Chapter 17

Microorganisms and Organic Pollutants

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17.1 INTRODUCTION

Global release of industrial and agricultural chemicals has resulted in widespread environmental pollution. The energy production industry alone generates large amounts of waste during the processing of coal and oil, and also nuclear energy production. As just one example, the United States Environmental Protection Agency (USEPA) estimates that more than 1 million underground storage tanks (USTs), predominantly employed for gasoline storage, have been in service in the United States. As of 2013, there have been over 510,000 confirmed accidental releases from these USTs (USEPA, 2013). This type of contamination is known as a point source. In contrast, the application of pesticides and fertilizers over vast land areas can result in what is called nonpoint source contamination. It has been reported that >90% of the monitored streams and >55% of shallow groundwater sites on agricultural and urban lands are contaminated with pesticides (Gilliom *et al.*, 2006).

Groundwater contamination is a critical issue from two perspectives. First, groundwater constitutes approximately 97% of all available fresh water on Earth. In the USA, groundwater sources account for around 37% of the water used for public potable water supplies, and around 98% of that used for private water supplies (e.g., rural residents) (Hutson *et al.*, 2004). Second, there is a hydrologic interchange between surface and subsurface water systems, such that there is a conduit for moving groundwater contamination into surface waters (see Information Box 17.1). As a result of accidental or intentional releases of hazardous waste in the United States, over 1.4 million acres of chemical plumes in groundwater require remediation (NRC, 2003). Furthermore, there is little doubt that these chemical plumes are contributing to the contamination of surface waters including streams and lakes.

The objective of this chapter is to examine microbial interactions with organic pollutants that can be harnessed

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Information Box 17.1 An Endangered River—Groundwater Connections

The Colorado River is currently impacted by two different groundwater contaminant plumes. The source of the first plume is a plant near Henderson, Nevada, where, in the 1950s the U.S. Navy commissioned a manufacturing plant to produce perchlorate, a rocket fuel component. Perchlorate is a health concern because it is a competitive inhibitor of iodide transport to the thyroid (Greer *et al.*, 2002). As a result of this operation, the groundwater under the plant now contains approximately 9.3 million kg of perchlorate dissolved in 34 billion liters of water (Hogue, 2003). This groundwater feeds into the Las Vegas Wash, a tributary of the Colorado River that flows into Lake Mead. The groundwater plume contributed between 226 and 453 kg/day of perchlorate into the wash in the 1990s, resulting in perchlorate levels as high as 24 parts per billion in Lake Mead (Hogue, 2003).

The second plume involves an abandoned uranium tailings site once operated by Atlas Corporation near Moab, Utah. The site is unlined and contains approximately 9.5 million metric tons of uranium wastes, including 1.6 billion liters of contaminated liquid. As a result, the uranium content in the groundwater near the Moab site exceeds the EPA groundwater standards at uranium tailings piles by 530-fold (ORNL, 1998). A study conducted by the Oak Ridge National Laboratory estimates that 36,520 liters/day or 25.4 liters/min of uranium-contaminated water enters the Colorado River at the Moab site (ORNL, 1998). Furthermore, the tailings at Moab contain high levels of ammonia, a marker of mill contamination, which is also leaching into the groundwater and eventually entering the Colorado River.

to help prevent contamination, and also to remediate contaminated sites. The United States has passed a series of environmental laws mandating the cleanup of such sites, but the cost of cleanup has been estimated to be in the trillions of dollars. Therefore, as a society we are examining the cleanup issue from several perspectives. The first is related to the cleanup target and the question "How clean is clean?" As you can imagine, the stricter the cleanup provision such as lower contaminant concentrations, the greater the attendant cleanup costs. It may require tens to hundreds of millions of dollars for complete cleanup of a large, complex, hazardous waste site. In fact, it may be impossible to clean many sites completely. It is very important, therefore, that the physical feasibility of cleanup and the degree of potential risk posed by the contamination be weighed against the economic impact, and the future use of the site. The second perspective being considered is whether natural microbial activities in the environment can aid in the cleanup of contaminated sites, and whether these activities will occur rapidly enough naturally, or whether they can and should be enhanced. These two perspectives are closely tied together because although microbial activities can reduce contamination significantly, they often do not completely remove it.

17.2 ENVIRONMENTAL LAW

Society began responding to environmental concerns long ago, beginning with the recognition that our environment is fragile, and that human activities can have a great impact on it. This led to the creation of Yellowstone National Park in 1872, and the assignment of the care of forested public domain lands to the U.S. Forest Service in 1897. After World War II, as the pollution impacts of industrialization began to be apparent, Congress began to legislate in the area of pollution control. This legislation culminated in the major federal pollution control statutes of the 1970s that now constitute a large body of law called environmental law. Federal environmental law consists of laws in the conservation and pollution control areas, along with key planning and coordination statutes.

Environmental law is constantly changing and evolving as we try to respond to shifting priorities and pressures on resources. Sometimes changes are made to the law to allow further contamination or risk of contamination to occur for the "good of society." An example is oil exploration in environmentally sensitive areas such as Alaska and the Atlantic and Pacific coasts. On the other hand, some laws can be made more stringent. Again, using an example from the oil industry, whereas refinery wastes are heavily regulated for disposal, the same types of wastes generated in an oil field were not regulated, and were routinely buried without treatment. When attention was drawn to this practice, laws were enacted to require the oil industry to implement proper oil field disposal practices. As these examples suggest, environmental law comprises a complex body of laws, regulations and decisions now established in the United States. This body of law, which evolved quite quickly in comparison with labor, tax, banking and communications laws, already ranks with these other areas in size and complexity (Arbuckle *et al.*, 1987).

The term "environmental law" came into being with the enactment of the National Environmental Policy Act (NEPA) in early 1970 (Information Box 17.2). Although environmental law can vary considerably from state to state and even from city to city, a series of major federal environmental protection laws have been enacted that pertain to the generation, use and disposition of hazardous waste. NEPA requires each agency, government or industry that proposes a major action which may have a significant

Information Box 17.2 U.S. Title I Congressional Declaration of National Environmental Policy Sec. 101[42 USC § 4331]

(a) The Congress, recognizing the profound impact of man's activity on the interrelations of all components of the natural environment, particularly the profound influences of population growth, high-density urbanization, industrial expansion, resource exploitation, and new and expanding technological advances and recognizing further the critical importance of restoring and maintaining environmental quality to the overall welfare and development of man, declares that it is the continuing policy of the Federal Government, in cooperation with State and local governments, and other concerned public and private organizations, to use all practicable means and measures, including financial and technical assistance, in a manner calculated to foster and promote the general welfare, to create and maintain conditions under which man and nature can exist in productive harmony, and fulfill the social, economic, and other requirements of present and future generations of Americans.

(b) In order to carry out the policy set forth in this Act, it is the continuing responsibility of the Federal Government to use all practicable means, consistent with other essential considerations of national policy, to improve and coordinate Federal plans, functions, programs, and resources to the end that the Nation may—

1. fulfill the responsibilities of each generation as trustee of the environment for succeeding generations;
2. assure for all Americans safe, healthful, productive, and aesthetically and culturally pleasing surroundings;
3. attain the widest range of beneficial uses of the environment without degradation, risk to health or safety, or other undesirable and unintended consequences;
4. preserve important historic, cultural, and natural aspects of our national heritage, and maintain, wherever possible, an environment which supports diversity, and variety of individual choice;
5. achieve a balance between population and resource use which will permit high standards of living and a wide sharing of life's amenities; and
6. enhance the quality of renewable resources and approach the maximum attainable recycling of depletable resources.

(c) The Congress recognizes that each person should enjoy a healthful environment and that each person has a responsibility to contribute to the preservation and enhancement of the environment.

effect on the human environment to prepare an environmental impact statement (EIS). The EIS must address the environmental impact of the proposed action and any reasonable alternatives that may exist. The types of projects that NEPA covers are landfills, roads, dams, building complexes, research projects and any private endeavor requiring a federal license that may affect the environment. *NEPA does not mandate particular results, and it does not require a federal agency to adopt the least environmentally damaging alternative*. Because of this, NEPA can be thought of as an "environmental full disclosure law," which requires the applicant to take a "hard look" at the environmental consequences of its action. Thus, an EIS allows environmental concerns and planning to be integrated into the early stages of project planning. Unfortunately, an EIS is often done as an afterthought and becomes a rationale for a project that may be a poor alternative.

A series of environmental laws have been passed since NEPA to protect our natural resources (Table 17.1). These include laws such as the Clean Air Act and the Clean Water Act, which protect air and water resources. There are also laws that govern the permitting of the sale of hazardous chemicals and laws that mandate specific action to be taken in the cleanup of contaminated sites. When the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA), more commonly known as Superfund, was enacted, it became clear that technology for cleaning up hazardous waste sites was needed. Early remedial actions for contaminated sites consisted of excavation and removal of the contaminated soil to a landfill. Very soon, it became apparent that this was simply moving the problem around, not solving it. As a result, the Superfund Amendments and Reauthorization Act (SARA) was passed in 1986. This act added several new dimensions to CERCLA. SARA stipulates cleanup standards, and mandates the use of the National Contingency Plan to determine the most appropriate action to take for site cleanup.

Two types of responses are available within Superfund: (1) removal actions in response to immediate threats, e.g., removing leaking drums, and (2) remedial actions, which involve cleanup of hazardous sites. The Superfund provisions can be used when a hazardous substance is released or there is a substantial threat of a release that poses imminent and substantial endangerment to public health and welfare. The first step is to place the potential site into the Superfund Site Inventory. After a preliminary assessment and site inspection, the decision is made as to whether or not the site will be placed on the National Priority List (NPL). Sites placed on this list are those deemed to require a remedial action. Currently, there are more than 1200 sites on the NPL. The National Contingency Plan (NCP) is the next component. The purpose of the NCP is to characterize

TABLE 17.1 History of Environmental Law

Law	Year Passed	Goals
Clean Air Act (CAA)	1970	Sets nationwide ambient air quality standards for conventional air pollutants. Sets standards for emissions from both stationary and mobile sources (e.g., motor vehicles).
Clean Water Act (CWA)	1972	Mandates "fishable/swimmable" waters wherever attainable. Provides for (1) a construction grants program for publicly owned water treatment plants and requires plants to achieve the equivalent of secondary treatment; (2) a permit system to regulate point sources of pollution; (3) areawide water quality management to reduce nonpoint sources of pollution; (4) wetlands protection, sludge disposal and ocean discharges; (5) regulation of cleanup of oil spills.
Surface Mining Control and Reclamation Act (SMCRA)	1977	Regulates coal surface mining on private lands and strip mining on public lands. Prohibits surface mining in environmentally sensitive areas.
Resource Conservation and Recovery Act (RCRA)	1976	Provides a comprehensive management scheme for hazardous waste disposal. This includes a system to track the transportation of wastes and federal performance standards for hazardous waste treatment, storage and disposal facilities. Open dumps are prohibited.
Toxic Substances Control Act (TOSCA)	1976	Requires premarket notification of EPA by the manufacturer of a new chemical. Based on testing information submitted by the manufacturer or premarket test ordered by EPA (including biodegradability and toxicity), a court injunction can be obtained barring the chemical from distribution or sale. EPA can also seek a recall of chemicals already on the market. It is this Act that prohibits all but closed-circuit uses of PCBs.
Comprehensive Environmental Response, Compensation and Liability Act (CERCLA)	1980	Commonly known as Superfund, this Act covers the cleanup of hazardous substance spills, from vessels, active or inactive facilities. Establishes a Hazardous Substances Response Trust Fund, financed by a tax on the sale of hazardous chemicals, to be used for removal and cleanup of hazardous waste releases. Cleanup costs must be shared by the affected state. Within certain limits and subject to a few defenses, anyone associated with the release is strictly liable to reimburse the fund for cleanup costs, including damage to natural resources.
Superfund Amendments and Reauthorization Act (SARA)	1986	SARA provides cleanup standards and stipulates rules through the National Contingency Plan for the selection and review of remedial actions. It strongly recommends that remedial actions use on-site treatments that "permanently and significantly reduce the volume, toxicity, or mobility of hazardous substances" and requires remedial action that is "protective of human health and the environment, that is cost-effective, and that utilizes permanent solutions and alternative treatment technologies or resource recovery technologies to the maximum extent practicable."
National Contingency Plan (NCP)	1988	A five-step process to use in evaluation of contaminated sites and suggest the best plan for remediation.

the nature and extent of risk posed by contamination, and to evaluate potential remedial options. The investigation and feasibility study components are normally conducted concurrently and with a "phased" approach. This allows feedback between the two components. The selection of the specific remedial action to be used at a particular site is a very complex process. The goals of the remedial action are that it be protective of human health and the environment, that it maintains protection over time and that it maximizes waste treatment.

How does microbiology fit into remedial action strategies? Biological remediation approaches have been found to be more economical and often have better public acceptance than traditional physical/chemical approaches. The remainder of this chapter deals with biodegradation of organic contaminants and ways in which these processes can be harnessed to remediate contaminated sites.

17.3 THE OVERALL PROCESS OF BIODEGRADATION

Biodegradation is the breakdown of organic contaminants that occurs due to microbial activity. As such, these organic contaminants can be considered as a microbial food source or substrate. Biodegradation of any organic compound can be thought of as a series of biological degradation steps or a pathway that ultimately results in the oxidation of the parent compound. Often, the degradation of these compounds results in the generation of energy as described in Chapter 3.

Complete biodegradation or mineralization involves oxidation of the parent compound to form carbon dioxide and water, a process that provides both carbon and energy for growth and reproduction of cells. Figure 17.1 illustrates the mineralization of an organic compound under either aerobic or anaerobic conditions. The series of degradation

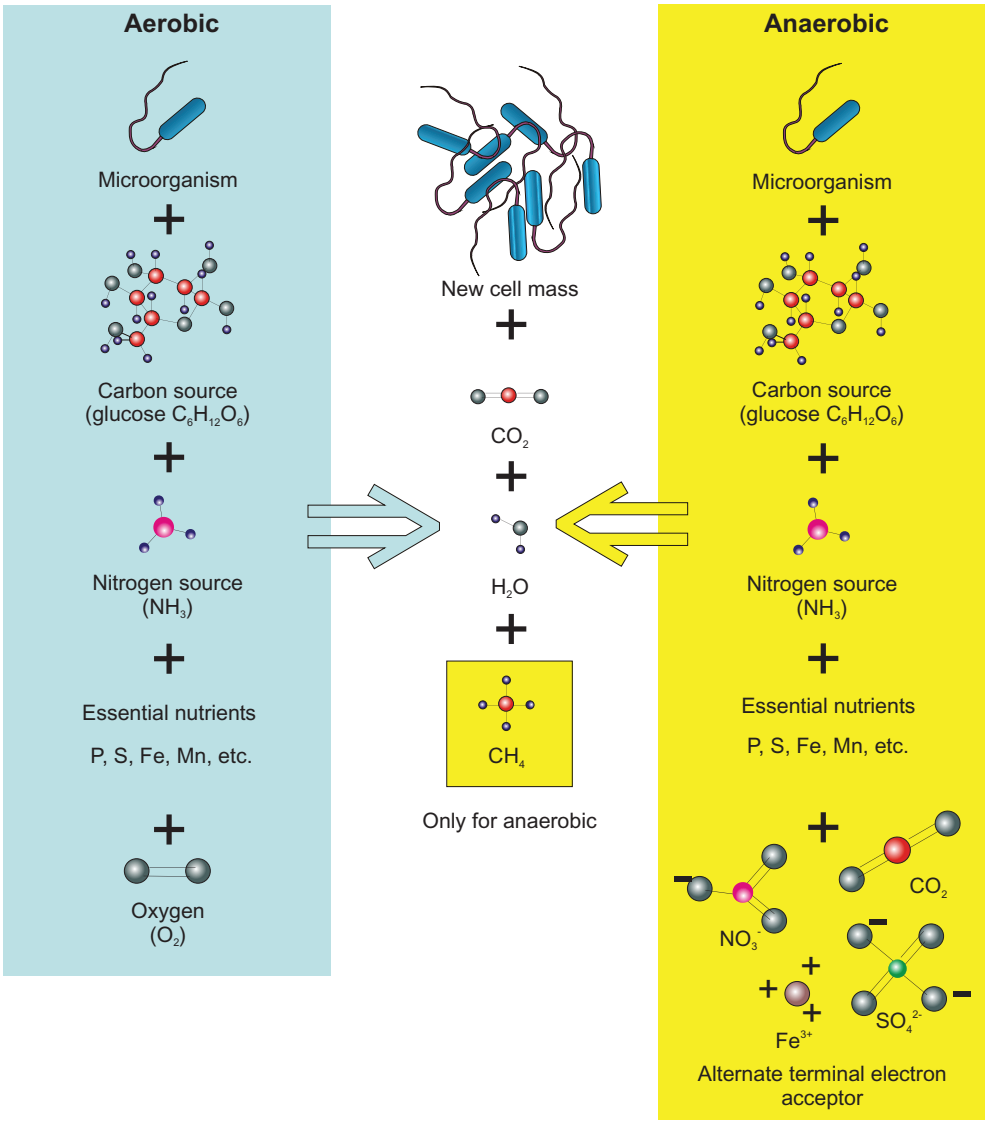


FIGURE 17.1 Aerobic (blue) or anaerobic (yellow) mineralization of an organic compound.

steps constituting mineralization is similar whether the carbon source is a simple sugar such as glucose, a plant polymer such as cellulose or a pollutant molecule. Each degradation step in the pathway is catalyzed by a specific enzyme made by the degrading cell. Enzymes are most often found within a cell, but are also made and released from the cell to help initiate degradation reactions. Enzymes found external to the cell are known as extracellular enzymes. Extracellular enzymes are important in the degradation of macromolecules such as the plant polymer cellulose (see Section 16.2.3.1). Macromolecules must be broken down into smaller subunits outside the cell to allow transport of the smaller subunits into the cell. Biotransformation may stop at any step in the biodegradation pathway if the appropriate enzyme, either internal or extracellular, is not present (Figure 17.2). In fact, lack of appropriate biodegrading enzymes is one common reason for persistence of organic contaminants, particularly those with unusual chemical structures that existing enzymes do not recognize. Thus, contaminant compounds that have structures similar to those of natural substrates are normally easily degraded. Those that are quite dissimilar to natural substrates are often degraded slowly or not at all.

Some organic contaminants are only partially degraded by environmental microorganisms. This can result from absence of the appropriate degrading enzyme as mentioned earlier. A second type of incomplete degradation is cometabolism, in which a partial oxidation of the substrate occurs but the energy derived from the oxidation is not used to support microbial growth. The process occurs when organisms possess one or more enzymes that coincidentally can degrade a particular contaminant in addition to its target substrate. Thus, such enzymes are nonspecific. Cometabolism can occur during periods of active growth, or can result from the interaction of resting (nongrowing) cells with an organic compound. Cometabolism is difficult

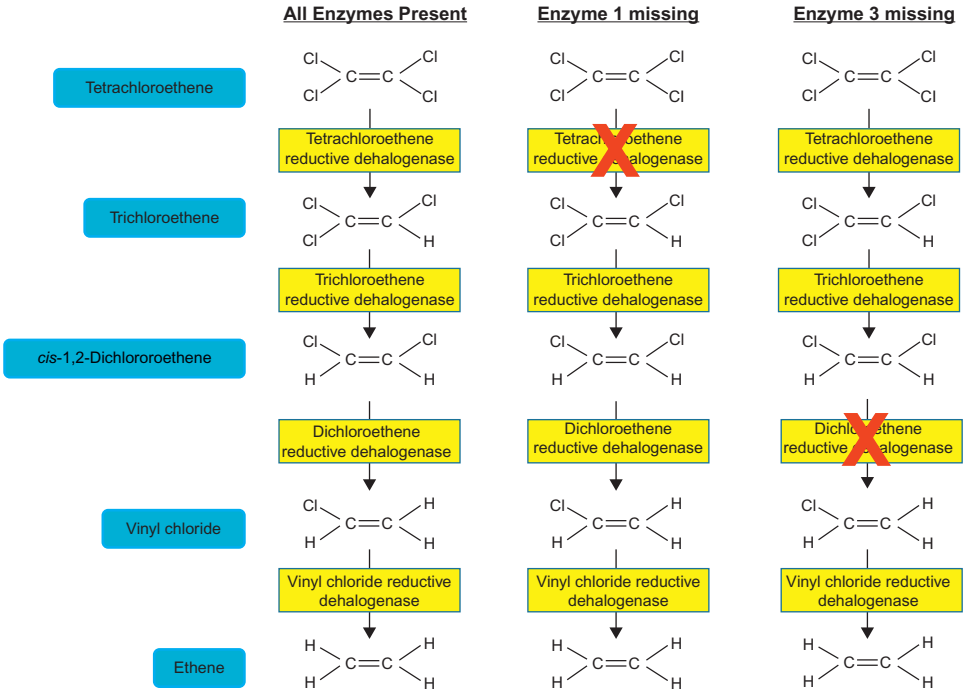


FIGURE 17.2 Stepwise transformation of tetrachloroethene (also known as perchloroethene; PCE). A different enzyme catalyzes each step of the pathway. Some microorganisms such as *Dehalococcoides ethenogenes* strain 195 are capable of using PCE as a terminal electron acceptor and completely dechlorinating it to the non-toxic compound ethene. In contrast, most other organisms either lack the ability to produce the enzymes needed to initiate transformation of PCE (e.g., missing enzyme 1) or can only transform it partially resulting in the accumulation of intermediate compounds such as *cis*-1,2-dichloroethene or vinyl chloride (e.g., missing enzyme 3). The potential accumulation of biodegradation intermediates is a concern since some of these chemicals, such as vinyl chloride, may be more toxic than the parent compound.

to measure in the environment, but has been demonstrated for some environmental contaminants. For example, the industrial solvent trichloroethene (TCE; also known as trichloroethylene) can be oxidized cometabolically by methanotrophic bacteria that grow on methane as a sole carbon source (Suttinun *et al.*, 2013). TCE is of great interest for several reasons. It is one of the most frequently reported contaminants at hazardous waste sites, it is a suspected carcinogen and it is generally resistant to biodegradation. As shown in Figure 17.3, the first step in the oxidation of methane by methanotrophic bacteria is catalyzed by the enzyme methane monooxygenase. This enzyme is so nonspecific that it can also cometabolically catalyze the first step in the oxidation of TCE when both methane and TCE are present. The bacteria receive no energy benefit from this cometabolic degradation step. The subsequent degradation steps shown in Figure 17.3 may be catalyzed spontaneously, by other bacteria, or in some cases by the methanotroph. This is an example of a cometabolic reaction that may have great significance in remediation. Research is currently investigating the application of these methanotrophs, as well as cometabolizing microorganisms that grow on toluene, ethylene, propylene, propane, butane and even ammonia, to TCE-contaminated sites.

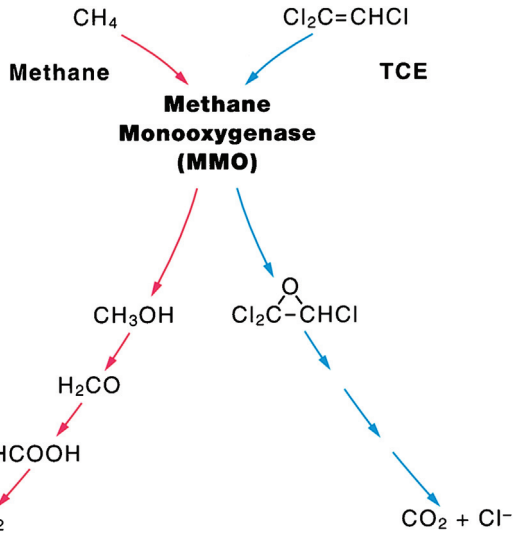


FIGURE 17.3 The oxidation of methane by methanotrophic bacteria is catalyzed by the enzyme methane monooxygenase. The same enzyme can act nonspecifically on trichloroethene (TCE). Subsequent TCE degradation steps may be catalyzed spontaneously, by other bacteria, or in some cases by the same methanotroph. From Pepper *et al.* (2006).

Partial or incomplete degradation can also result in polymerization or synthesis of compounds that are more complex and stable than the parent compound. This occurs when

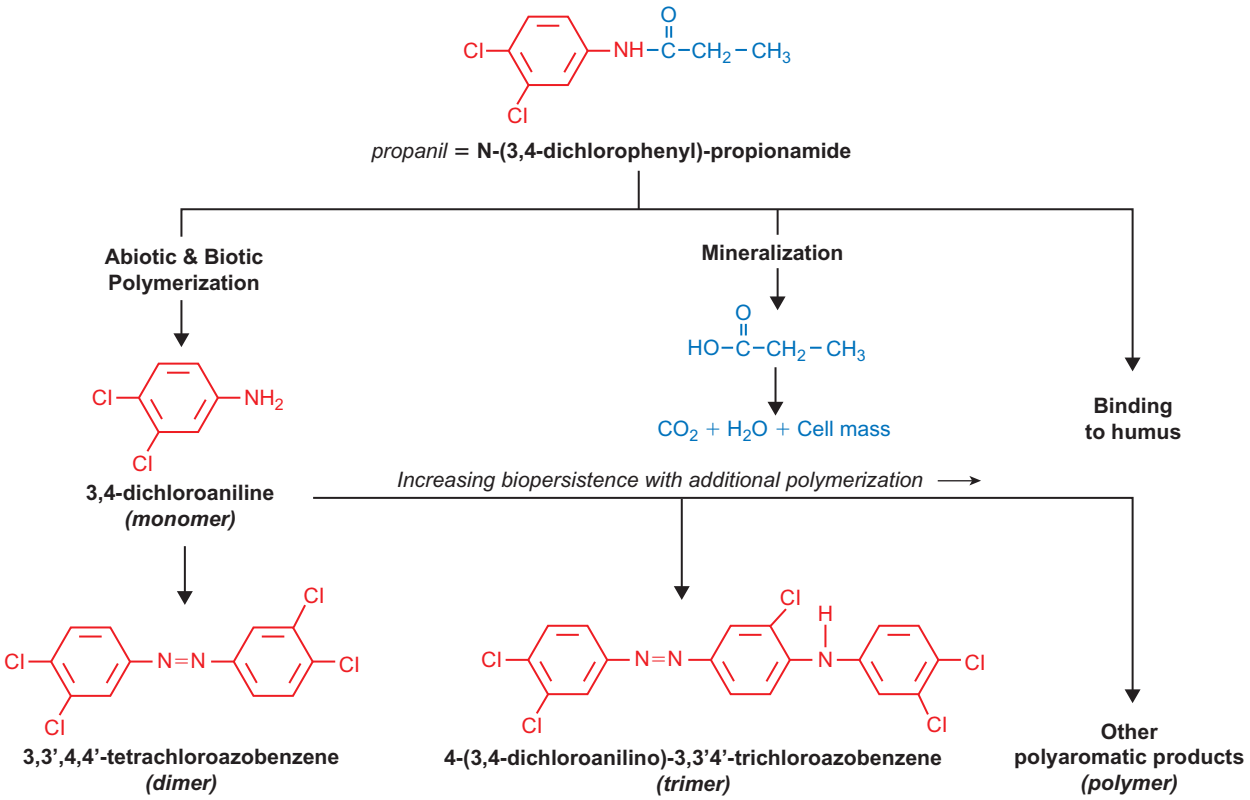


FIGURE 17.4 Polymerization reactions that occur with the herbicide propanil during biodegradation. Propanil is a selective post-emergence herbicide used in growing rice. It is toxic to many annual and perennial weeds. The environmental fate of propanil is of concern because it, like many other pesticides, is toxic to most crops except for cereal grains. It is also toxic to fish. Care is used in propanil application to avoid contamination of nearby lakes and streams. From Pepper *et al.* (2006).

initial degradation steps, often catalyzed by extracellular enzymes, create reactive intermediate compounds. These highly reactive intermediate compounds can then combine with each other or with other organic matter present in the environment. This is illustrated in Figure 17.4, which shows some possible polymerization reactions that occur with the herbicide propanil during biodegradation. These include formation of dimers or larger polymers, which are quite stable in the environment. Stability is due to low bioavailability (high sorption and low solubility), lack of degrading enzymes and the fact that some of these residues become chemically bound to the soil organic matter fraction.

17.4 CONTAMINANT STRUCTURE, TOXICITY AND BIODEGRADABILITY

The vast majority of the organic carbon available to microorganisms in the environment is material that has been photosynthetically fixed (plant material). Of concern are environments that receive large additional inputs of carbon from agriculture or industry (petroleum products, organic solvents, pesticides). Although many of these chemicals can be readily degraded because of their structural similarity to naturally occurring organic carbon, the amounts added may exceed the existing carrying capacity of the environment. Carrying capacity is defined here as the maximum level of microbial activity that can be expected under existing environmental conditions. Microbial activity may be limited by both biological and physical–chemical factors. These factors include low numbers of microbes, insufficient oxygen or nutrient availability, and suboptimal temperature or water availability. These factors are discussed further in Section 17.5. Microbial activity, whether degradation occurs and the rate of degradation, also depend on several factors related to the structure and physical–chemical properties of the contaminant (Miller and Herman, 1997) (see Information Box 17.3).

17.4.1 Genetic Potential

The onset of contaminant biodegradation generally follows a period of adaptation or acclimation of indigenous microbes, the length of which depends on the contaminant structure. The efficient cycling of plant-based organic matter by soil microorganisms can promote the rapid degradation of organic contaminants that have a chemical

Information Box 17.3 Genetic and Contaminant Structure Factors that Impact Biodegradation

- Phenotypic genetic potential. The presence and expression of appropriate degrading genes by the indigenous microbial community.
- Toxicity. The inhibitory effect of the contaminant on cellular metabolism.
- Bioavailability. The effect of limited water solubility and sorption on the rate at which a contaminant is taken up by a microbial cell.
- Contaminant structure. Includes both steric and electronic effects. Steric effects involve the extent to which substituent groups on a contaminant molecule sterically hinder recognition by the active site of the degrading enzyme. Electronic effects involve the extent to which substituent groups electronically interfere with the interaction between the active site of the enzyme and the contaminant. Electronic effects can also alter the energy required to break critical bonds in the molecule.

Information Box 17.4 Enhanced Degradation of Atrazine in Soil—An Example of Microbial Adaptation Through Gene Transfer

The herbicide atrazine has been used extensively to control broadleaf and grass weeds in several major crops including corn. Due partly to its environmental persistence, contamination of surface water and groundwater with atrazine has been a major concern over the past few decades. However, recent studies have found that atrazine is degraded rapidly in many fields where it has been applied repeatedly over several years. In many of these locations, the half-life of atrazine has been reduced to <5 days in comparison to the 26 to 142 day half-lives found in sites where atrazine has not previously been applied (Krutz *et al.*, 2010). This enhanced degradation appears to be due to adaptation of the soil microbial communities, largely through acquisition of plasmid-encoded catabolic genes via horizontal gene transfer processes. While this enhanced degradation may be beneficial for reducing the potential environmental impacts of atrazine, it can greatly diminish atrazine's residual activity against weeds thereby necessitating the use of other herbicides or alternative weed control strategies, in order to maintain current levels of crop production.

structure similar to those of natural soil organic compounds (Section 3.3.1). Previous exposure to a contaminant through repeated pesticide applications or through frequent spills will create an environment in which a biodegradation pathway is maintained within an adapted community. Adaptation of microbial populations most commonly occurs by induction of enzymes necessary for biodegradation followed by an increase in the population of biodegrading organisms (Leahy and Colwell, 1990).

Naturally occurring analogues of certain contaminants may not exist, and previous exposure may not have occurred. Degradation of these contaminants requires a second type of adaptation that involves a genetic change such as a mutation or a gene transfer (Information Box 17.4). This results in the development of new metabolic capabilities. The time needed for an adaptation requiring a genetic change, or for the selection and development of an adapted community, is not yet predictable, but it may require weeks to years or may not occur at all (van der Meer, 2006).

17.4.2 Toxicity

Chemical spills and engineered remediation projects, such as landfarming of petroleum refinery sludges, can involve extremely high contaminant concentrations. In these cases, toxicity of the contaminant to microbial populations can slow the remediation process. One common type of toxicity is that associated with nonionic organic contaminants such as petroleum hydrocarbons or organic solvents. This toxicity is mainly due to a nonspecific narcotic-type mode of action, which is based on the partitioning of a dissolved contaminant into the lipophilic layer of the cell membrane, which causes a disruption of membrane integrity (Sikkema *et al.*, 1995). This effect is important because, due to hydrophobic interactions, the cell membrane is a major site of organic contaminant accumulation in microorganisms. In addition, functional groups such as halogens and even the molecular weight of a compound influence its toxicity to microbial cells (Kenawy *et al.*, 2007).

Models have been developed that relate bioconcentration (the accumulation of a hydrophobic contaminant by a cell or organism) and toxicity to the physicochemical attributes or descriptors of the organic contaminant. These models are referred to as quantitative structure–activity relationship (QSAR) models. A number of models have been developed based on different attributes such as structure, functional groups and metabolic pathways of degradation (Pavan and Worth, 2006). Specific descriptors can include hydrophobicity and molecular connectivity (which represents the surface topography of a compound). As might be expected, no one QSAR works for all

compounds, in fact some studies show that such models correctly predict biodegradability 68 to 91% of the time depending on the model used and the set of contaminants used to test the models (Tunkel *et al.*, 2005; Pavan and Worth, 2006). Refinement of these models is currently an international effort because the use of QSAR is expected to increase in the future due to: (1) the high costs associated with experimental determination of contaminant persistence, bioconcentration and toxicity, and (2) international pressure to reduce the use of animal testing (Pavan and Worth, 2006).

17.4.3 Bioavailability

For a long time biodegradation was thought to occur if the appropriate microbial enzymes were present. As a result, most research focused on the actual biodegradative process, specifically the isolation and characterization of biodegradative enzymes and genes. There are, however, two steps in the biodegradative process. The first is the uptake of the substrate by the cell, and the second is the metabolism or degradation of the substrate. Assuming the presence of an appropriate metabolic pathway, degradation of a contaminant can proceed rapidly if the contaminant is available in a water-soluble form. However, degradation of contaminants with limited water solubility or those that are strongly sorbed to soil or sediments can be limited due to their low bioavailability (Maier, 2000).

Growth on an organic compound with limited water solubility poses a unique problem for microorganisms. Most microorganisms obtain substrate from the surrounding aqueous phase, but the opportunity for contact between the degrading organism and an organic compound with low water solubility is limited. Such a compound may be present in a liquid or solid state, both of which can form a two-phase system with water. Liquid hydrocarbons can be less or more dense than water, forming a separate phase above or below the water surface. For example, polychlorinated biphenyls (PCBs) and chlorinated solvents such as TCE are denser than water, and form a separate phase below the water surface. Solvents less dense than water, such as benzene and other petroleum constituents, form a separate phase above the water surface. There are three possible modes of microbial uptake of a liquid organic (Figure 17.5):

1. Utilization of the solubilized organic compound (Figure 17.5A).
2. Direct contact of cells with the organic compound. This can be mediated by cell modifications, such as fimbriae (Rosenberg *et al.*, 1982), or cell surface hydrophobicity (Zhang and Miller, 1994), which increase attachment of the cell to the organic compound (Figure 17.5B).

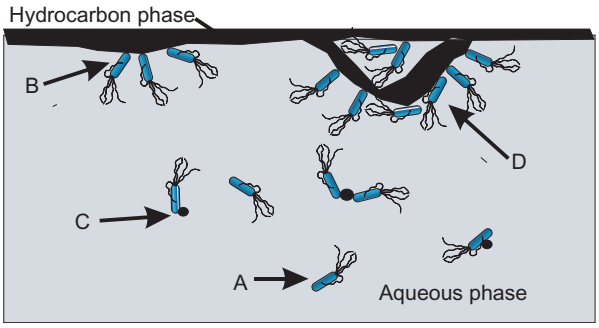


FIGURE 17.5 A water environment with a petroleum oil phase floating on the surface. This is typical of what might occur when oil is spilled in the ocean. There are several ways in which microbes reach the oil phase in this type of situation. (A) Microbes taking up hydrocarbons dissolved in the aqueous phase surrounding degrading cells. This uptake mode becomes limiting as the aqueous solubility of the hydrocarbon decreases. (B) Uptake via direct contact of degrading cells at the aqueous–hydrocarbon interface of large oil drops in water. This uptake mode is limited by the interfacial area between the water and hydrocarbon phase. (C) Uptake through direct contact of degrading cells with fine or submicrometer-size oil droplets dispersed in the aqueous phase. This uptake mode is limited by the formation of such droplets. In the ocean environment, wave action can create substantial dispersion of oil. In a soil environment, such dispersion is more limited. (D) Enhanced uptake as a result of production of biosurfactants or emulsifiers that effectively increase the apparent aqueous solubility of the hydrocarbon, or allow better attachment of cells to the hydrocarbon.

3. Direct contact with fine or submicrometer size substrate droplets dispersed in the aqueous phase (Figure 17.5C).

The mode that predominates depends largely on the water solubility of the organic compound. In general, direct contact with the organic compound plays a more important role (modes 2 and 3) as water solubility decreases.

Some microbes can enhance the rate of uptake and biodegradation as a result of production of biosurfactants or emulsifiers (Figure 17.5D). There are two effects of biosurfactants. First, they can effectively increase the aqueous solubility of the hydrocarbon through formation of micelles or vesicles that associate with hydrocarbons (Figure 17.6). Second, they can facilitate attachment of cells to the hydrocarbon by making the cell surface more hydrophobic, and thus better able to stick to a separate oil phase (Figure 17.7). This makes it possible to achieve greatly enhanced biodegradation rates in the presence of biosurfactants (Herman *et al.*, 1997).

For organic compounds in the solid phase, e.g., waxes, plastics or polyaromatic hydrocarbons (PAHs), there are only two modes by which a cell can take up the substrate:

1. Direct contact with the substrate
2. Utilization of solubilized substrate

Available evidence suggests that for solid-phase organic compounds, utilization of solubilized substrate is

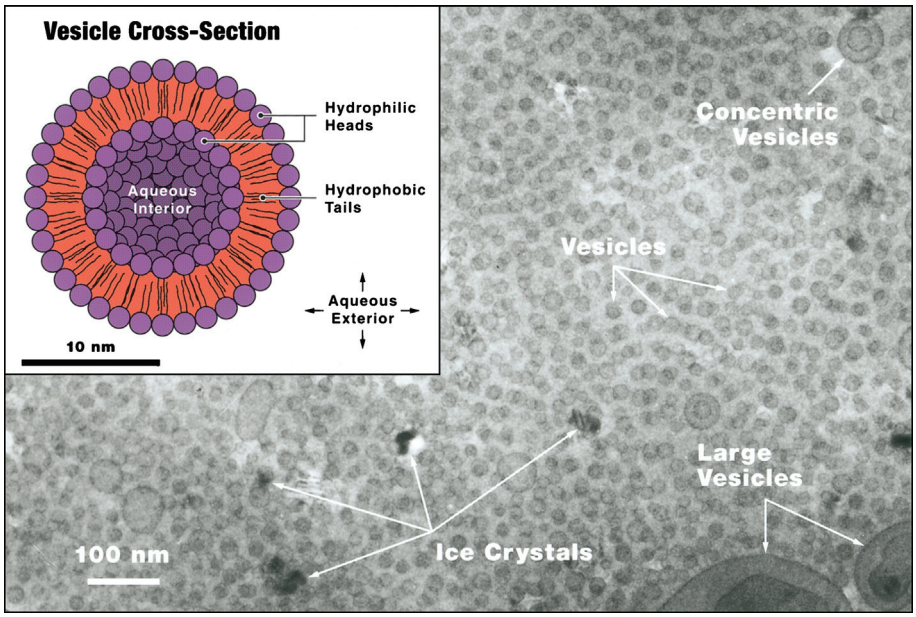


FIGURE 17.6 Cryo-transmission electron micrograph of a microbially produced surfactant, rhamnolipid. In water, this compound spontaneously forms aggregates such as the vesicles shown here. Hydrocarbons like to associate with the lipid-like layer formed by the hydrophobic tails of the surfactant vesicles. These tiny surfactant–hydrocarbon structures are soluble in the aqueous phase. From Pepper *et al.* (2006).

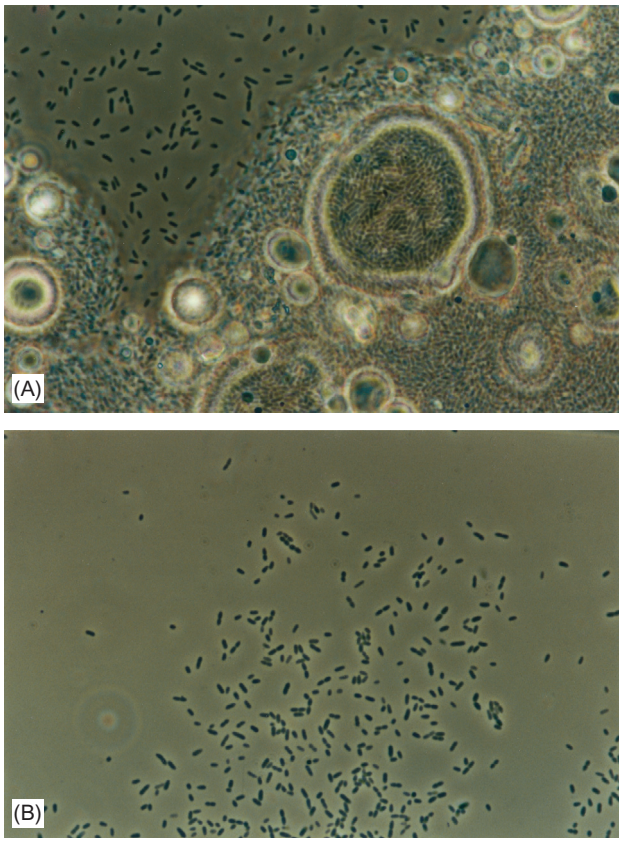


FIGURE 17.7 Phase-contrast micrographs showing the effect of a biosurfactant on the ability of *Pseudomonas aeruginosa* ATCC 15524 to stick to hexadecane droplets (magnification ×1000). (A) Addition of rhamnolipid biosurfactant (0.1 mM) causes cells to clump and to stick to oil droplets. (B) No biosurfactant is present and individual cells do not clump, and do not stick to oil droplets in the solution. Photos courtesy D.C. Herman.

most important. Thus, low water solubility has a greater impact on degradation of solid-phase organic compounds than on liquid-phase organics.

Another factor that affects bioavailability of an organic compound is sorption of the compound by soil or sediment (Novak *et al.*, 1995). Depending on the sorption mechanism, organic compounds can be weakly (hydrogen bonding, van der Waals forces, hydrophobic interactions) or strongly (covalent bonding) bound to soil. Sorption of weakly bound or labile residues is reversible, and when a sorbed residue is released back into solution it becomes available for microbial utilization (Scow, 1993). Bioavailability can also be reduced by the diffusion of contaminants into soil matrix microsites that are inaccessible to bacteria because of pore-size exclusion (Alexander, 1995). There is evidence that the proportion of labile residues made available by desorption decreases with the length of time the residues are in the soil. Thus, as contaminants age and become sequestered more deeply within inaccessible microsites (Figure 4.7), bioavailability, and therefore biodegradation, can be expected to decrease.

Finally, some contaminants may be incorporated into soil organic matter by the catalytic activity of a wide variety of oxidative enzymes that are present in the soil matrix. The incorporation of contaminants into soil organic matter is called humification, a process that is usually irreversible and that may be considered as one factor in the aging process (Bollag, 1992). These bound or humified residues are released and degraded only very slowly as part of the normal turnover of humic material in soil (see Section 16.2.3.2).

17.4.4 Contaminant Structure

17.4.4.1 Steric Effects

Some types of contaminant structures can lead to low degradation rates even if the contaminant structure is similar to naturally occurring molecules. The presence of branching or functional groups can slow degradation by changing the chemistry of the degradation reaction site. The reaction site is the contact area between a degradative enzyme and the contaminant substrate where a transformation step occurs. When the reaction site is blocked by branching or a functional group, contact between the contaminant and enzyme at the reaction site is hindered. This is known as a steric effect and is illustrated in Figure 17.8, which compares two structures, an eight-carbon *n*-alkane (A) and the same eight-carbon backbone with four methyl branches (B). Whereas octane is readily degradable by the pathway shown in Figure 17.12, the four methyl substituents in 3,3,6,6-tetramethyl octane inhibit degradation at both ends of the molecule. Branching or functional groups can also affect transport of the substrate across the cell membrane, especially if the transport is enzyme assisted. Steric effects usually increase as the size of the functional group increases (Pitter and Chudoba, 1990).

17.4.4.2 Electronic Effects

Functional groups may also contribute electronic effects that hinder biodegradation by affecting the interaction between the contaminant and the enzyme. Functional groups can be electron donating (e.g., CH_3) or electron withdrawing (e.g., Cl), and therefore can change the electron density of the reaction site. In general, functional groups which add to the electron density of the reaction site increase biodegradation rates, and functional groups that decrease the electron density of the reaction site decrease biodegradation rates. To illustrate the relationship between functional group electronegativities and rate of biodegradation, Pitter and Chudoba (1990) compared the electronegativity of a series of ortho-substituted phenols with their biodegradation rates. Five different functional groups were tested, and it was found that as the electronegativity of the substituents increased, biodegradation rates decreased (Figure 17.9).

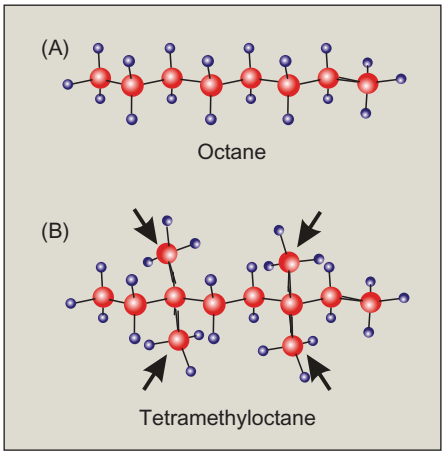


FIGURE 17.8 The structure of (A) octane, which is readily degradable, and (B) a tetramethyl-substituted octane, which is not degradable because the methyl groups block the enzyme–substrate catalysis site.

17.5 ENVIRONMENTAL FACTORS AFFECTING BIODEGRADATION

A number of parameters influence the survival and activity of microorganisms in any given environment. One factor that has great influence on microbial activity is organic matter, the primary source of carbon for heterotrophic microorganisms in most environments. Surface soils have a relatively high and variable organic matter content, and therefore are characterized by high microbial numbers and diverse metabolic activity (see Chapter 4). In contrast, the subsurface unsaturated (vadose) zone and saturated zone usually have a much lower content and diversity of organic matter, resulting in lower microbial numbers and activity. Exceptions to this rule are some areas of the saturated zone that have high flow or recharge rates, which can lead to numbers and activities of microorganisms similar to those found in surface soils.

Occurrence and abundance of microorganisms in an environment are determined not only by available carbon but also by various physical and chemical factors. These include oxygen availability, nutrient availability, temperature, pH, salinity and water activity. Inhibition of biodegradation can be caused by a limitation imposed by any one of these factors, but the cause of the persistence of a contaminant is sometimes difficult to determine. Perhaps the most important factors controlling contaminant biodegradation in the environment are oxygen availability, organic matter content, nitrogen availability and contaminant bioavailability. Interestingly, the first three of these factors can change considerably depending on the location of the contaminant. Figure 17.10 shows the relationship between organic carbon, oxygen and microbial activity in a profile of the terrestrial ecosystem including surface soils, the vadose zone and the saturated zone.

17.5.1 Redox Conditions

Redox conditions are very important in determining the extent and rate of contaminant biodegradation. For most contaminants, aerobic biodegradation rates are much higher than anaerobic biodegradation rates. For example, petroleum-based hydrocarbons entering the aerobic zones of freshwater lakes and rivers are generally susceptible to microbial degradation, but oil accumulated in anaerobic

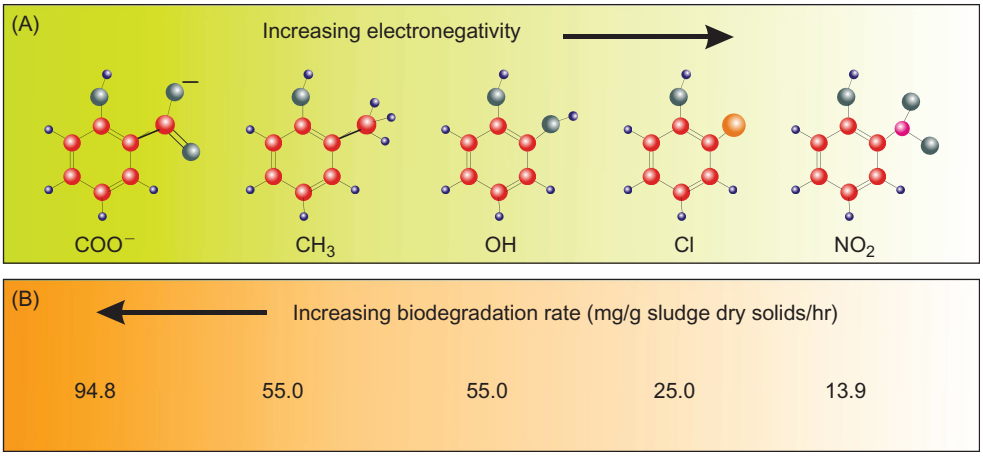


FIGURE 17.9 Various ortho-substituted phenols and their respective biodegradation rates. Adapted from Pitter and Chudoba (1990).

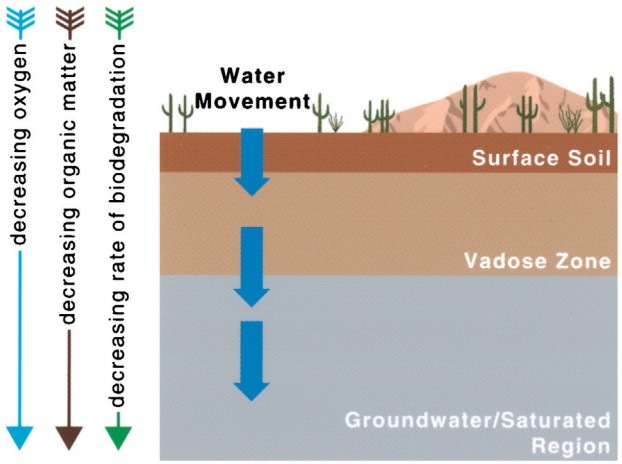


FIGURE 17.10 There are three major locations where contamination can occur in terrestrial ecosystems: surface soils, the vadose zone and the saturated zone. The availability of both oxygen and organic matter varies considerably in these zones. As indicated, oxygen and organic matter both decrease with depth, resulting in a decrease in biodegradation activity with depth. From Pepper *et al.* (2006).

sediments can be highly persistent. Oxygen is especially important for degradation of highly reduced hydrocarbons such as the alkanes. For example, low molecular weight alkanes such as methane do not degrade anaerobically. Higher molecular weight alkanes, such as hexadecane ($C_{16}H_{34}$), can occur, but degradation is very limited, and usually is only found in historically petroleum-contaminated sites. In contrast, there are some highly chlorinated compounds (e.g., perchloroethene (PCE)) that are recalcitrant under aerobic conditions, but amenable to biotransformation under anaerobic conditions.

17.5.2 Organic Matter Content

Surface soils have large numbers of microorganisms. Bacterial numbers commonly range from 10^7 to 10^{10} per gram of soil with somewhat lower fungal numbers, 10^5 to 10^6 per gram of soil. In contrast, microbial populations in deeper regions such as the deep vadose zone and groundwater region are often lower by two orders of magnitude or more (see Chapter 4). This large decrease in microbial numbers with depth is due primarily to differences in organic matter content. Both the vadose zone and the groundwater region have low amounts of organic matter. One result of low total numbers of microorganisms is that a low population of contaminant degraders may be present initially. Thus, biodegradation of a particular contaminant may be slow until a sufficient biodegrading population has been built up. A second reason for slow biodegradation in the vadose zone and groundwater region is that because a low amount of organic matter is present, the organisms in this region are often dormant. This can cause their response to an added carbon source to be slow, especially if the carbon source is a contaminant molecule that has low bioavailability, or to which the organisms have not had prior exposure.

Because of these trends in oxygen availability and organic matter content, several generalizations can be made with respect to surface soils, the vadose zone and the groundwater region (Figure 17.10 and Information Box 17.5).

17.5.3 Nitrogen

Microbial utilization of organic contaminants, particularly hydrocarbons composed primarily of carbon and hydrogen, creates a demand for essential nutrients such as nitrogen and phosphorus. Thus, biodegradation can often be improved simply by the addition of nitrogen fertilizers. This is particularly true in the case of biodegradation of petroleum oil spills, in which nitrogen shortages can become acute. In general, microbes have an average C:N ratio within their biomass of about 5:1 to 10:1 depending on the type of microorganism. Therefore, a ratio of

Information Box 17.5 Biodegradation in Surface and Subsurface Environments

- Biodegradation in surface soils is primarily aerobic and rapid. Zones of anaerobic activity develop in areas of high microbial activity.
- Biodegradation in the vadose zone is also primarily aerobic. Low organic matter content results in low microbial numbers leading to significant acclimation times to allow biodegrading populations to build up.
- Biodegradation in the deep groundwater region is also initially slow because of low microbial numbers. Conditions can rapidly become anaerobic because of lack of available dissolved oxygen. Biodegradation in shallow groundwater regions is initially more rapid because of higher microbial numbers but is similarly slowed by low dissolved oxygen availability.

approximately 100:10:1 (C:N:P) is often used in such sites. However, in some instances, quite different ratios have been used. For example, Wang and Bartha (1990) found that effective remediation of hydrocarbons in soil required the addition of nitrogen and phosphorus to maintain a C:N ratio of 200:1 and a C:P ratio of 1000:1. Why were the C:N and C:P ratios maintained at levels so much higher than the cell C:N and C:P ratios? As discussed in Section 16.3.3, it is because much of the hydrocarbon that is metabolized is released as carbon dioxide, so that much of the carbon is lost from the system. In contrast, almost all of the nitrogen and phosphorus metabolized is incorporated into microbial biomass, and thus is conserved in the system.

17.5.4 Other Environmental Factors

17.5.4.1 Temperature

Hydrocarbon degradation has been reported to occur at a range of temperatures from close to freezing to more than 30°C. Bacteria can adapt to temperature extremes in order to maintain metabolic activity; however, seasonal temperature fluctuations in the natural environment have been shown to affect the rate at which degradation occurs (Palmisano *et al.*, 1991). For example, the degradation rates of hexadecane and naphthalene in a river sediment were reduced approximately 4.5-fold and 40-fold, respectively, in winter (0–4°C) compared with summer (8–21°C) samples (Wyndham and Costerton, 1981).

17.5.4.2 pH

In soils, the rate of hydrocarbon degradation is influenced by pH with the highest rates generally observed at neutral pH. However, microorganisms have been isolated from historically contaminated sites that have adapted to growth on hydrocarbons even at very acidic pH levels (pH 2–3). It has been observed that the diversity of these microorganisms is lower than for their counterparts that grow at neutral pH (Uyttebroek *et al.*, 2007).

17.5.4.3 Salinity

In typical terrestrial or freshwater ecosystems, co-contamination with moderate-to-high levels of salinity tends to slow hydrocarbon degradation (Ulrich *et al.*, 2009). In marine ecosystems, hydrocarbons are frequently introduced naturally from oil seeps and natural gas deposits as well as anthropogenically from oil tanker spills and discharges; therefore, marine environments tend to contain microbial populations adapted for degradation of hydrocarbons under the salinity levels typically found in these ecosystems (see also Chapter 31). However, elevated salinity levels may also slow degradation even in marine ecosystems (Mille *et al.*, 1991). Also, in contrast to hydrocarbon degraders found in soil which exhibit great diversity, marine hydrocarbon degraders seem to be a small group of specialized obligate hydrocarbon utilizers that have been named marine obligate hydrocarbonoclastic bacteria (OHCB). The OHCB respond rapidly to the addition of hydrocarbons, and have been shown to transiently increase up to 90% of the total microbial community in response to added hydrocarbon (Yakimov *et al.*, 2007). Recognized OHCB genera include *Alcanivorax*, *Cycloclasticus*, *Marinobacter*, *Thallassolituus* and *Oleispira*. Related bacteria were found to dominate the bacterial community within a "cloud" of dispersed oil in the Gulf of Mexico following the BP *Deepwater Horizon* oil leak in 2010 (see Case Study 31.1; Hazen *et al.*, 2010). Which genera predominate in any given environment depends on environmental conditions. For example, *Oleispira* are psychrophiles that dominate in cold waters.

17.5.4.4 Water Activity

Optimal conditions for activity of aerobic soil microorganisms occur when between 38% and 81% of the soil pore space is filled with water (also referred to as percent saturation). In this range of water content, water and oxygen availability are maximized. At higher water contents, the slow rate of oxygen diffusion through water limits oxygen replenishment, thereby limiting aerobic activity. At lower water contents, water availability becomes limiting. Why is the optimal percent saturation range so

broad? It is because optimal activity really depends upon a combination of factors including water content and available pore space. Available pore space is measured as bulk density, which is defined as the mass of soil per unit volume (g/cm^3). This means that in any given soil, increasing bulk density indicates increasing compaction of the soil. In a soil that is loosely compacted (lower bulk density), a water saturation of 70% represents more water (more filled small pores and pore throats) than in a highly compacted soil. Therefore, in a soil with low bulk density oxygen, diffusion constraints become important at lower water saturation than for highly compacted soils.

17.6 BIODEGRADATION OF ORGANIC POLLUTANTS

17.6.1 Pollutant Sources and Types

In 2011, the United States used more than 6.8 billion barrels of oil for heating, generation of electricity and transportation (USEIA, 2012). Other sources of energy are coal, natural gas and nuclear energy. Large amounts of waste, including solvents, acids, bases and metals, are also produced by the paper, transportation, electronics, defense and metals industries. The EPA estimates that the global market for pesticides in 2007 was over $39 billion of which the United States accounted for 32% of the market (Grube *et al.*, 2011). As these figures demonstrate, both industry and agriculture produce large amounts of chemicals. Inevitably, some of these find their way into the environment as a result of normal handling procedures and accidental spills.

Figure 17.11 shows various contaminant molecules that are added to the environment in significant quantities by anthropogenic activities (see Information Box 17.6). The structure of most contaminant molecules is based on one of the first three structures shown in Figure 17.11: aliphatic, alicyclic or aromatic. By combining or adding to these structures, a variety of complex molecules can be formed that have unique properties useful in industry and agriculture. The objective of this section is to become familiar with these structures and their biodegradation pathways so that given a structure, a reasonable biodegradation pathway can be predicted. An excellent resource for obtaining biodegradation information for a variety of compounds under both aerobic and anaerobic conditions is the University of Minnesota Biocatalysis/Biodegradation Database (http://umbbd.msi.umn.edu/).

17.6.2 Aliphatics

There are several common sources of aliphatic hydrocarbons that enter the environment as contaminants. These include: straight-chain and branched-chain structures found in petroleum hydrocarbons; the linear alkyl benzenesulfonate (LAS) detergents; and the one- and two-carbon halogenated compounds such as chloroform and TCE that are commonly used as industrial solvents. Some general rules for aliphatic biodegradation are presented in Information Box 17.7, and specific biodegradation pathways are summarized in the following sections for alkanes/alkenes and chlorinated aliphatics.

17.6.2.1 Alkanes

Aerobic Conditions

Because of their structural similarity to fatty acids and plant paraffins, which are ubiquitous in nature, many microorganisms in the environment can utilize *n*-alkanes (straight-chain alkanes) as a sole source of carbon and energy. In fact, it is easy to isolate alkane-degrading microbes from any environmental sample. As a result, alkanes are usually considered to be the most readily biodegradable type of hydrocarbon. Biodegradation of alkanes occurs with a high biological oxygen demand (BOD) using one of the two pathways shown in Figure 17.12. The more common pathway is the direct incorporation of one atom of oxygen onto one of the end carbons of the alkane by a monooxygenase enzyme resulting in the formation of a primary alcohol. Alternatively, a dioxygenase enzyme can incorporate both oxygen atoms into the alkane to form a hydroperoxide. The end result of both pathways is the production of a primary fatty acid. There are also examples in the literature of diterminal oxidation, with both ends of the alkane oxidized, and of subterminal oxidation, with an interior carbon oxidized (Britton, 1984).

Fatty acids are common metabolites found in all cells. They are used in the synthesis of membrane phospholipids and lipid storage materials. The common pathway used to catabolize fatty acids is known as β-oxidation, a pathway that cleaves off consecutive two-carbon fragments (Figure 17.12). Each two-carbon fragment is removed by coenzyme A as acetyl-CoA, which then enters the tricarboxylic acid (TCA) cycle for complete mineralization to CO_2 and H_2O. If you think about this process, it becomes apparent that if one starts with an alkane that has an even number of carbons, the two-carbon fragment acetyl-CoA will be the last residue. If one starts with an alkane with an odd number of carbons, the three-carbon fragment propionyl-CoA will be the last residue. Propionyl-CoA is then converted to succinyl-CoA, a four-carbon molecule that is an intermediate of the TCA cycle.

What types of alkanes do microbes most prefer? In general, midsize straight-chain aliphatics (*n*-alkanes C_{10} to C_{18} in length) are utilized more readily than *n*-alkanes with either shorter or longer chains. Long-chain *n*-alkanes are utilized more slowly because of low bioavailability

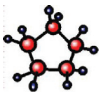
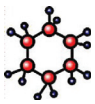
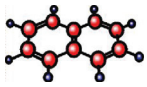
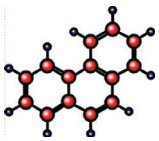
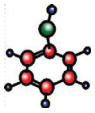
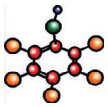
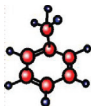
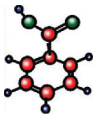
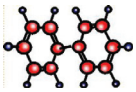
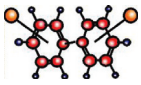
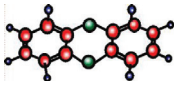
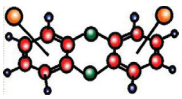
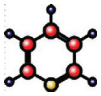
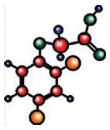
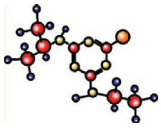
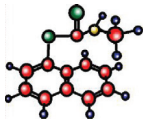
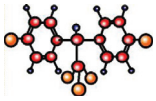
Hydrocarbon Type	Structure	Name	Physical state at room temp.	Source and uses
Aliphatics		propane n=1	gas	Petroleum contains both linear and branched aliphatics. The gasoline fraction of crude oil is 30–70% aliphatic depending on the source of the crude oil.
		hexane n=4	liquid	
		hexatriacontane n=34	solid	
Alicyclics		cyclopentane	liquid	Petroleum contains both unsubstituted and alkyl substituted alicyclics. The gasoline fraction of crude oil is 20–70% alicyclic depending on the source of the crude oil.
		cyclohexane	liquid	
Aromatics		benzene	liquid	Petroleum contains both unsubstituted and alkyl substituted aromatics. The gasoline fraction of crude oil is 10–15% depending on the source of the crude oil.
		naphthalene	solid	
		phenanthrene	solid	
Substituted aliphatics		chloroform	liquid	Anthropogenically manufactured and used as solvents and degreasing agents, and in organic syntheses.
		trichloroethene (TCE)	liquid	
Substituted aromatics		phenol	liquid	Found in coal tar or manufactured and used as a disinfectant; and in manufacture of resins, dyes and industrial chemicals.
		pentachlorophenol	liquid	Manufactured and used as an insecticide, defoliant, and wood preservative.
		toluene	liquid	Found in tar oil, used in manufacture of organics, explosives, and dyes. Also used as a solvent.
		benzoate	liquid	Found in plants and animals and manufactured for use as a food preservative and dye component, and in curing tobacco.

FIGURE 17.11 Representative pollutant structures. (*Continued*)

Biaryl hydrocarbons		biphenyl	solid	Biphenyl is the parent compound of variously chlorinated biphenyl mixtures known as the PCBs. PCBs are used as transformer oils and plasticizers.
		polychlorinated biphenyls (PCBs)	liquid	
Heterocyclics		dibenzodioxin	solid	Dioxins are created during incineration processes and are contaminants associated with the manufacture of herbicides including 2,4-D and 2,4,5-T.
		chlorinated dioxins	solid	
		pyridine	liquid	Found in coal tar. Used as a solvent and synthetic intermediate.
		thiophene	liquid	Found in coal tar, coal gas and crude oil. Used as a solvent and in manufacture of resins, dyes, and pharmaceuticals.
Pesticides Organic acids		2,4-dichlorophenoxy acetic acid	solid	Broadleaf herbicide
Organophosphates		chlorpyrifos	solid	Used as an insecticide and an acaricide
Triazenes		atrazine	solid	Selective herbicide
Carbamates		carbaryl	solid	Contact insecticide
Chlorinated hydrocarbons		1,1,1-trichloro-2,2-bis-(4-chlorophenyl)-ethane (DDT)	solid	Contact insecticide
		methyl bromide	gas	Used to degrease wool, extract oil from nuts, seeds and flowers, used as an insect and soil fumigant.

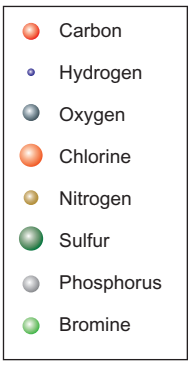


FIGURE 17.11 (*Continued*).

Information Box 17.6 The 2011 Agency for Toxic Substances Disease Registry (ATSDR) Top Twenty Pollutants

By U.S. law, the ATSDR and EPA are required to prepare a list, in order of priority, of substances that are most commonly found at facilities on the National Priorities List (NPL) and which are determined to pose the most significant potential threat to human health due to their known or suspected toxicity, and potential for human exposure at these NPL sites. This list is revised every two years as additional information becomes available.

1 Arsenic
2 Lead
3 Mercury
4 Vinyl Chloride
5 Polychlorinated Biphenyls
6 Benzene
7 Cadmium
8 Benzo(a)pyrene
9 Polycyclic Aromatic Hydrocarbons
10 Benzo(b)fluoranthene
11 Chloroform
12 Aroclor 1260
13 DDT
14 Aroclor 1254
15 Dibenzo(a,h)anthracene
16 Trichloroethene
17 Chromium, Hexavalent
18 Dieldrin
19 Phosphorus, White
20 Hexachlorobutadiene

From: the Agency for Toxic Substances & Disease Registry, 2011.

Information Box 17.7 General Rules for Degradation of Non-halogenated Aliphatic Compounds

- Aerobic conditions:
  - aliphatics are readily degraded
  - midsize straight-chain aliphatics (*n*-alkanes C_{10} to C_{18} in length) are utilized more readily than *n*-alkanes with either shorter or longer chains
  - saturated aliphatics and alkenes are degraded similarly
  - hydrocarbon branching decreases biodegradability
- Anaerobic conditions:
  - degradation is very limited for low molecular weight alkanes; higher molecular weight alkanes, e.g., hexane, are biodegraded following "activation" through the addition of fumarate
  - "activated" aliphatics including alkenes, alchohols, or acids are readily degraded

resulting from extremely low water solubilities. For example, the water solubility of decane (C_{10}) is 0.052 mg/L, and the solubility of octadecane (C_{18}) is almost 10-fold less (0.006 mg/L). Solubility continues to decrease with increasing chain length. In contrast, short-chain *n*-alkanes have higher aqueous solubility, e.g., the water solubility of butane (C_4) is 61.4 mg/L, but they are toxic to cells. Short-chain alkanes are toxic to microorganisms because their increased water solubility results in increased uptake of the alkanes, which are then dissolved in the cell membrane. The presence of these short alkanes within the cell membrane can alter the fluidity and integrity of the cell membrane.

The toxicity of short-chain *n*-alkanes can be mediated in some cases by the presence of free-phase oil droplets. Protection occurs because the short-chain alkanes partition into the oil droplets. This results in reduced bioavailability because the aqueous phase concentration is decreased. Thus, *n*-alkane degradation rates will differ depending on whether the substrate is present as a pure compound or in a mixture of compounds.

Biodegradability of aliphatics is also negatively influenced by branching in the hydrocarbon chain. The degree of resistance to biodegradation depends on both the number of branches and the positions of methyl groups in the molecule. Compounds with a quaternary carbon atom (four carbon−carbon bonds) such as that shown in Figure 17.8B are extremely stable because of steric effects, as discussed in Section 17.4.4.1.

Alkenes are hydrocarbons that contain one or more double bonds. The majority of alkene biodegradability studies have used 1-alkenes as model compounds (Britton, 1984). These studies have shown that alkenes and alkanes have comparable biodegradation rates. The initial step in 1-alkene degradation can involve attack at the terminal or a subterminal methyl group as described for alkanes. Alternatively, the initial step can be attack at the double bond, which can yield a primary or secondary alcohol or an epoxide. Each of these initial degradation products is further oxidized to a primary fatty acid, which is degraded by β-oxidation as shown in Figure 17.12 for alkanes.

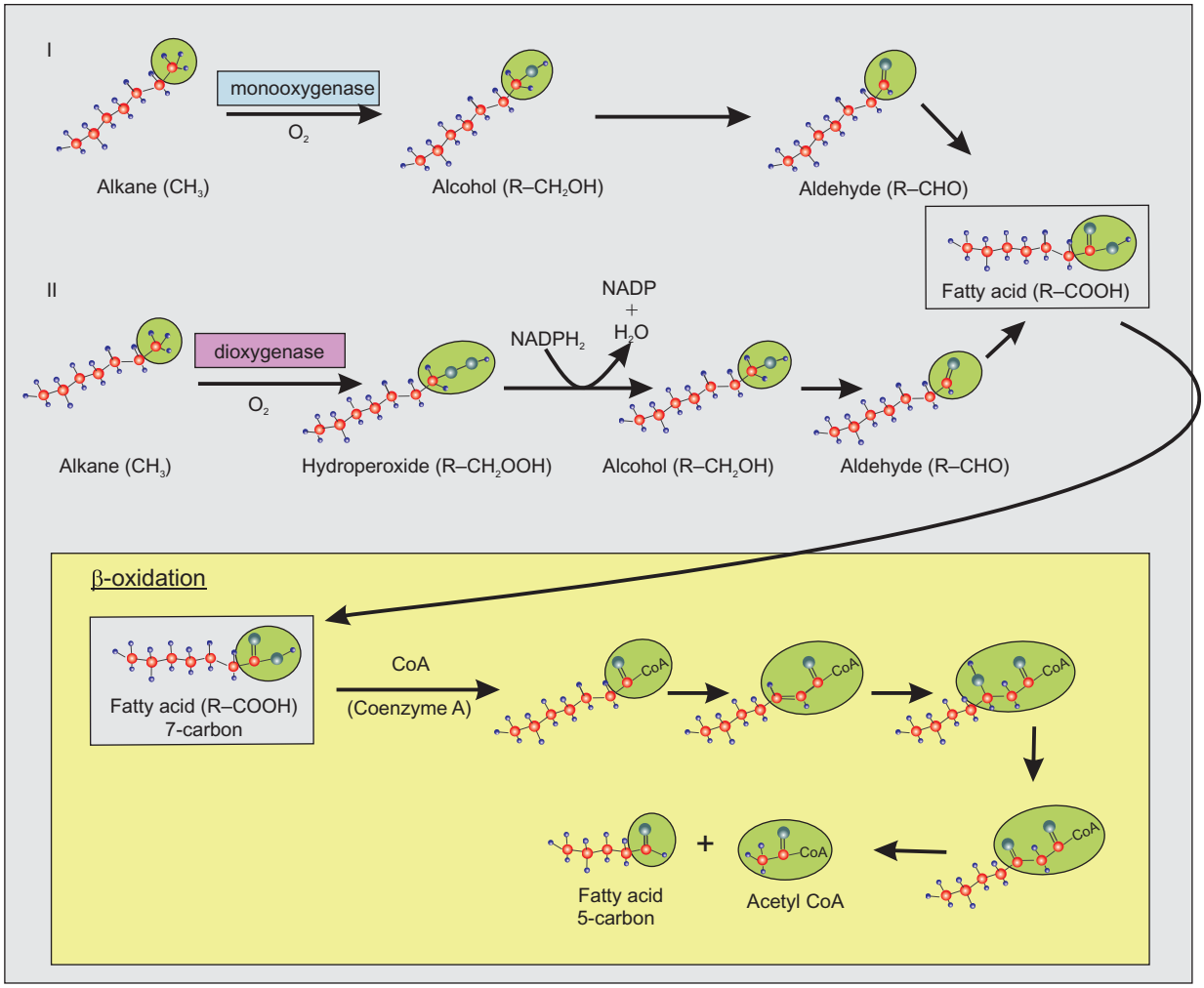


FIGURE 17.12 Aerobic biodegradation of alkanes.

Anaerobic Conditions

In comparison to aerobic conditions, aliphatic hydrocarbons, which are highly reduced molecules, are degraded slowly, if at all, anaerobically (Figure 17.13). This is supported by the fact that hydrocarbons in natural underground reservoirs of oil (which are under anaerobic conditions) are not degraded despite the presence of microorganisms. The current view is that low molecular weight alkanes (e.g., methane) do not energetically support anaerobic degradation. This is because they must be activated or functionalized prior to degradation. In contrast, higher molecular weight aliphatics have been shown to undergo degradation using a unique pathway, where the first step in biodegradation involves activation through the addition of a four-carbon oxygen-containing moiety, fumarate, into the alkane (Figure 17.14). Once oxygen has been introduced into the molecule through the addition of fumarate, it is mineralized, although the exact pathway has not been elucidated (Widdel and Rabus, 2001). Aliphatics that are already activated, including both alkenes and aliphatics containing oxygen (aliphatic alcohols and ketones), are readily biodegraded anaerobically.

17.6.2.2 Halogenated Aliphatics

Chlorinated solvents such as trichloroethene (TCE, $Cl_2{=}CHCl$) and perchloroethene (PCE, $Cl_2C{=}CCl_2$) have been extensively used as industrial solvents. As a result of improper use and disposal, these solvents are among the most frequently detected types of organic contaminants in groundwater. The need for efficient and cost-effective remediation of solvent-contaminated sites has stimulated interest in the biodegradation of these C_1 and C_2 halogenated aliphatics. General rules for biodegradation of chlorinated aliphatics are provided in Information Box 17.8.

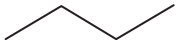
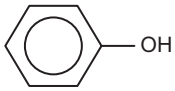
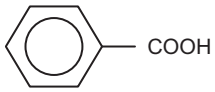
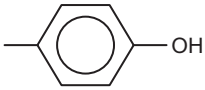
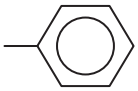
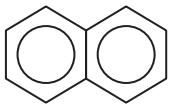
Substrate	Structure	Aerobic Biodegradation	Anaerobic Biodegradation
Lower MW alkanes		+++	−/+, only denitrifying conditions
Higher MW alkanes		+++	+/−, only in historically petroleum contaminated sites
Alkenes		+++	+/−, only in historically petroleum contaminated sites
Phenols		+++	++
Aromatic Acids		+++	++
Alkylphenols		+++	++
BTEX (benzene, toluene, xylenes)		+++	+/−, only in historically petroleum contaminated sites
PAH (polyaromatic hydrocarbons) up to three rings		+++	+/−, only in historically petroleum contaminated sites
Lignin/Humus		++	

FIGURE 17.13 A comparison of biodegradability of aliphatic and aromatic hydrocarbons under aerobic and anaerobic conditions. A (−) means no biodegradation; (+) means increasing ease of biodegradation. An increasing number of (+) means increasing ease of biodegradation. Courtesy James A. Field.

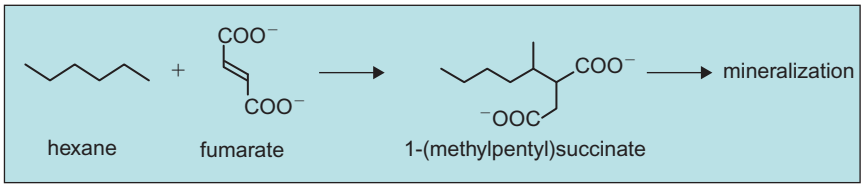


FIGURE 17.14 Anaerobic biodegradation of alkanes.

Aerobic Conditions

Under aerobic conditions, halogenated aliphatics are generally degraded more slowly than aliphatics without halogen substitution. For example, although 1-chloroalkanes ranging from C_1 to C_{12} are degraded as a sole source of carbon and energy in pure culture, they are degraded more slowly than their nonchlorinated counterparts. The presence of two or three chlorines bound to the same carbon atom inhibits aerobic degradation (Janssen *et al.*, 1990). For example, while TCE is degraded under aerobic conditions, PCE is not. These results can be explained by the decreasing electronic effects of the chlorine atom on the enzyme−carbon reaction center as the alkane chain length increases (see Section 17.4.4.2).

Biodegradation of halogenated aliphatics occurs by one of two basic types of reactions (Figure 17.15). Substitution

Information Box 17.8 General Rules for Degradation of Chlorinated Aliphatics

- Aerobic conditions
  - aliphatics with one or two chlorines are generally mineralized
  - as the number of halogens increases, biodegradation rates decrease and reactions tend to become cometabolic
  - highly chlorinated aliphatics, e.g. PCE, are recalcitrant
- Anaerobic conditions
  - most chlorinated aliphatics undergo partial transformation, not mineralization
  - aliphatics with two or more chlorines are transformed either through cometabolism (slow) or halorespiration (rapid)

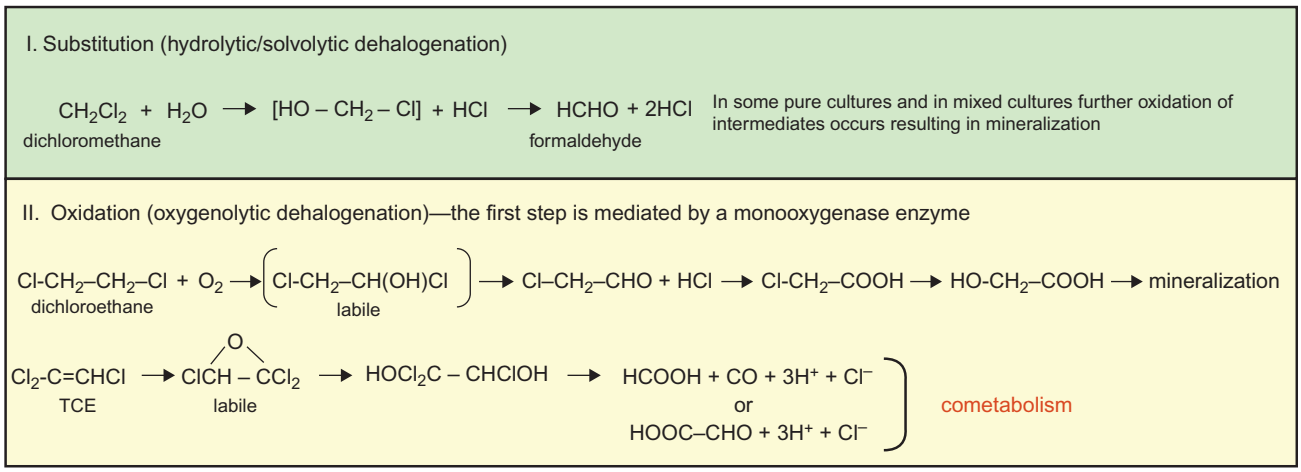


FIGURE 17.15 Aerobic degradation pathways for chlorinated aliphatics via (I) substitution or (II) oxidation.

is a nucleophilic reaction (the reacting species brings an electron pair) in which the halogens on a mono- or dihalogenated compound are substituted by a hydroxy group. Oxidation reactions are catalyzed by a select group of monooxygenase and dioxygenase enzymes that have been reported to oxidize highly chlorinated C_1 and C_2 compounds (e.g., TCE). These monooxygenase and dioxygenase enzymes are produced by bacteria and oxidize a variety of nonchlorinated compounds including methane, ammonia, toluene and propane. These enzymes do not have exact substrate specificity, and thus they can also participate in either the metabolic or the cometabolic degradation of chlorinated aliphatics (Bhatt *et al.*, 2007). Figure 17.15 shows an example of a metabolic oxidation that supports growth (dichloroethane) and an example of a cometabolic oxidation (TCE). For cometabolic reactions, usually, a large ratio of enzyme substrate to chlorinated aliphatic is required to achieve cometabolic degradation of the chlorinated aliphatic (Section 17.3, Figure 17.3).

Anaerobic Conditions

In some very limited instances, C_1 chlorinated aliphatics such as chloromethane and dichloromethane can serve as a source of carbon and energy to support growth; however, chlorinated aliphatics are generally metabolized under anaerobic conditions primarily through two processes: (1) cometabolism; and (2) use as a terminal electron acceptor to support growth, a process called halorespiration. Both of these processes usually result in partial transformation of the substrate rather than complete mineralization. In general, the process of removing chlorines under anaerobic conditions is referred to as reductive dehalogenation. Reductive dehalogenation can be mediated by reduced transition metal complexes found in coenzymes such as vitamin B12 or in enzymes that can participate in dehalogenation. As shown in Figure 17.16, in the first step, electrons are transferred from the reduced metal to the halogenated aliphatic, resulting in an alkyl radical and free halogen. Then the alkyl radical can either scavenge a hydrogen atom (1), or lose a second halogen to form an alkene (2). Conversely, a small group of bacteria, specifically *Dehalococcoides* spp. including *D. ethenogenes* strain 195, can use even highly chlorinated compounds such as PCE and TCE directly as terminal electron acceptors and sequentially dechlorinate them completely to form ethene (Fennel *et al.*, 2004; Figure 17.2).

It is now clearly recognized that reductive dehalogenation is a very important process in contaminated environments for highly chlorinated aliphatics and chlorinated

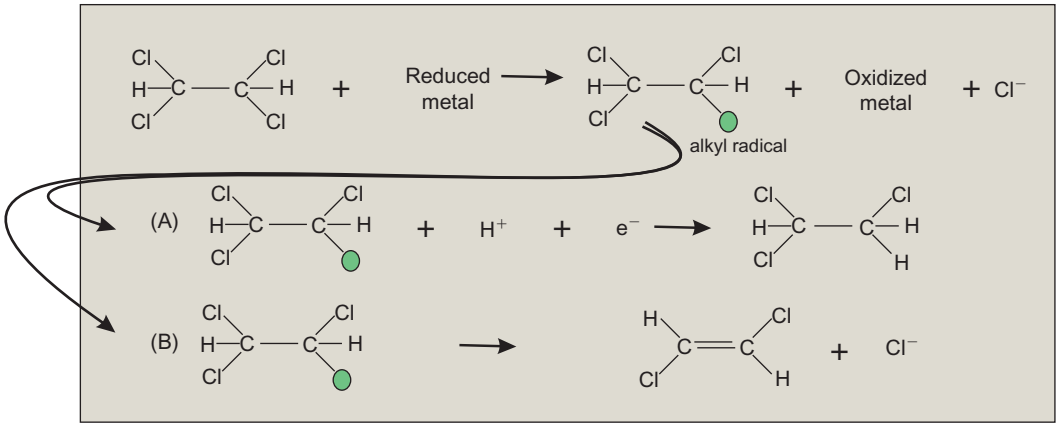


FIGURE 17.16 Reductive dehalogenation of tetrachloroethane to trichloroethane (A) or dichloroethene (B).

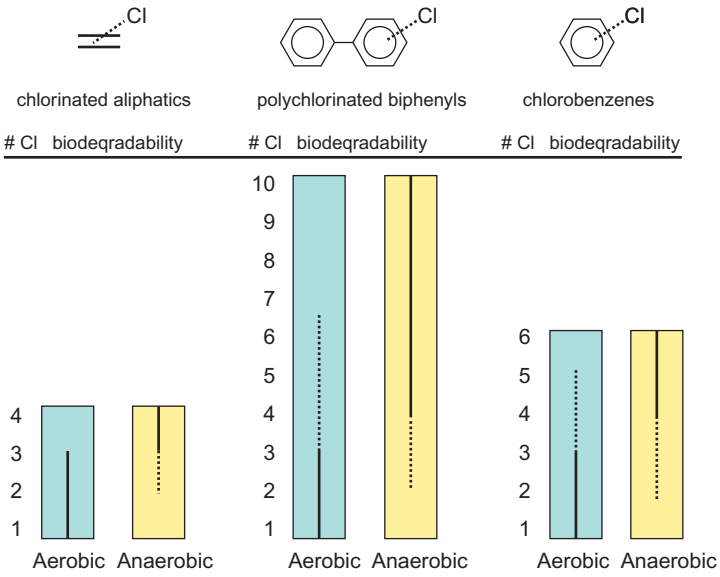


FIGURE 17.17 The effect of increasing numbers of chlorines on biodegradability under aerobic and anaerobic conditions. Solid lines indicate ready biodegradation, dashed lines indicate biodegradation has been observed on some occasions, no line indicates no biodegradation. Courtesy James A. Field.

compounds in general. This is because anaerobic conditions favor the degradation of highly chlorinated compounds, whereas aerobic conditions favor the degradation of mono- and di-substituted halogenated compounds (Figure 17.17). Recall that TCE and PCE are among the most common groundwater contaminants. Under aerobic conditions, PCE is inert, and while TCE can be cometabolized aerobically, this process requires optimization of the electron donor to TCE ratio. However, under anaerobic conditions, halorespiration of both TCE and PCE can occur quite rapidly resulting in lesser chlorinated metabolites that become amenable for aerobic biodegradation. If appropriate populations of microorganisms (e.g., *Dehalococcoides* spp.) are present, TCE and PCE can even be completely dechlorinated to ethene through use of the chlorinated organic as a terminal electron acceptor during metabolism of a corresponding electron donor (e.g., H_2 or a C_1 or C_2 organic compound such as ethanol). Interestingly, the chlorinated, inorganic compound perchlorate (ClO_4^-) can also be degraded by a similar mechanism (Information Box 17.9).

17.6.3 Alicyclics

Alicyclic hydrocarbons (Figure 17.11) are major components of crude oil, 20 to 70% by volume. They are commonly found elsewhere in nature as components of plant oils and paraffins, microbial lipids and pesticides (Trudgill, 1984). The various components can be simple, such as cyclopentane and cyclohexane, or complex, such as trimethylcyclopentane and various cycloparaffins. The use of alicyclic compounds in the chemical industry, and the release of alicyclics to the environment through industrial processes, other than oil processing and utilization, is more limited than for aliphatics and aromatics. Consequently, the issue of health risks associated with human exposure to

Information Box 17.9 Microbial Degradation of Perchlorate—Implications for Microbial Life on Mars?

Perchlorate is a contaminant of emerging concern—especially in groundwater. It exists as an anion consisting of a chlorine atom surrounded by four oxygen atoms (ClO_4^-), and has been used in a variety of products including rocket fuel and fireworks. It has now been detected as a contaminant in water supplies in over 20 U.S. States (Information Box 17.1). Since perchlorate can interfere with thyroid activity in humans, the USEPA has decided to regulate perchlorate under the Safe Drinking Water Act, and is developing a proposed national primary drinking water regulation for perchlorate. Although perchlorate is an inorganic compound, its degradation pathway shares some similarities with the degradation of the chlorinated solvents TCE and PCE. For example, a wide array of bacteria are now known to be capable of using perchlorate as a terminal electron acceptor, under anaerobic conditions, during the metabolism of electron donors including hydrogen, organic acids, alcohols and reduced iron, and ultimately converting the perchlorate into chloride (Coates and Achenbach, 2004). The knowledge of these microbial transformations of perchlorate along with the discovery of high levels of perchlorate on Mars have recently led some to speculate about the potential for the perchlorate to enable the existence of microbial life on Mars (McKay *et al.*, 2013). Obviously, one way that perchlorate could potentially support microbial life on Mars is by serving as an electron acceptor during the microbial reduction of ferrous iron or other electron donors. In addition, perchlorate could indirectly support microbial life through its ability to depress the freezing-point of water thus extending the temperature range under which life may be possible.

Information Box 17.10 General Rules for Degradation of Alicyclics

- Aerobic conditions
  - alicyclics are readily degradable
  - unsubstituted alicyclics typically form a lactone intermediate
  - a consortium is usually required for mineralization of unsubstituted alicyclics
- Anaerobic conditions
  - alicyclics are readily degradable under sulfate-reducing conditions
  - degradation is more limited under methanogenic conditions

alicyclics has not reached the same level of importance as for the other classes of compounds, especially the aromatics. As a result, far less research has focused on the study of alicyclic biodegradation (see Information Box 17.10).

Aerobic Conditions

It is difficult to isolate pure cultures that degrade alicyclic hydrocarbons using enrichment techniques. Although microorganisms with complete degradation pathways have been isolated (Trower *et al.*, 1985), alicyclic hydrocarbon degradation is thought to occur primarily by commensalistic and cometabolic reactions as shown for cyclohexane in Figure 17.18. In this series of reactions, one organism converts cyclohexane to cyclohexanol (step 1) cometabolically during the oxidation of propane, but is unable to further transform the compound. A second organism that is unable to oxidize cyclohexane to cyclohexanol can perform the subsequent transformations (step 2 onward) including lactonization, ring opening and mineralization of the remaining aliphatic compound (Perry, 1984).

Cyclopentane and cyclohexane derivatives that contain one or two OH, C=O or COOH groups are readily metabolized, and such degraders are easily isolated from environmental samples. In contrast, degradation of alicyclic derivatives containing one or more CH_3 groups is inhibited. This is reflected in the decreasing rate of biodegradation for the following series of alkyl derivatives of cyclohexanol: cyclohexanol > methylcyclohexanol > dimethylcyclohexanol (Pitter and Chudoba, 1990).

Anaerobic Conditions

Anaerobic biodegradation of complex mixtures of alicyclic compounds in gas condensate has been demonstrated under methanogenic and sulfate-reducing conditions. Gas condensate is the mixture of hydrocarbons in raw natural gas. It is a mixture containing primarily aliphatics (C_2–C_{12}), cyclopentanes, cyclohexanes and aromatics (BTEX = benzene, toluene, ethylbenzene, xylene). In examining the fate of the alicyclic components of gas condensate, sulfate-reducing conditions were found to support the anaerobic biodegradation of unsubstituted cyclopentanes and cyclohexanes as well as those with one methyl or ethyl substitution. In contrast, biodegradation under methanogenic conditions was much less extensive (Townsend *et al.*, 2004). Some alicyclics showed recalcitrance under both methanogenic and sulfate-reducing conditions including dimethyl-substituted cyclopentanes and cyclohexanes.

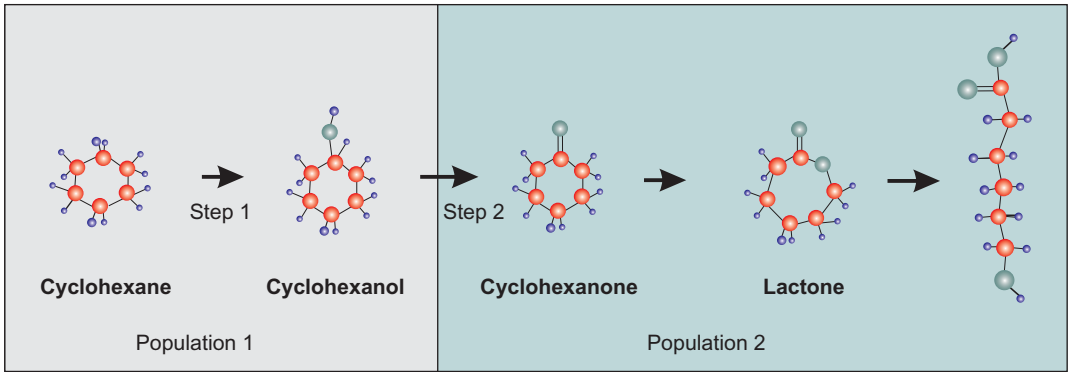


FIGURE 17.18 Degradation of cyclohexane.

17.6.4 Aromatics

Aromatic compounds contain at least one unsaturated ring system with the general structure C_6R_6, where R is any functional group (Figure 17.11). Benzene (C_6H_6) is the parent hydrocarbon of this family of unsaturated cyclic compounds. Compounds containing two or more fused benzene rings are called polyaromatic hydrocarbons (PAHs; also known as polycyclic aromatic hydrocarbons). Aromatic hydrocarbons are natural products; they are part of lignin and are formed as organic materials are burned, for example, in forest fires. However, the addition of aromatic compounds to the environment has increased dramatically through activities such as fossil fuel processing, and utilization and burning of wood and coal.

The quantity and composition of the aromatic hydrocarbons are of major concern when evaluating a contaminated site because several components of the aromatic fraction have been shown to be carcinogenic to humans. Aromatic compounds also have demonstrated toxic effects toward microorganisms.

17.6.4.1 Unsubstituted Aromatics

Aerobic Conditions

A wide variety of bacteria and fungi can carry out aromatic transformations, both partial and complete, under a variety of environmental conditions (see Information Box 17.11; Johnsen *et al.*, 2005). Under aerobic conditions, the most common initial transformation is a hydroxylation that involves the incorporation of molecular oxygen. The enzymes involved in these initial transformations are either monooxygenases or dioxygenases. In general, prokaryotic microorganisms transform aromatics by an initial dioxygenase attack to *cis*-dihydrodiols. The *cis*-dihydrodiol is rearomatized to form a dihydroxylated intermediate, catechol. The catechol ring is cleaved by a second dioxygenase either between the two hydroxyl groups (ortho pathway) or next to one of the hydroxyl groups (meta pathway) and further degraded to completion (Figure 17.19).

Most eukaryotic microorganisms do not mineralize aromatics; rather, they are processed for detoxification and excretion. This is done by an initial oxidation with a cytochrome P-450 monooxygenase, which incorporates one atom of molecular oxygen into the aromatic compound, and reduces the second to water, resulting in the formation of an arene oxide. This is followed by the enzymatic addition of water to yield a *trans*-dihydrodiol (Figure 17.20). Alternatively, the arene oxide can be isomerized to form phenols, which can be conjugated with sulfate, glucuronic acid and glutathione. These conjugates are similar to those formed in higher organisms, which are used in the elimination of aromatic compounds.

A small group of eukaryotes, the lignolytic fungi, can completely mineralize aromatic compounds in a process known as lignolytic degradation. The lignin structure is based on two aromatic amino acids, tyrosine and phenylalanine. In order to degrade an amorphous aromatic-based structure such as lignin, the white rot fungi release nonspecific extracellular enzymes such as laccase or H_2O_2-dependent lignin peroxidase. These enzymes generate oxygen-based free radicals that react with the lignin polymer to release residues that are taken up by the cell and degraded. Since the lignin structure is based on an aromatic structure and the initial enzymes used to degrade lignin are nonspecific, the white rot fungi are able to use the same activity to degrade a variety of aromatic contaminants. The most famous of the white rot fungi is *Phanerochaete chrysosporium*, which has been demonstrated to degrade a variety of aromatic compounds (see Information Box 2.6)

Often the capacity for aromatic degradation is plasmid mediated (Ghosal *et al.*, 1985). Plasmids can carry both individual genes and operons encoding partial or complete biodegradation of an aromatic compound. An example of the latter is the NAH7 plasmid, which codes for the entire naphthalene degradation pathway. The NAH7 plasmid was obtained from *Pseudomonas putida*, and contains genes that encode the enzymes for the first 11 steps of naphthalene oxidation. This plasmid or closely related plasmids are frequently found in sites that are contaminated with

Information Box 17.11 General Rules for Degradation of Aromatics

- Aerobic conditions
  - aromatics are degraded by a wide variety of bacteria, fungi, and algae
  - bacteria use an initial dioxygenase attack to form cis-dihydrodiols and then a catechol intermediate. In this case the aromatic is mineralized and used as a source of carbon and energy
  - eukaryotes use an initial monooxygenase attack to form trans-dihydrodiols, via an arene oxide. In this case the aromatic is being processed for detoxification and excretion. Alternatively, some fungi use lignolytic enzymes and have been shown to mineralize aromatics but not as a sole source of carbon and energy
  - low MW aromatics are degraded much more rapidly than high MW aromatics. Aromatics > three rings are resistant to biodegradation and may not serve as a sole source of carbon and energy
  - microbial adaptation occurs from chronic aromatic exposure
  - many of the genes involved in aromatic, especially PAH, degradation are plasmid encoded.
- Anaerobic conditions
  - mineralization generally requires a consortium
  - benzoyl-CoA is the common degradation intermediate

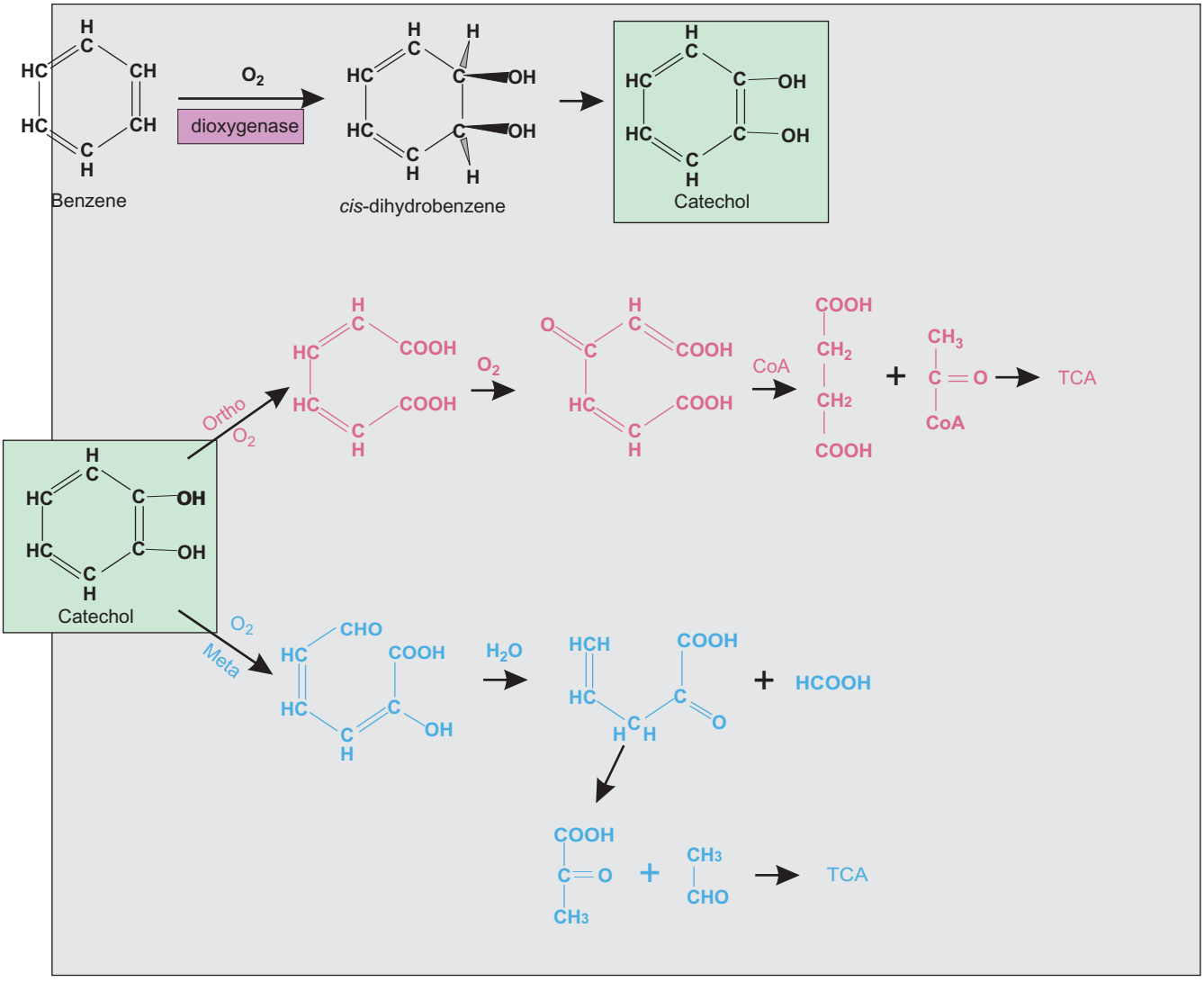


FIGURE 17.19 Incorporation of oxygen into the aromatic ring by the dioxygenase enzyme, followed by meta or ortho ring cleavage.

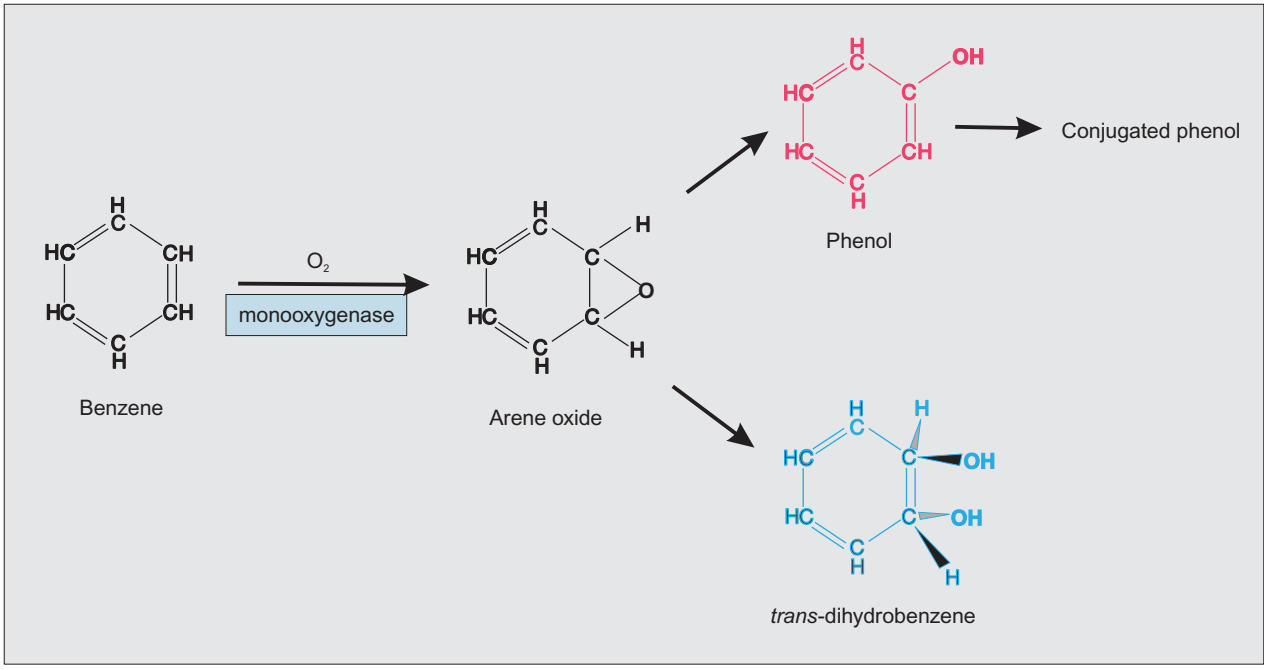


FIGURE 17.20 Fungal monooxygenase incorporation of oxygen into the aromatic ring.

PAHs (Ahn *et al.*, 1999). This plasmid has also been used to construct a luminescent bioreporter gene system. Here the lux genes that cause luminescence have been inserted into the *nah* operon in the NAH plasmid. When the *nah* operon is induced by the presence of naphthalene, both naphthalene-degrading genes and the lux gene are expressed. As a result, naphthalene is degraded and the reporter organism luminesces. Such reporter organisms are currently being used as sensors to study the temporal and spatial activity of the reporter in soil systems.

In general, aromatics composed of one, two or three condensed rings are transformed rapidly and often completely mineralized, whereas aromatics containing four or more condensed rings are transformed much more slowly, often as a result of a cometabolic attack. This is due to the limited bioavailability of these high-molecular-weight aromatics. Such PAHs have very limited aqueous solubility, and sorb strongly to particle surfaces in soil and sediments. However, it has been demonstrated that chronic exposure to aromatic compounds will result in increased transformation rates because of adaptation of an indigenous population to growth on aromatic compounds.

Anaerobic Conditions

Like aliphatic hydrocarbons, aromatic compounds can be completely degraded under anaerobic conditions. Anaerobic mineralization of aromatics produces benzoyl CoA as the common degradation intermediate (Figure 17.21). If the aromatic is oxygenated such as for benzoate, biodegradation occurs rapidly and even at rates comparable to aerobic conditions (Figure 17.13). However, under anaerobic conditions, a mixed microbial community works together even though each of the microbial components requires a different redox potential. For example, mineralization of benzoate can be achieved by growing an anaerobic benzoate degrader in co-culture with a methanogen and sulfate reducer. The initial transformations in such a system are often carried out fermentatively, and this results in the formation of aromatic acids, which in turn are transformed to methanogenic precursors such as acetate, carbon dioxide and formate. These small molecules can then be utilized by methanogens (Figure 17.22). Such a mixed community is called a consortium. It is not known how this consortium solves the problem of requiring different redox potentials in the same vicinity in a soil system. Clearly, higher redox potentials are required for degradation of the more complex substrates such as benzoate, leaving smaller organic acid or alcohol molecules that are degraded at lower redox potentials. To ultimately achieve degradation may require that the organic acids and alcohols formed at higher redox potential be transported by diffusion or by movement with water (advection), to a region of lower redox potential. On the other hand, it may be that biofilms form on the soil surface, and that redox gradients are formed within the biofilm allowing complete degradation to take place.

17.6.4.2 Substituted Aromatics

One group of aromatics of special interest is the chlorinated aromatics. These compounds have been used

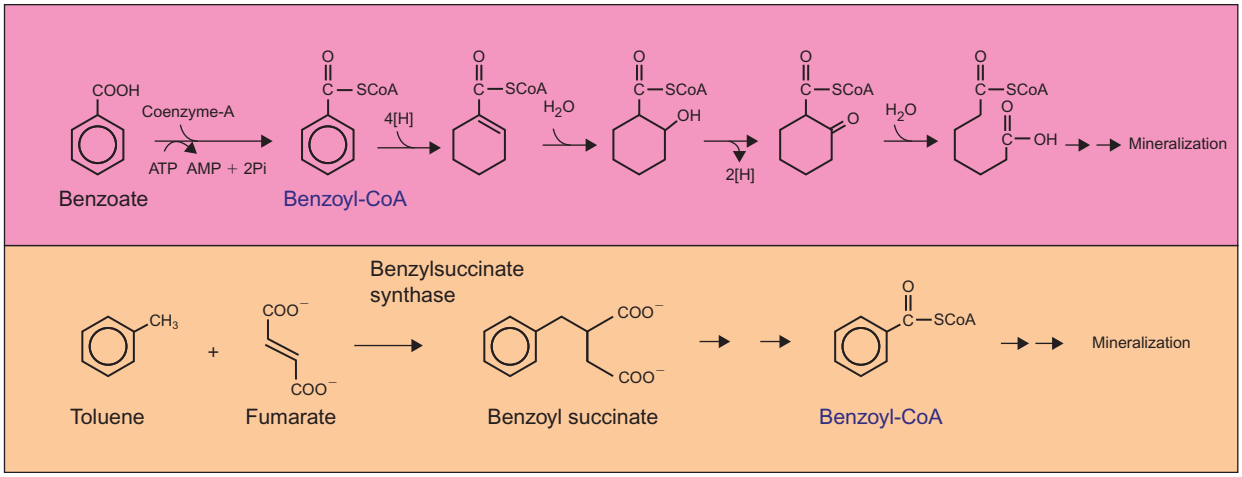


FIGURE 17.21 Anaerobic biodegradation of benzoate and toluene showing the common benzoyl-CoA intermediate.

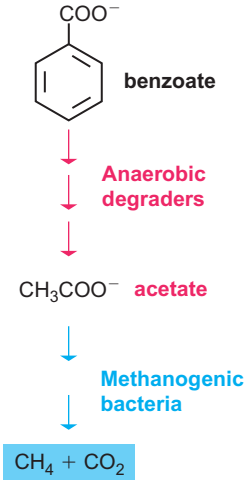


FIGURE 17.22 Anaerobic biodegradation of aromatic compounds by a consortium of anaerobic bacteria. From Pepper *et al.* (2006).

extensively as solvents and fumigants (e.g., dichlorobenzene), and wood preservatives (e.g., pentachlorophenol (PCP)), and are parent compounds for pesticides such as 2,4-dichlorophenoxyacetic acid (2,4-D) and DDT. The difficulty for aerobic microbes in the degradation of chlorinated aromatics is that the common intermediate in aromatic degradation is catechol (see Figure 17.19). Catechol formation requires two adjacent unsubstituted carbons so that hydroxyl groups can be added. Chlorine substituents can block these sites. Some bacteria solve this problem by removing a chlorine using a dehalogenase or monooxygenase enzyme.

Chlorinated phenols are particularly toxic to microorganisms. In fact, phenol itself is very toxic and is used as a disinfectant. Chlorination adds to toxicity, which increases with the degree of chlorination. For example, van Beelen and Fleuren-Kemilä (1993) quantified the effect of PCP and several other pollutants on the ability of soil microorganisms to mineralize [^{14}C]acetate in soil. The amount of PCP required to reduce the initial rate of acetate mineralization by 10% ranged between 0.3 and 50 mg/kg dry soil, depending on the soil type. High concentrations of PCP also have inhibitory effects on PCP-degrading microorganisms. For example, Alleman *et al.* (1992) investigated the effect of PCP on six species of PCP-degrading fungi. They showed that increasing the PCP concentration from 5 to 40 mg/L decreased fungal growth, and decreased the ability of the fungi to degrade PCP.

Methylated aromatic derivatives, such as toluene, constitute another common group of substituted aromatics. These are major components of gasoline and are commonly used as solvents. These compounds can initially be attacked either on the methyl group or directly on the ring as shown in Figure 17.23. This can be compared to anaerobic degradation of toluene shown in Figure 17.21. Alkyl derivatives of aromatics are attacked first at the alkyl chain, which is shortened by β-oxidation to the corresponding benzoic acid or phenylacetic acid, depending on the number of carbon atoms. This is followed by ring hydroxylation and cleavage.

17.6.5 Dioxins and PCBs

Dioxins and dibenzofurans are created during waste incineration and are part of the released smoke stack effluent. Once thought to be one of the most potent carcinogens known, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is associated with the manufacture of 2,4-D and 2,4,5-trichlorophenoxy acetic acid (2,4,5-T), hexachlorophene and other pesticides that have 2,4,5-T as a precursor. Current thinking is that TCDD is less dangerous in terms of carcinogenicity and teratogenicity than once thought, but that noncancer risks including diabetes, reduced IQ

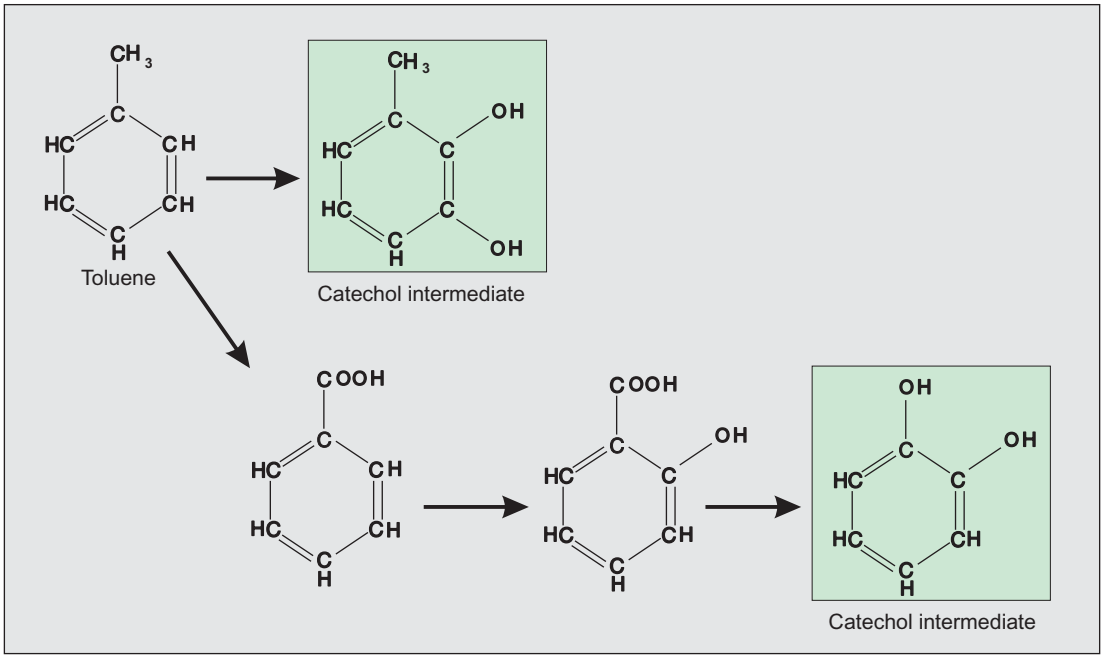


FIGURE 17.23 Aerobic biodegradation of toluene.

and behavioral impacts may be more important. The structure of TCDD (Figure 17.11) and its low water solubility, 0.002 mg/L, result in great stability of this molecule in the environment.

Although bacterial and fungal biodegradation of TCDD has been demonstrated, the extent of biodegradation is very minimal. For example, a mixture of six bacterial strains isolated from TCDD-contaminated soil obtained from Seveso, Italy, was able to produce a metabolite presumed to be 1-hydroxy-TCDD (Phillippi *et al.*, 1982). However, less than 1% of the original TCDD was degraded in 12 weeks. Other work has focused on the anaerobic reductive dechlorination of TCDD and more highly chlorinated isomers (Kao *et al.*, 2001).

Biphenyl is the unchlorinated analogue or parent compound of the polychlorinated biphenyls (PCBs), which were first described in 1881 (Waid, 1986). The PCBs consist of different chlorine-substituted biphenyls of which, in theory, there are 209 possible isomers. Only approximately 100 actually exist in commercial formulations. The aqueous solubility of biphenyl is 7.5 mg/L, and any chlorine substituent decreases the water solubility. In general, the water solubility of monochlorobiphenyls ranges from 1 to 6 mg/L, compared to 0.08 to 5 mg/L for dichlorobiphenyls. In contrast, for hexachlorobiphenyl, the aqueous solubility is just 0.00095 mg/L.

By 1930, because of their unusual stability, PCBs were widely used as nonflammable heat-resistant oils in heat transfer systems, as hydraulic fluids and lubricants, as transformer fluids, in capacitors, as plasticizers in food packaging materials, and as petroleum additives. PCBs were used as mixtures of variously chlorinated isomers and marketed under various trade names, e.g., Aroclor (U.S.A.), Clophen (Germany), Phenoclor (Italy), Kanechlor (Japan), Pyralene (France), and Soval (Russia). The U.S. domestic sales of Aroclors 1221−1268 (where the last two numbers indicated the percent chlorination) went from 32 million pounds in 1957 to 80 million pounds in 1970, with the most popular blend being 1242. PCB use in transformers and capacitors accounted for about 50% of all Aroclors used.

Past use of PCBs has resulted in their accumulation in the environment from waste dumps and spills and as a result of PCB manufacturing processes (see Case Study 17.1). Although some PCB degradation occurs, it is limited by low bioavailability, by the recalcitrance of highly chlorinated PCB congeners under aerobic conditions, and by incomplete degradation under anaerobic conditions. The extensive research that has been performed to understand PCB degradation has suggested several strategies for promoting biodegradation. Of these, the most promising is the use of a sequential anaerobic−aerobic process first to allow removal of chlorines using halorespiration, and then allow mineralization of the less chlorinated congeners.

17.6.6 Heterocyclic Compounds

Heterocyclics are cyclic compounds containing one or more heteroatoms (nitrogen, sulfur or oxygen) in addition to carbon atoms. The dioxins already discussed as well as other compounds shown in Figure 17.11 fall into this

Case Study 17.1 Polychlorinated Biphenyls

A rice oil factory accident in Japan in 1968 brought PCBs international attention. In the factory, the heat exchanger pipes used to process rice oil contained PCBs as the heat exchange fluid. Unnoticed, a heat exchange pipe broke and leaked PCBs into a batch of rice oil, which was then packaged and consumed by the local population. The contaminated rice oil poisoned over 1000 people, producing a spectrum of symptoms including chloracne, gum and nail bed discoloration, joint swelling, emission of waxy secretions from eyelid glands, and lethargy. As a result, the U.S. Food and Drug Administration (FDA) issued tolerance levels for PCBs in food and packaging products, and the Environmental Protection Agency (EPA) under TOSCA, issued rules governing the use of PCBs. This has drastically reduced domestic production and use of PCBs. Despite decreased use, PCBs still pose an environmental problem because they are not only chemically stable, they also resist biodegradation. Because past use of PCBs has been high, PCBs have accumulated in the environment.

category. In general, heterocyclic compounds are more difficult to degrade than analogous aromatics that contain only carbon. This is probably due to the higher electronegativity of the nitrogen and oxygen atoms compared with the carbon atom, leading to deactivation of the molecule toward electrophilic substitution. Heterocyclic compounds with five-membered rings and one heteroatom are readily biodegradable, probably because five-membered ring compounds exhibit higher reactivity toward electrophilic agents, and hence are more readily biologically hydroxylated. The susceptibility of heterocyclic compounds to biodegradation decreases with increasing number of heteroatoms in the molecules.

17.6.7 Pesticides

Pesticides are the biggest nonpoint source of chemicals added to the environment. The majority of the currently used organic pesticides are subject to extensive mineralization within the time of one growing season or less. Synthetic pesticides show a bewildering variety of chemical structures, but most can be traced to relatively simple aliphatic, alicyclic and aromatic base structures already discussed. These base structures bear a variety of halogen, amino, nitro, hydroxyl, carboxyl and phosphorus substituents. For example, the chlorophenoxyacetates, such as 2,4-D and 2,4,5-T, have been released into the environment as herbicides over the past 50 years. Both of these structures are biodegradable and aerobic pathways are presented in Figure 17.24.

As an exercise, examine the pesticide structures presented in Figure 17.25. For each set of pesticides, predict which is more easily degraded under aerobic conditions. You are correct if you predicted 2,4-D for the first set. It is rapidly degraded by soil microorganisms and although 2,4,5-T is also degraded, the degradation is much slower. For the second set of pesticides, propham is more degradable. In propachlor, the extensive branching so close to the ring structure blocks biodegradation. In the third set, carbaryl is more degradable because of the extensive chlorination and complex ring structures of aldrin. In fact, the estimated half-life of carbaryl in soil is 30 days, compared with 1.6 years for aldrin. Half-life is a term used to express the time it takes for 50% of the compound to be degraded. Generally, five half-lives are believed sufficient for the compound to be completely degraded. Finally, in the fourth set, methoxychlor is more degradable than DDT. In this case, the half-lives are even longer, 1 year for methoxychlor and 15.6 years for DDT.

17.7 BIOREMEDIATION

The objective of bioremediation is to exploit naturally occurring biodegradative processes to clean up contaminated sites (NRC, 1993). There are several types of bioremediation. *In situ* bioremediation is the in-place treatment of a contaminated site. *Ex situ* bioremediation may be implemented to treat contaminated soil or water that is removed from a contaminated site. Biostimulation, which is the modification of environmental conditions (e.g., addition of oxygen, nitrogen) to enhance the biodegradation activity of indigenous microorganisms, is often used to increase the speed and effectiveness of bioremediation. In contrast, intrinsic bioremediation or natural attenuation is the indigenous level of contaminant biodegradation that occurs without any stimulation or treatment. All of these types of bioremediation continue to receive increasing attention as viable remediation alternatives for several reasons. These include generally good public acceptance and support, good success rates for some applications and a comparatively low cost of bioremediation when it is successful. As with any technology, there are also drawbacks. Success can be unpredictable because a biological system is being used. A second consideration is that bioremediation rarely restores an environment completely. Often the residual contamination left after treatment is strongly sorbed and not available to microorganisms for degradation. Over a long period of time (years), these residuals

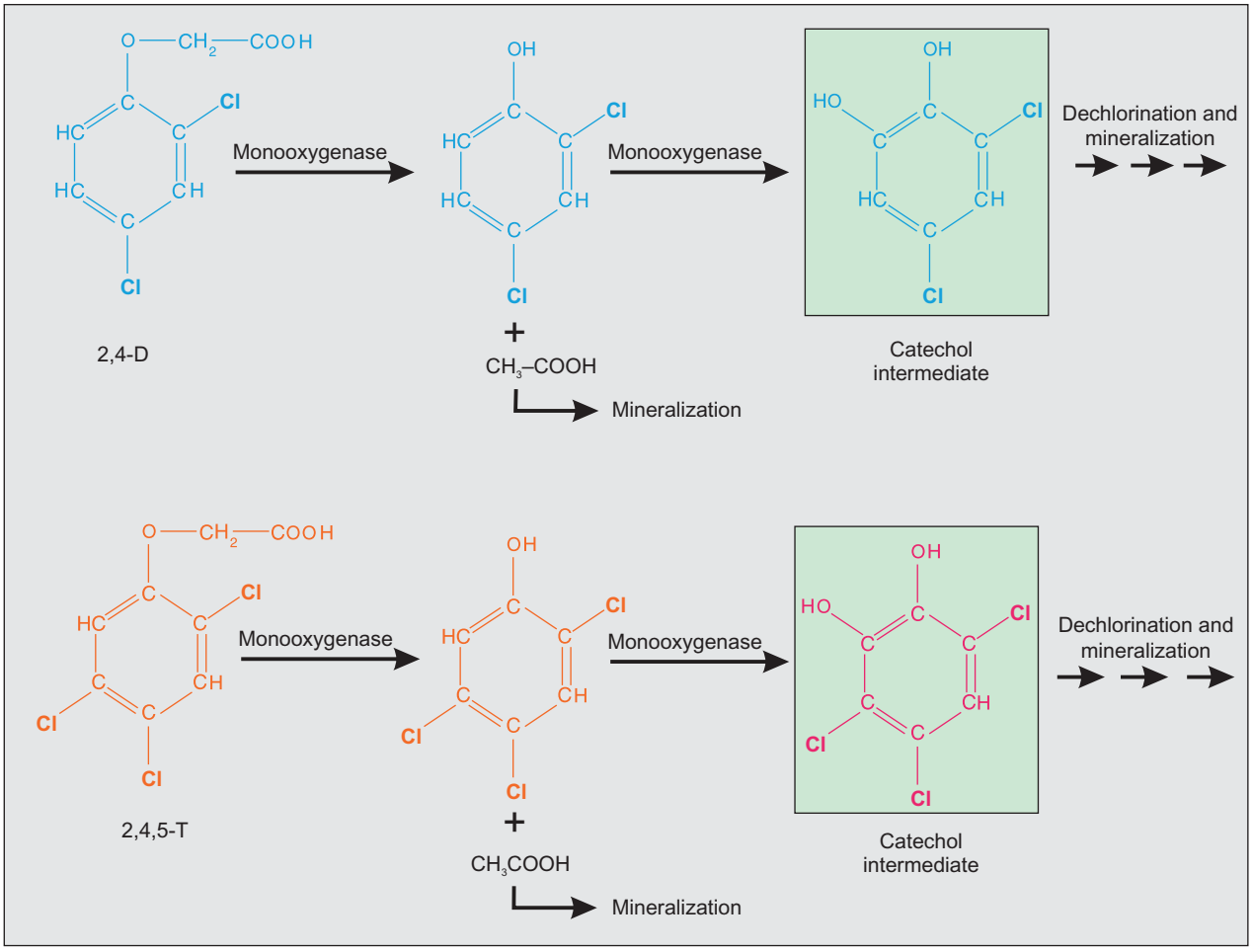


FIGURE 17.24 Aerobic biodegradation of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T).

can be slowly released. There is little research concerning the fate and potential toxicity of such released residuals, and therefore there is both public and regulatory concern about the importance of residual contamination.

Although it is often not thought of as bioremediation, domestic sewage waste has been treated biologically for many years with good success. Interestingly, even sewage treatment is undergoing reexamination in the light of detection of trace levels of endocrine disrupting compounds (EDCs) in treated wastewater. These compounds mimic hormone activities in mammalian endocrine systems and arise from pharmaceuticals and personal care products (PPCPs) that are in sewage but are not completely removed by conventional drinking and wastewater treatment plants (Snyder *et al.*, 2003). Removal of EDCs and PPCPs is incomplete for two reasons; diverse chemical structures that require acclimation, and low concentrations which may fail to induce biodegradation pathways (Information Box 17.12).

In application of bioremediation to problems other than sewage treatment, it must be kept in mind that biodegradation is dependent on the pollutant structure and bioavailability. Therefore, bioremediation success will depend on the type of pollutant or pollutant mixture present, and the type of microorganisms present. The first successful application of bioremediation outside sewage treatment was the cleanup of oil spills, and success in this area is now well documented (see Chapter 31). In the past few years, many new bioremediation technologies have emerged that are being used to address other types of pollutants including (USEPA, 2001):

- Volatile organic compounds (including chlorinated VOCs)
- Polyaromatic hydrocarbons
- Pesticides, herbicides
- Explosives

Several key factors are critical to successful application of bioremediation: environmental conditions, contaminant and nutrient availability, and the presence of degrading microorganisms. If biodegradation does not occur, the first thing that must be done is to isolate the

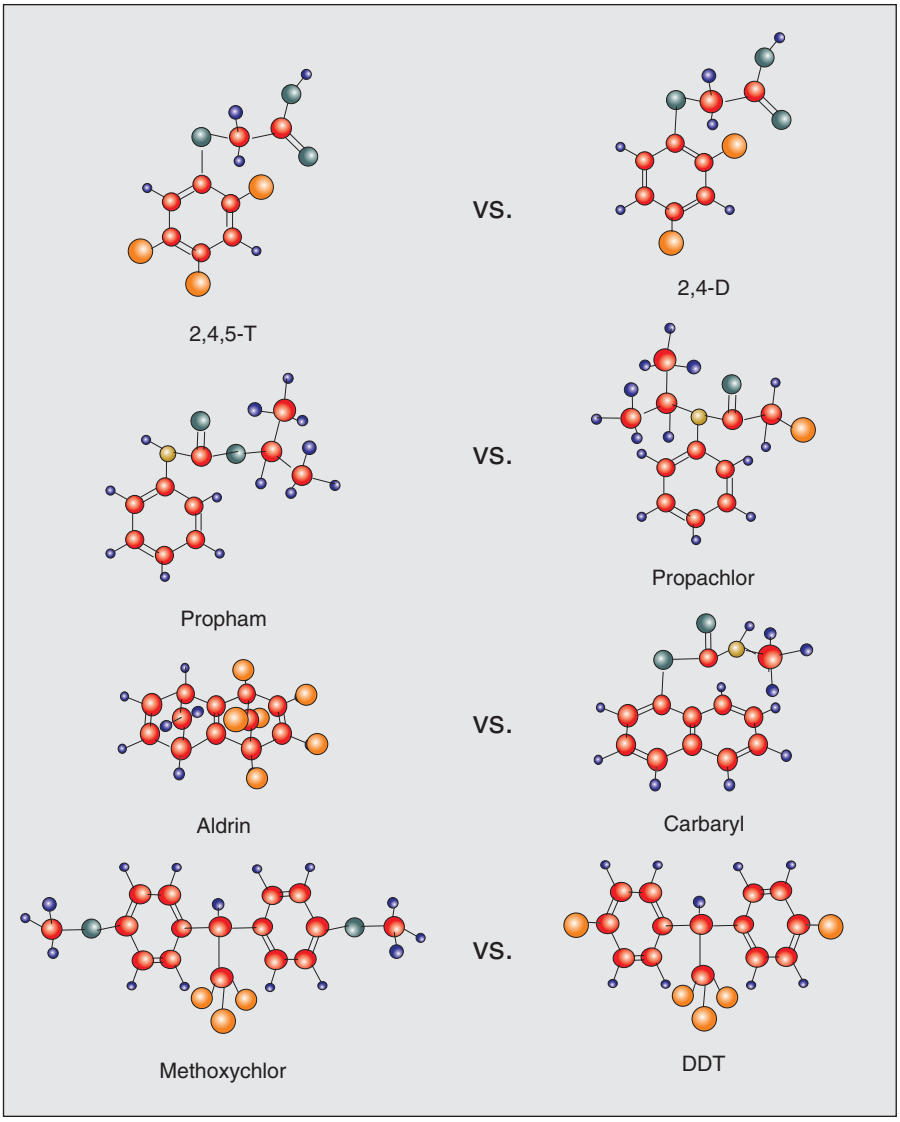


FIGURE 17.25 Comparison of four sets of pesticides. Examine the structure and functional groups of each set of pesticides and then predict which of each set is more easily biodegraded.

Information Box 17.12 Contaminant Concentration, Biodegradation and Growth

Assuming a given contaminant is biodegradable, the contaminant concentration will dictate whether biodegradation pathways are induced and at what rate biodegradation occurs. Four cases can be defined:

Contaminant concentration	Biodegradation behavior
very low	no induction, no biodegradation, no growth
low	induction, biodegradation does not support growth, energy used for maintenance, biodegradation rate is steady state
medium	induction, biodegradation, growth with increasing biodegradation rates until some limiting condition is reached
high	toxicity, no biodegradation, no growth

factor limiting bioremediation, and sometimes this can be a very difficult task. Initial laboratory tests using soil or water from a polluted site can usually determine whether degrading microorganisms are present and whether there is an obvious environmental factor that limits biodegradation, such as extremely low or high pH or lack of nitrogen and/or phosphorus. However, sometimes the limiting factor is not easy to identify. Often pollutants are present as mixtures, and one component of the pollutant mixture can have toxic effects on the growth and activity of degrading microorganisms. Low bioavailability due to sorption and aging is another factor that can limit bioremediation and can be difficult to evaluate in the environment.

Bioremediation was not considered as an option for cleanup of contaminated sites until the 1980s, but has since become an established alternative for remediation of many sites worldwide including numerous Superfund sites in the United States (USEPA, 2001). Overall, from

1982 to 2008, bioremediation has been used as a remedy for control of source contamination zones at 13% of Superfund sites and has been used for treatment of groundwater at 38% of Superfund sites (USEPA, 2010).

17.7.1 Addition of Oxygen or Other Gases

One of the most common limiting factors in bioremediation is availability of oxygen. Oxygen is an element required for aerobic biodegradation. In addition, oxygen has low solubility in water, and a low rate of diffusion (movement) through both air and water. The combination of these three factors makes it easy to understand that inadequate oxygen supplies will slow bioremediation. Several technologies have been developed to overcome a lack of oxygen. A typical bioremediation system used to treat a contaminated aquifer as well as the contaminated zone above the water table is shown in Figure 17.26A. This system contains a series of injection wells or galleries, and a series of recovery wells that comprise a two-pronged approach to bioremediation. First, the recovery wells remove contaminated groundwater, which is treated above ground, in this case using a bioreactor containing

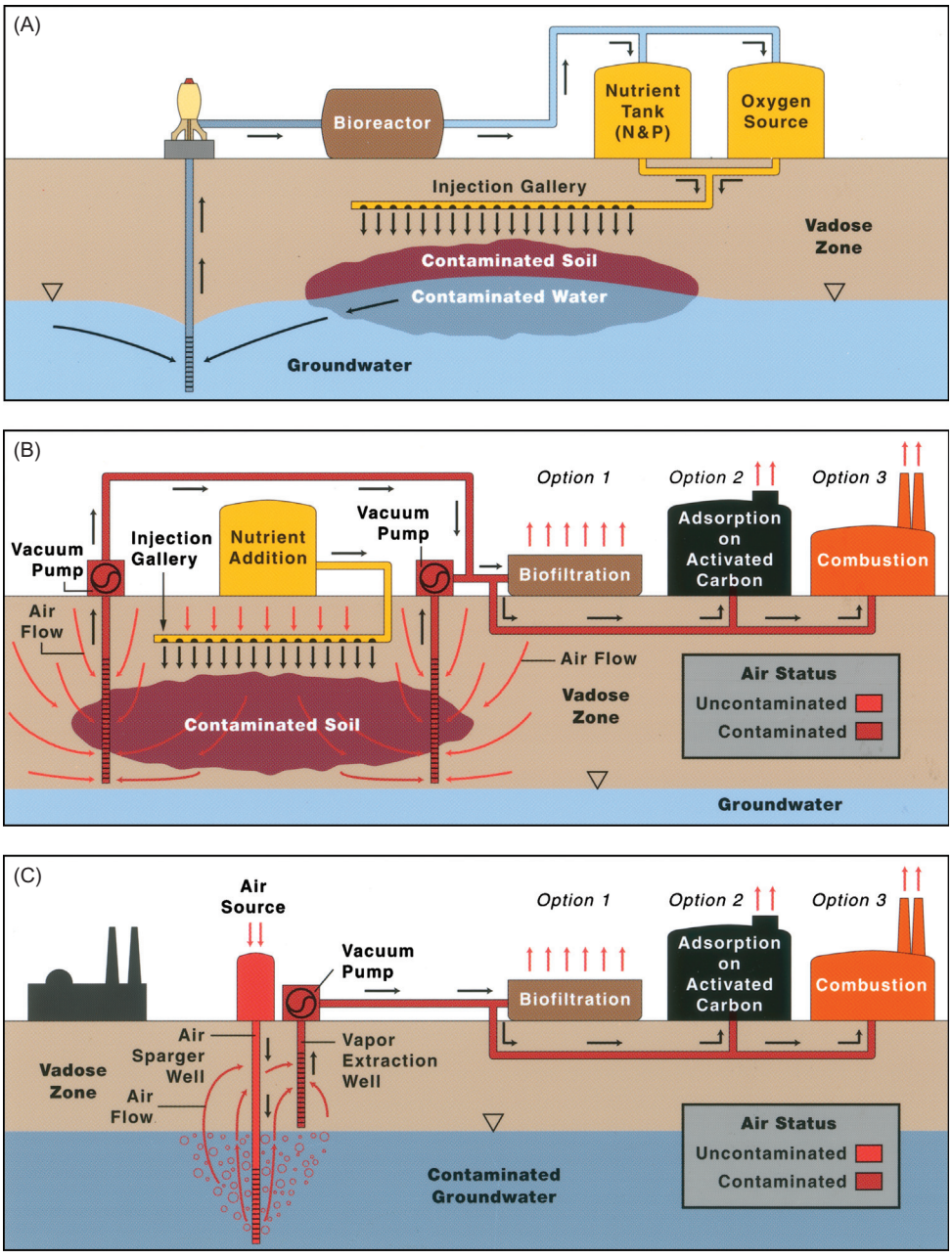


FIGURE 17.26 Bioremediation approaches: (A) *In situ* bioremediation in the vadose zone and groundwater. Nutrient and oxygen are pumped into the contaminated area to promote *in situ* processes. This figure also shows *ex situ* treatment. *Ex situ* treatment is for water pumped to the surface and uses an aboveground bioreactor, as shown, to biodegrade contaminants. Alternatively, other nonbiological methods can be used to remove contaminants from the water, e.g., air stripping, activated carbon, oil–water separation or oxidation. Following treatment, an injection well returns the contaminant-free water to the aquifer. (B) Bioventing and biofiltration in the vadose zone. Air is slowly drawn through the contaminated site (bioventing), which stimulates *in situ* aerobic degradation. Volatile contaminants removed with the air can be treated biologically using a biofilter as shown or by adsorption on activated carbon, or by combustion. (C) Bioremediation in groundwater by air sparging. Air is pumped into the contaminated site to stimulate aerobic biodegradation. Volatile contaminants brought to the surfaced are treated by biofiltration, activated carbon or combustion.

microorganisms that are acclimated to the contaminant. This would be considered *ex situ* treatment. Following bioreactor treatment, the clean water is supplied with oxygen and nutrients, and then it is reinjected into the site. The reinjected water provides oxygen and nutrients to stimulate *in situ* biodegradation. In addition, the reinjected water flushes the vadose zone to aid in removal of the contaminant for aboveground bioreactor treatment. This remediation scheme is a very good example of the use of a combination of physical, chemical and biological treatments to maximize the effectiveness of the remediation treatment.

Bioventing is a technique used to add oxygen directly to a site of contamination in the vadose zone (unsaturated zone). In bioventing alone, air is injected at very low flow rates into the contaminated zone to promote biodegradation. Alternatively in some cases, flow rates can be increased to combine soil vapor extraction technology and bioremediation. In this case, extracted vapor-phase contaminants are treated aboveground either biologically or chemically, and in addition, *in situ* bioremediation is stimulated. The bioventing zone is highlighted by red arrows in Figure 17.26B and includes the vadose zone and contaminated regions just below the water table. As shown in this figure, a series of wells have been constructed around the zone of contamination. To initiate bioventing, a vacuum is drawn on these wells to force accelerated air movement through the contamination zone. This effectively increases the supply of oxygen throughout the site, and thus the rate of contaminant biodegradation. If the rate of air movement is increased further, contaminants are volatilized and removed as air is forced through this system. This contaminated air can be treated biologically by passing the air through aboveground soil beds in a process called biofiltration, as shown in Figure 17.27 (Jutras *et al.*, 1997).

In contrast, air sparging is used to add oxygen to the saturated zone (Figure 17.26C). In this process, an air sparger well is used to inject air under pressure below the water table. The injected air displaces water in the soil matrix, creating a temporary air-filled porosity. This causes oxygen levels to increase, resulting in enhanced biodegradation rates. In addition, volatile organics will volatilize into the airstream, and can be removed by a vapor extraction well.

Methane is another gas that can be added with oxygen in extracted groundwater and reinjected into the saturated zone. Methane is used specifically to stimulate methanotrophic activity and cometabolic degradation of chlorinated solvents. As described in Chapter 16, methanotrophic organisms produce the enzyme methane monooxygenase to degrade methane, and this enzyme also cometabolically degrades several chlorinated solvents. Cometabolic degradation of chlorinated solvents is presently being tested in field trials to determine the usefulness of this technology.

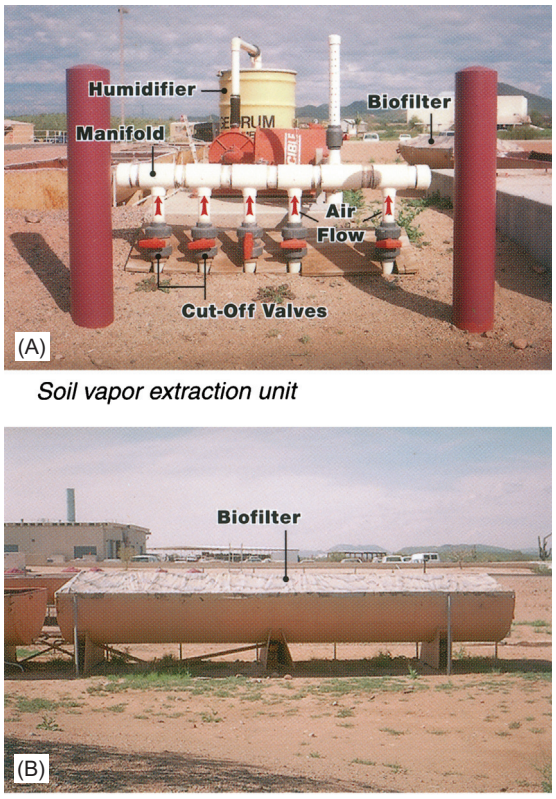


FIGURE 17.27 Bioremediation of gasoline vapors from a leaking underground storage tank. (A) Red arrows denote the direction of airflow out of the ground and through the manifold, which controls the airflow. The air then passes through the humidifier, a tank containing water, and into the biofilter (the large red vertical cylinders serve as protective barriers to prevent damage to the manifold). (B) Air flows from the manifold into the soil in the biofilter through a perforated pipe running lengthwise along the bottom of the biofilter.

17.7.2 Nutrient Addition

A common bioremediation treatment is the addition of nutrients, in particular nitrogen and phosphorus. Many contaminated sites contain organic wastes that are rich in carbon but contain minimal amounts of nitrogen and phosphorus. Nutrient addition is illustrated in the bioremediation schemes shown in Figure 17.26A and B. Injection of nutrient solutions takes place from an aboveground batch-feed system. The goal of nutrient injection is to optimize the ratio of carbon, nitrogen and phosphorus (C:N:P) in the site to approximately 100:10:1. However, sorption of added nutrients can make it difficult to achieve the optimal ratio accurately.

17.7.3 Sequential Anaerobic–Aerobic Degradation

The rapid biodegradation of many priority pollutants requires both anaerobic and aerobic stages. As already

discussed, aerobic conditions favor the biodegradation of compounds with fewer halogen substituents, and anaerobic conditions favor the biodegradation of compounds with a high number of halogen substituents. However, complete biodegradation of highly halogenated aliphatics under anaerobic conditions often does not take place. Therefore, some researchers have proposed the use of a sequential anaerobic and aerobic treatment. Initial incubation under anaerobic conditions is used to decrease the halogen content, and subsequent addition of oxygen creates aerobic conditions to allow complete degradation to proceed aerobically. This approach was used successfully to treat a groundwater plume containing TCE, DCE, vinyl chloride and petroleum hydrocarbons (Morkin *et al.*, 2000).

17.7.4 Addition of Surfactants

Surfactant addition has been proposed as a technique for increasing the bioavailability and hence biodegradation of contaminants (see Section 17.4.3). Surfactants can be synthesized chemically and are also produced by many microorganisms, in which case they are called biosurfactants. Surfactants work similarly to industrial and household detergents that effectively remove oily residues from machinery, clothing or dishes. As shown in the inset in Figure 17.6, individual contaminant molecules can be "solubilized" inside surfactant micelles. These micelles range from 5 to 10 nm in diameter. Alternatively, surfactant molecules can coat oil droplets and emulsify them into solution—a property that makes them useful in dispersants for "breaking up" oil spills (Information Box 17.13). In addition, surfactants can enhance the ability of microbes to stick to oil droplets. There have been extensive laboratory and field tests performed with both synthetic- and bio-surfactants. While these materials definitely increase the bioavailability of organic contaminants, they do not always stimulate biodegradation. However, enough successful tests have been performed to indicate that, if chosen carefully, surfactants can be used to enhance the remediation process (Maier and Soberon-Chavez, 2000; Martienssen and Schirmer, 2007).

17.7.5 Addition of Microorganisms or DNA

If appropriate biodegrading microorganisms are not present in soil or if microbial populations have been reduced because of contaminant toxicity, specific microorganisms can be added as "introduced organisms" to enhance the existing populations. This process is known as bioaugmentation. Scientists are also capable of creating "superbugs," organisms that can degrade pollutants at extremely rapid rates. Such organisms can be developed through successive adaptations under laboratory conditions, or can be genetically engineered.

Although bioaugmentation, with naturally occurring or engineered organisms, has been demonstrated to increase contaminant degradation in numerous lab-based studies, it generally has not been successful for remediation in actual field sites (Gentry *et al.*, 2004; Stroo *et al.*, 2013). The problem is that introduction of a microorganism to a contaminated site may fail for at least two reasons. First, the introduced microbe often cannot establish a niche in the environment. In fact, these introduced organisms often do not survive in a new environment beyond a few weeks. Second, there are difficulties in delivering the introduced organisms to the site of contamination, because microorganisms, like contaminants, can be strongly sorbed by solid surfaces.

Information Box 17.13 Use of Dispersants to Reduce Environmental Impacts from the BP *Deepwater Horizon* Oil Leak

In 2010, the BP *Deepwater Horizon* oil leak released an estimated 780 million liters of oil into the Gulf of Mexico. In order to help prevent the generation of large oil slicks and reduce the amount of oil reaching the shoreline, dispersants, including Corexit 9500A®, were applied to the water above the leak. Dispersants generally contain a mixture of chemicals, including surfactants and solvents, that are used to emulsify oil into droplets which are more likely to remain suspended in the water column. The application of dispersants to the BP *Deepwater Horizon* oil leak succeeded in reducing the amount of oil that reached the surface, and likely helped to reduce the impact of oil slicks on the shoreline ecosystem. In addition, the increased surface area of the dispersed oil likely increased its potential for biodegradation. This is supported by studies which found greatly increased microbial numbers in the cloud of dispersed oil (Atlas and Hazen, 2011). However, in the process of containing the oil leak, millions of liters of dispersants were ultimately applied to the Gulf waters thus raising potential concerns about the impacts of the dispersants themselves on marine ecosystems. For example, one study reported that although the toxicity of the dispersant Corexit 9500A® to a marine rotifer was similar to that of the oil being treated, the toxicity of the oil mixed with the dispersant was increased over 52-fold in comparison to the toxicity of the oil alone (Rico-Martínez *et al.*, 2013). It remains to be seen whether the benefits of using such dispersants to reduce the impacts of oil spills on shoreline ecosystems is being offset by negative impacts on other marine ecosystems. In addition, research is ongoing to cost-effectively produce less toxic dispersants for use in such oil spills. There is great interest in the use of biosurfactants for this purpose (see also Chapter 31).

Despite these obstacles, one case in which bioaugmentation has been successful at the field scale is the remediation of soil and groundwater contaminated with chlorinated solvents such as TCE and PCE (Case Study 17.2; Stroo *et al*., 2013). Why has bioaugmentation succeeded for remediation of these compounds when it so frequently fails for other applications? Two possible reasons are: (1) the unique nature of the chemicals and organisms involved, and (2) the alteration of site conditions to create a niche for the added microorganisms. Unlike petroleum hydrocarbons and many other contaminants, PCE and TCE are used only by a very select group of microorganisms as electron acceptors to support growth under anaerobic conditions (e.g., reductive dehalogenation, see Section 17.6.2.2). Thus, there is less competition for the chemical substrate between the added and indigenous organisms than occurs for many other compounds, e.g., petroleum. In addition, these sites are usually converted from aerobic to anaerobic conditions, through addition of electron donors, prior to addition of the bioaugmentation culture. This creates an immediate niche for the added microorganisms to establish and begin reductive dehalogenation. Since anaerobic conditions generally result in lower microbial numbers, diversity and activity than that found in aerobic conditions, this likely further decreases competition from indigenous microorganisms.

Currently, very little is known about microbial transport and establishment of environmental niches. These are areas of active research, and in the next few years scientists may gain a further understanding of microbial behavior in soil ecosystems. However, until we discover how to successfully deliver and establish introduced microorganisms, bioaugmentation will not be a viable bioremediation option for the majority of contaminated sites.

If bioaugmentation does not work, one way to take advantage of the superbugs that have been developed is to use them in bioreactor systems under controlled conditions. Extremely efficient biodegradation rates can be achieved in bioreactors that are used in aboveground treatment systems.

Another bioaugmentation strategy is to add specific genes that can confer a specific degradation capability to indigenous microbial populations. The addition of degradative genes relies on the delivery and uptake of the genetic material by indigenous microbes. There are two approaches that can be taken in delivery of genes. The first is to use microbial cells to deliver the DNA via conjugation. The second is to add "naked" DNA to the soil to allow uptake via transformation. This second approach may reduce the difficulty of delivery since DNA alone is much smaller than a whole cell. However, little is known as yet about these two approaches. As discussed in Case Study 3.1, Di Giovanni *et al*. (1996) demonstrated that

Case Study 17.2 Bioaugmentation-based Remediation of Groundwater Contaminated with Chlorinated Ethenes

Although bioaugmentation has been shown to increase the degradation of many compounds in lab-bases studies, it often fails when applied to field sites under environmental conditions. However, one area in which bioaugmentation has been successfully demonstrated at the field scale is in the remediation of sites contaminated with chlorinated ethenes such as trichloroethene (TCE) and tetrachloroethene (PCE). A classic example of this is the remediation of a site at Kelly Air Force Base in San Antonio, TX, U.S.A. This site was contaminated with up to 1 mg of PCE per liter of groundwater and also lower amounts of TCE and *cis*-1,2-dichloroethene (cDCE) (Major *et al*., 2002). The scientists first conducted laboratory microcosm tests using soil and groundwater from the site to determine the potential for biostimulation of the indigenous microbial community to dechlorinate the PCE and TCE. Amendment of the microcosms with various electron donors (e.g., methanol and lactate) expedited dehalogenation to cDCE, but did not further dehalogenate it to vinyl chloride or ethene (see Figure 17.2 for details on the degradation pathway). Since biostimulation alone was not effective, microcosms were also bioaugmented with a dechlorinating, enrichment culture (KB-1). This culture, containing populations of *Dehalococcoides* spp., was originally obtained from a TCE-contaminated site in southern Ontario, Canada. In contrast to the biostimulated microcosms, the bioaugmented microcosms demonstrated complete dechlorination of TCE to ethene. Based upon these results, the scientists then conducted a field-scale test of bioaugmentation to remediate the contaminated soil and groundwater *in situ*. For the first 89 days of the experiment, water was recirculated through the site without electron donor addition in order to equilibrate and verify the system hydraulics. After day 89, methanol and acetate were added as electron donors for generation of anaerobic conditions. On day 176, bioagumentation began with addition of the KB-1 culture. Similar to the laboratory microcosm tests, concentrations of PCE began to decrease following biostimulation with a corresponding accumulation of cDCE, but little conversion to vinyl chloride or ethene. However, once the site was bioaugmented with the KB-1 culture, the cDCE began also to disappear with a corresponding accumulation of ethene. Concentrations of PCE, TCE and cDCE all ultimately decreased to <5 μg/L. A combination of PCR- and DNA sequencing-based approaches were used to verify that the bioaugmentation culture was spread throughout the treatment zone where the dechlorination occurred, thus providing further evidence that dechlorination of the PCE and TCE was due to the presence and activity of the added microbial culture.

gene transfer can occur in soil, resulting in 2,4-D degradation activity. However, whether such transfer is common, and whether conditions are conducive or inhibitory to such transfer, is not known.

QUESTIONS AND PROBLEMS

1. Why is there concern about the presence of organic contaminants in the environment?
2. Describe the different factors that can limit biodegradation of organic contaminants in the environment.
3. Draw and name an aliphatic, alicyclic and aromatic structure, each with six carbons.
4. Outline the biodegradation pathway for each of the structures that you just drew under aerobic conditions.
5. Why are aerobic conditions usually preferred for biodegradation of organic contaminants? Under what conditions might anaerobic biodegradation be preferred?
6. Compare the advantages and disadvantages of intrinsic, *ex situ* and *in situ* bioremediation.
7. You have been hired to bioremediate a site in which the groundwater is contaminated with petroleum. Groundwater samples have a strong sulfide smell and gas chromatographic analysis of the samples shows negligible biodegradation of the petroleum has occurred. What is your recommendation?
8. Kleen Co. is in charge of a site in Nevada that was used for pesticide preparation. As a result of years of operation, the groundwater below this site has elevated levels of pesticides (up to 20 mg/L). Your initial investigation shows that: (1) the pesticide-containing plume is neither growing nor shrinking in size; (2) there are pesticide degraders in the plume; (3) and the dissolved oxygen levels in the plume range from 2 to 4 mg/L. This site is not being used presently, and the groundwater is not used for drinking water purposes. What is your best recommendation based on these site characteristics and on your knowledge of cost of remediation?

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