (e g , transfer from organism to organism)

Bioaccumulation is a characteristic shared by many different types of organisms (Table 6 6) Hazardous compounds that accumulate in the tissues of larger animals and plants are generally those that are environmentally persistent—in many cases, the recalcitrant molecules However, hazardous materials other than those that are persistent may be bioaccumulated (e g , benzene, toluene, naphthalene) Because of bioaccumulation, many of these compounds can have deleterious effects on higher organisms, including domestic animals and humans

Bioaccumulation by phototrophic organisms (algae, cyanobacteria, photosynthetic bacteria, and higher plants) has limited application to hazardous-waste treatment and removal processes Phototrophs, which use sunlight for energy, can sustain relatively large populations without organic matter at levels high enough to serve as carbon and electron donors (Kobayashi and Rittmann 1982) Under laboratory conditions, some algae can store hydrophobic compounds in large lipid stores (Table 6 6) Once compounds accumulate in the tissues, the algae can be harvested for destructive treatment or safe disposal elsewhere Higher plants also may bioaccumulate hazardous materials from soils

TABLE 6.6 Examples of Bioaccumulation of Synthetic Compounds by Microorganisms[a]

Microorganism	Compound
Fungi	
Aphanomyces euteiches, Fusarium solani, Pythium ultimum, Rhozoctonia solani	DDT, dieldrin, PCNB (pentachloronitrobenzene)
Aspergillus sp	Toxaphene, methoxychlor, DDT
Actinomycetes	
Streptomyces albus, S aurefaciens, S griseus, S viridochromogenes	Dieldrin

TABLE 6.6 (Contd)

Microorganism	Compound
Bacteria	
Bacillus subtilus, Serratia marcesans, Agrobacterium tumefaciens	DDT, dieldrin, methoxychlor
Aerobacter aerogenes	DDT
Flavobacterium harrisoni	Toxaphene, methoxychlor
Cyanobacteria (blue-green algae)	
Microcystis aeruginosa	Benzene, toluene, chlorobenzene, 1,2-dichlorobenzene, nitrobenzene, naphthalene, 2,6-dinitrotoluene, phenanthrene, di-N-butylphthalate, pyrene
Anacystis nidulans	Malathion, carbaryl, parathion, DDT, aldrin, dieldrin
Anabaena cylindrica	Aldrin, dieldrin
Nostoc muscorum	Dieldrin
Algae	
Chlorella	Toxaphene, methoxychlor
Chloroccuum sp ,[b] *Dunaliella teriolecta*,[b] *Chlamydamonas* sp ,[c] *Thalassiosira pseudomana*,[d] *Porphyridium cruentum*[e]	Mirex
Nitzschia[d]	Mirex, methoxychlor, 2,4-DBE
Monoraphidium[d]	Methoxychlor
Euglena gracilus,[c] *Scenedesmus obliquis*[b]	Parathion, DDT
Cylindrothea closterium[d]	DDT
Selanastrum capricornatum[b]	Benzene, toluene, chlorobenzene, 1,2-dichlorobenzene, nitrobenzene, naphthalene, 2,6-dinitrotoluene, phenathrene, di-n-butylphthalate, pyrene

(a) Adapted from Kobayashi and Rittmann (1982)
(b) Green alga
(c) Flagellate alga
(d) Diatom
(e) Rhodophyte (red algae)

6.5 ACTIVATION

Enzymatic conversion of certain innocuous chemicals to products that are hazardous to other organisms (animals, plants, and humans) is one possible consequence of biotreatment Generation of toxicants from harmless chemical precursors is called activation Activation is usually a mineralization sequence but may also occur during co-metabolism, although the toxic intermediates usually do not persist over long periods (Alexander 1981). Activation is a relatively uncommon phenomenon, but it must be considered in biotreatment of complex wastes or mixtures of complex wastes

Four possibilities may occur as a result of microbial activation

- a relatively non-toxic compound becomes toxic
- relatively short-lived compound becomes persistent
- a compound becomes both more toxic and more persistent
- a toxicant for one type of organism becomes toxic to another type of organism

Microbial activation is evident in the methylation of inorganic mercury in aquatic sediments to yield monodimethylmercury compounds which are more hazardous and more readily bioaccumulated by aquatic animals Inorganic arsenic may also be methylated, and the products of the microbial transformation are the far more toxic methyl arsines (Cox and Alexander 1973, Cheng and Focht 1979)

In some cases, innocuous inorganic compounds may be converted to potential carcinogens The reactions are apparently partly enzymatic and partly nonenzymatic, and are related to microorganism activities An example is the N-nitrosation of secondary amines, which produces the relatively persistent and toxic nitrosamines The organic precursor is nitrite, which is continually generated in soil and water from ammonium (or nitrate) during nitrification Although the N-nitroso derivatives may endure in the environment for some time, the dialkylamine precursors are readily transformed by microorganisms (Ayanaba, Verstraete and Alexander 1973, Mills and Alexander 1976, Tate and Alexander 1976)

A compound toxic to one type of organism may also be changed into products that affect other organisms An example is the dehalogenation and oxidation of pentachlorobenzyl alcohol, a compound used in Japan to control a fungus that harms rice When plant residues containing the fungicide enter the soil, tri- and tetrachlorinated benzoic acids are formed, which suppress the growth of subsequent rice crops (Ishida 1972)

The phenomena of increased toxicity can occur with chemical detoxification For example, the pesticide dimethoate can be entirely degraded by alkaline hydrolysis, but the degradation product, mercaptoacetic acid, is almost as toxic as the dimethoate (SCS Engineers 1979a)

6.6 METALS—SPECIAL CASES

Microorganisms have a minor role in converting metals from one form to another in the environment Along with larger organisms, they sometimes participate in uptake of metals Transfer of metals through soil and water depends primarily on their interaction with other metals and their chemical form (Epstein and Chaney 1978, Chaney 1981, 1982, Chaney et al 1982) The form of metals can be altered in soils, and metals can be removed chemically from municipal sludges (Jenkins et al 1981), or removed physically and chemically from liquid waste streams (NATO 1981)

Copper, nickel, cadmium, lead, zinc, and, to a lesser extent, manganese and cobalt behave similarly in soils In acid soils, these heavy metals can exist as divalent cations, for example Cu^{2+} and Zn^{2+} In alkaline or neutral soils, they may combine with a hydroxyl ion, for example $Zn(OH)^{+}$ The hydrous oxide of manganese and iron can control the availability of heavy metals by sorption and desorption Organic materials bind metals more strongly at a soil pH below 7 5 Arsenic reacts in the soil with iron, aluminum, calcium, and magnesium ions, and its concentration in solution decreases with time Chromium generally is oxidized or reduced to Cr^{3+} and precipitates as an insoluble hydroxide

The acidity of soil and water affects the solubility of heavy metals and trace elements Solubility generally increases as pH decreases (e g , acidity increases) For each unit rise in soil pH, there is about a 100-fold decrease in the activity of zinc and copper Iron, manganese, and aluminum may be dissolved and become mobile in soil at low (acidic) pH As the solubility and mobility of metals increases, the potential for their uptake and bioaccumulation by plants also increases

Organic soils have a higher cation-retention capacity than do mineral soils Thus, organic material tends to bind, or chelate, metal ions Chelation may be more important than the simple cation exchange role of the organic matter, and may involve greater metal selectivity Compared to mineral soils, metal availablity is reduced in organic soils at lower pH

The cation exchange capacity (CEC) is important in binding all cations, including the toxic metal cations A soil with a high CEC is safer for disposal of wastes contaminated by metals than is soil with a low CEC

Toxic metals revert with time to chemical forms less available to plants Reversion is usually rapid for zinc and, for metals in general, more rapid in calcareous soils Soil pH, phosphate, organic material, and the amount of toxic metal added can affect the rate and extent of reversion Cobalt reversion is related to the amount of manganese oxides in soil, but evidence on reversion of other toxic metals is meager Reversion can be stopped with some metals by prolonged soil submergence and especially by a decreased pH

Metals at hazardous-waste disposal sites are usually difficult to transform through conventional biological treatments The reasons are fourfold

- Metals are generally not degraded (biologically or chemically) and tend to persist for long periods of time in the environment
- Some metals are toxic to plants and animals, or cause adverse effects, at low concentrations
- Some metals tend to be biologically concentrated by living organisms
- Some metals can be converted to a more toxic form in the environment by bioprocesses

Both toxicity and biological concentration are highly species-dependent for individual metals, and vary from relatively toxic to relatively innocuous for different metals Similarly, different metals behave differently in the environment Thus, identification of the metals present is important to planning biotreatment

One feature common to all metals is their environmental persistence Unlike organic pollutants, metals cannot undergo complete biological or chemical degradation in nature (Buhler 1973) Since metals are stable, they may be transported for considerable distances by air and water, they may also pass through, and accumulate in, food chains Consequently, metallic wastes tend to remain indefinitely in the environment and can become long-term hazards In terms of biotreatment at hazardous-waste sites, high levels of metals in the soil may inhibit the breakdown of organic compounds by microorganisms (Pajak et al 1977, Loveless and Painter 1968; Lamb et al 1964)

Inorganic material such as metals may be partially removed from soils and liquids and concentrated in biomass by adsorption during biological treatment Such inorganics, however, are not transformed or destroyed by biological processes At higher concentrations, they may also inhibit microbial activity and will remain a disposal problem

When land treatment is used to biotransform hazardous materials, the availability of heavy metals, their uptake, and their accumulation will depend upon a number of factors (Epstein and Chaney 1978), including

- Soil pH - toxic metals are generally more available to plants below pH 6 5
- Organic matter - organic matter can chelate and complex heavy metals so that they are less available to plants
- Soil phosphorus - phosphorus interacts with certain metal cations to decrease their biological availability
- Cation exchange capacity - CEC is important for the binding of metal cations Soils with high CEC are safer than soils with low CEC for land disposal of sludge or other treated wastes containing metal residues
- Moisture, aeration, temperature - these factors affect activity and growth of plants and, hence, uptake of metals
- Metal identity - Zn, Cu, Ni and other metals differ in their toxicity to plants and their reactivity in soils

- Plant species - plant species and varieties differ widely in tolerance to uptake of heavy metals Generally, vegetable crops are more sensitive than grasses
- Plant parts - grains and fruits generally accumulate smaller amounts of heavy metals than leafy tissues
- Plant age - the older leaves of plants will contain larger amounts of metals than young leaves, and maximum metallic content occurs at the end of the growing season

6.7 ENVIRONMENTAL INFLUENCES

Most hazardous-waste materials are degraded or transformed by heterotrophic microorganisms The organisms may coexist with other organisms and partially depend upon them Heterotrophic organisms obtain some of their oxygen from algae in waste stabilization ponds, as the algae photosynthesize organic compounds and release oxygen Nitrifying bacteria, which are present in activated sludge systems, are autotrophic and able to chemosynthesize nitrate from ammonia

All microorganisms involved in degradation or transformation require carbon as a food source Some organisms can use a wide range of organic substances as their primary food source, while others are more specific All microorganisms require certain ratios of nutrients The essential nutrients are nitrogen, phosphorus, sulfur, potassium, calcium, and magnesium These are called macronutrients because of the quantity in which they are required Micronutrients are as essential as macronutrients, but they are needed only in trace amounts Micronutrients essential for growth include iron, boron, copper, manganese, zinc, chromium, and cobalt

Water is the medium of life for most microorganisms The organisms synthesize water-soluble organics Water is essential for plant life because it controls temperature, dissolves nutrients, maintains correct pH values, and controls osmotic pressure, hydrolysis reactions, oxidation reactions, and transport of ions and compounds

In field situations, interactions among environmental factors such as dissolved oxygen (aerobic or anaerobic metabolism), water, temperature, pH, presence of other chemical compounds, and salinity often control biotransformation processes (Alexander 1981, Kobayashi and Rittmann 1982) In addition, the physical or chemical characteristics of a chemical compound (solubility, volatility, hydrophobicity, and octanol-water partition coefficient) affect the ability of microorganisms to transform that compound Compounds that are insoluble in water are less readily available to microorganisms than are soluble compounds

Because of environmental conditions that differ, some chemicals are readily biodegradable in one environment and persistent in another For example, the absence of oxygen is associated with the resistance of carbohydrates to microbial destruction This resistance is shown by peats, which are extremely durable under water in anaerobic deposits In addition, certain classes of

chemicals are not readily transformed by microorganisms when attached to the surface of colloidal materials Aeration, pH, soil moisture, and soil temperature affect the solubility and, hence, the availability and uptake of heavy metals by plants (Epstein and Chaney 1978)

Most studies on biotransformation of chemical compounds have been conducted in the laboratory (in vitro) under controlled conditions, with pure microbe cultures and single substrates This approach is necessary to identify biochemical pathways However, laboratory studies reflect only the potential for degradation that would occur in the field situations The question remains, "Will degradation occur in the field as it does in the laboratory?"

Biotransformation or degradation studies have also been conducted with soil and sediment microcosms under representative environmental conditions (in situ) for the following reasons

- Substances persisting in a pure culture will often be changed under mixed-culture conditions (e g , waste treatment lagoons) due to co-metabolism Also, mixed cultures are more apt to contain the required type of microorganism or combinations of microorganisms
- Initial products from the activity of one microorganism may be broken down further by a series of different microorganisms acting in sequence (e g , products of co-metabolism undergo further enzymatic transformation and are mineralized)
- A compound in the field may be present at such low levels that the available nutrients are insufficient to initiate or sustain microbial activity (e g , there is too little energy flux)
- Physical conditions in the field may inhibit microorganisms from changing a compound (e g , anaerobic conditions prevail where aerobic conditions are necessary, pH is too high or too low, insufficient moisture)
- Antagonistic interactions between different species of microorganisms can restrict the activity of the microorganism that will transform a chemical compound (e g , bacteria are usually antagonistic to fungi)
- A compound that is readily changed or degraded experimentally may be rendered resistant if complexed with resistant organic materials in the field
- Substances may be present in the field that inhibit microbial activity on a specific compound or different compounds (e g , toxic levels of a metal)

If the factors controlling biotransformation of specific chemical compounds in field situations are adequately known, it may be possible to modify them to enhance microbial activity However, modification of environmental conditions is done most efficiently in bioreactor systems

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