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Chapter 24

Wastewater Treatment and Biosolids Reuse

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24.1 THE NATURE OF WASTEWATER (SEWAGE)

The Cloaca Maxima, the "biggest sewer" in Rome, had enough capacity to serve a city of 1 million people. This sewer, and others like it, simply collected wastes and discharged them into the nearest lake, river, or ocean. This made cities more habitable, but its success depended on transferring the pollution problem from one place to another. Although this worked reasonably well for the Romans, it does not work well today. Current population densities are too high to permit a simple dependence on transference. Thus, modern-day sewage is treated before it is discharged into the environment. In the latter part of the nineteenth century, the design of sewage systems allowed collection with treatment to lessen the impact on natural waters. Today, more than 15,000 wastewater treatment plants treat approximately 150 billion liters of wastewater per day in the United States alone. In addition, septic tanks, which were also introduced at the end of the nineteenth century, serve approximately 25% of the U.S. population, largely in rural areas.

 Domestic wastewater is primarily a combination of human feces, urine, and gray water. Gray water results from washing, bathing, and meal preparation. Water from various industries and businesses may also enter the system. People excrete 100–500 grams wet weight of feces and 1–1.3 liters of urine per person per day (Bitton, 2005). Major organic and inorganic constituents of untreated domestic sewage are shown in Table 24.1 .

 The amount of organic matter in domestic wastes determines the degree of biological treatment required. Three tests are used to assess the amount of organic matter: biochemical oxygen demand (BOD), chemical oxygen demand (COD), and total organic carbon (TOC).

 The major objective of domestic waste treatment is the reduction of BOD, which may be either in the form of solids (suspended matter) or soluble. BOD is the amount of dissolved oxygen consumed by microorganisms during the biochemical oxidation of organic (carbonaceous BOD) and inorganic (ammonia) matter. The methodology for measuring BOD has changed little since it was developed in the 1930s.

The 5-day BOD test (written $BOD₅$) is a measure of the amount of oxygen consumed by a mixed population of heterotrophic bacteria in the dark at 20°C over a period of 5 days. In this test, aliquots of wastewater are placed in a 300-ml BOD bottle (Fig. 24.1) and diluted in phosphate buffer (pH 7.2) containing other inorganic elements (N, Ca, Mg, Fe) and saturated with oxygen. Sometimes acclimated microorganisms or dehydrated cultures of microorganisms, sold in capsule form, are added to municipal and industrial wastewaters, which may not have a sufficient microflora to carry out the BOD test. In some cases a nitrification inhibitor is added to the sample to determine only the carbonaceous BOD.

 Dissolved oxygen concentration is determined at time 0 and after a 5-day incubation by means of an oxygen electrode,

From Pepper, Gerba, and Brusseau, 2006.

^Five-day test, (BOD5, 20°C).

chemical procedures (e.g., Winkler test), or a manometric BOD apparatus. The BOD test is carried out on a series of dilutions of the sample, the dilution depending on the source of the sample. When dilution water is not seeded with bacteria, the BOD value is expressed in milligrams per liter, according to the following equation (APHA, 1998):

$$
BOD(mg/L) = \frac{D_1 - D_5}{P}
$$
 (Eq. 24.1)

where:

 D_1 = initial dissolved oxygen (DO),

 D_5 = DO at day 5, and

 $P =$ decimal volumetric fraction of wastewater utilized. If the dilution water is seeded,

$$
BOD(mg/l) = \frac{(D_1 - D_5) - (B_1 - B_5)f}{P}
$$
 (Eq. 24.2)

where:

 D_1 = initial DO of the sample dilution (mg/l),

 D_5 = final DO of the sample dilution (mg/l),

- $P =$ decimal volumetric fraction of sample used,
- B_1 = initial DO of seed control (mg/l),
- B_5 = final DO of seed control (mg/l), and
- f = ratio of seed in sample to seed in control = $(\%$ seed in D_1 / $(\%$ seed in B_1).

 Because of depletion of the carbon source, the carbonaceous BOD reaches a plateau called the ultimate carbonaceous BOD (Fig. 24.2). The BOD_5 test is commonly used for several reasons:

- To determine the amount of oxygen that will be required for biological treatment of the organic matter present in a wastewater
- To determine the size of the waste treatment facility needed
- To assess the efficiency of treatment processes
- To determine compliance with wastewater discharge permits

FIGURE 24.1 BOD bottles used for the determination of BOD.

The typical BOD_5 of raw sewage ranges from 110 to 440 mg/l (see Example Calculation 24.1). Conventional sewage treatment will reduce this by 95%.

 Chemical oxygen demand (COD) is the amount of oxygen necessary to oxidize all of the organic carbon completely to CO_2 and H_2O . COD is measured by oxidation with potassium dichromate $(K_2Cr_2O_7)$ in the presence of sulfuric acid and silver, and is expressed in milligrams per liter. In general, 1 g of carbohydrate or 1 g of protein is approximately equivalent to 1 g of COD. Normally, the ratio BOD/COD is approximately 0.5. When this ratio falls below 0.3, it means that the sample contains large amounts of organic compounds that are not easily biodegraded.

 Another method of measuring organic matter in water is the TOC or total organic carbon test. TOC is determined by oxidation of the organic matter with heat and oxygen, followed by measurement of the $CO₂$ liberated with an infrared analyzer. Both TOC and COD represent the concentration of both biodegradable and nonbiodegradable organics in water.

 Pathogenic microorganisms are almost always present in domestic wastewater (Table 24.2). This is because large

FIGURE 24.2 Carbonaceous and nitrogenous BOD.

numbers of pathogenic microorganisms may be excreted by infected individuals. Both symptomatic and asymptomatic individuals may excrete pathogens. For example, the concentration of rotavirus may be as high as 10^{10} virions per gram of stool, or 10^{12} in $100 g$ of stool (Table 24.3). Infected individuals may excrete enteric pathogens for several weeks to months. The concentration of enteric pathogens in raw wastewater varies depending on the following:

- The incidence of the infection in the community
- The socioeconomic status of the population
- The time of year
- The per-capita water consumption

TABLE 24.2 Types and Numbers of Microorganisms Typically Found in Untreated Domestic Wastewater

 From Pepper, Gerba, and Brusseau, 2006.

Example Calculation 24.1 Calculation of BOD

Determine the 5-day BOD (BOD₅) for a wastewater sample when a 15-ml sample of the wastewater is added to a BOD bottle containing 300 ml of dilution water, and the dissolved oxygen is 8 mg/l. Five days later the dissolved oxygen concentration is 2 mg/l. Using Eq. 24.1:

$$
BOD(mg/l) = \frac{D_1 - D_5}{P}
$$

\n
$$
D_1 = 8 \text{ mg/l}
$$

\n
$$
D_5 = 2 \text{ mg/l}
$$

\n
$$
P = \frac{15 \text{ ml}}{300 \text{ ml}} = 5\% = 0.05
$$

\n
$$
BOD_5 = \frac{8 - 2}{0.05} = 120 \text{ mg/l}
$$

 0.05

TABLE 24.3 Incidence and Concentration of Enteric Viruses and Protozoa in Feces in the United States

a *Children in day care centers.*

TABLE 24.4 Estimated Levels of Enteric Organisms in Sewage and Polluted Surface Water in the United States

 The peak incidence of many enteric infections is seasonal in temperate climates. Thus, the highest incidence of enterovirus infection is during the late summer and early fall. Rotavirus infections tend to peak in the early winter, and *Cryptosporidium* infections peak in the early spring and fall. The reason for the seasonality of enteric infections is not completely understood, but several factors may play a role. It may be associated with the survival of different agents in the environment during the different seasons. *Giardia*, for example, can survive winter temperatures very well. Alternatively, excretion differences among animal reservoirs may be involved, as is the case with *Cryptosporidium*. Finally, it may well be that greater exposure to contaminated water, as in swimming, is the explanation for increased incidence in the summer months.

 Concentrations of enteric pathogens are much greater in sewage in the developing world than the industrialized world. For example, the average concentration of enteric viruses in sewage in the United States has been estimated to be $10³$ per liter (Table 24.4), whereas concentrations as high as $10⁵$ per liter have been observed in Africa and Asia.

24.2 MODERN WASTEWATER TREATMENT

 The primary goal of wastewater treatment is the removal and degradation of organic matter under controlled conditions. Complete sewage treatment comprises three major steps, primary, secondary, and tertiary treatment, as shown in Figure 24.3 .

24.2.1 Primary Treatment

Primary treatment is the first step in municipal sewage treatment and it involves physically separating large solids from the waste stream. As raw sewage enters the treatment plant, it passes through a metal grating that removes large debris, such as branches and tires (Fig. 24.4). A moving screen then filters out smaller items such as diapers and bottles (Fig. 24.5), after which a brief residence in a grit tank allows sand and gravel to settle out. The waste stream is then pumped into the primary settling tank (also known as a sedimentation tank or clarifier), where about half the suspended organic solids settle to the bottom as sludge (Fig. 24.6). The resulting sludge is referred to as primary sludge. Microbial pathogens are not effectively removed from the effluent in the primary process, although some removal occurs.

24.2.2 Secondary Treatment

Secondary treatment consists of biological degradation, in which the remaining suspended solids are decomposed by microorganisms and the number of pathogens is reduced. In this stage, the effluent from primary treatment usually undergoes biological treatment in a trickling filter bed (Fig. 24.7), an aeration tank (Fig. 24.8), or a sewage lagoon (see Section 24.3). A disinfection step is generally included at the end of the treatment.

24.2.2.1 Trickling Filters

 In modern wastewater treatment plants, the trickling filter is composed of plastic units (Fig. 24.9). In older plants, or in developing countries, the filter is simply a bed of stones or corrugated plastic sheets through which wastewater drips (see Fig. 24.7). This is one of the earliest systems introduced for biological waste treatment. The effluent is pumped through an overhead sprayer onto the filter bed, where bacteria and other microorganisms have formed a biofilm on the filter surfaces. These microorganisms intercept the organic material as it trickles past and decompose it aerobically.

 The media used in trickling filters may be stones, ceramic material, hard coal, or plastic media. Plastic media of polyvinyl chloride (PVC) or polypropylene are used today in high-rate trickling filters. As the organic matter passes through the trickling filter it is converted to microbial

FIGURE 24.3 Schematic of the treatment processes typical of modern wastewater treatment.

biomass, which forms a thick biofilm on the filter medium. The biofilm that forms on the surface of the filter medium is called a zooleal film. It is composed of bacteria, fungi, algae, and protozoa. Over time, the increase in biofilm thickness leads to limited oxygen diffusion to the deeper layers of the biofilm, creating an anaerobic environment near the filter medium surface. As a result, the organisms eventually slough from the surface and a new biofilm is formed. BOD removal by trickling filters is approximately 85% for low-rate filters (U.S. EPA, 1977). Effluent from the trickling filter usually passes into a final clarifier to further separate solids from effluent (Fig. 24.6).

FIGURE 24.4 Removal of large debris from sewage via a bar screen. From Pepper, Gerba, and Brusseau, 2006.

FIGURE 24.7 A trickling filter bed. Here, rocks provide a matrix supporting the growth of a microbial biofilm that actively degrades the organic material in the wastewater under aerobic conditions. Photo C.P. Gerba.

FIGURE 24.5 Removal of small debris via a moving screen. From Pepper, Gerba, and Brusseau, 2006.

FIGURE 24.8 Secondary treatment: an aeration basin. From Pepper, Gerba, and Brusseau, 2006.

FIGURE 24.6 Three clarifiers (foreground—blue) where suspended organic solids settle out as primary sludge. Also see the two anaerobic sludge digesters in the background (white). From Pepper, Gerba, and Brusseau, 2006.

24.2.2.2 Conventional Activated Sludge

Aeration-tank digestion is also known as the activated sludge process. In the United States, wastewater is most commonly treated by this process. Effluent from primary treatment is pumped into a tank and mixed with a bacteria-rich slurry known as activated sludge. Air or pure oxygen pumped through the mixture encourages bacterial growth and decomposition of the organic material. The material then goes to a secondary settling tank, where water is siphoned off the top of the tank and sludge is removed from the bottom. Some of the sludge is used as an inoculum for primary effluent. The remainder of the sludge, known as secondary sludge, is removed. This secondary sludge is added to primary sludge from primary treatment, and subsequently indigenous anaerobic digestion occurs (Fig. 24.6). The end product of this process is known as biosolids (see Section 24.8). The concentration of pathogens is reduced in the activated sludge process by antagonistic microorganisms as well as by adsorption to or incorporation in the secondary sludge.

FIGURE 24.9 (A) A unit of plastic material used to create a biofilter (trickling filter). The diameter of each hole is approximately 5 cm. (B) A trickling biofilter or biotower. This is composed of many plastic units stacked upon each other. Dimensions of the biofilter may be 20 m diameter by 10–30 m depth. From Pepper, Gerba, and Brusseau, 2006.

 An important characteristic of the activated sludge process is the recycling of a large proportion of the biomass. This results in a large number of microorganisms that oxidize organic matter in a relatively short time (Bitton, 2005). The detention time in the aeration basin varies from four to eight hours. The content of the aeration tank is referred to as the mixed-liquor suspended solids (MLSS). The organic part of the MLSS is called the mixed-liquor volatile suspended solids (MLVSS), which is the nonmicrobial organic matter as well as dead and living microorganisms and cell debris. The activated sludge process must be controlled to maintain a proper ratio of substrate (organic load) to microorganisms or food-to-microorganism ratio (F/M) (Bitton, 2005). This is expressed as BOD per kilogram per day and is calculated as

$$
\frac{F}{M} = \frac{Q \cdot BOD}{MLSS \cdot V}
$$
 (Eq. 24.3)

where

 $Q =$ flow rate of sewage in million gallons per day (MGD),

 $BOD_5 = 5$ -day biochemical oxygen demand (mg/l),

 $MLSS = mixed-liquor suspended solids (mg/l), and$

 $V =$ volume of aeration tank (gallons).

 F/M is controlled by the rate of activated sludge wasting. The higher the wasting rate, the higher the F/M ratio. For conventional aeration tanks the F/M ratio is 0.2–0.5 lb $BOD₅/day/lb$ MLSS, but it can be higher (up to 1.5) for activated sludge when high-purity oxygen is used. A low F/M ratio means that the microorganisms in the aeration tank are starved, leading to more efficient wastewater treatment.

 The important parameters controlling the operation of an activated sludge process are organic loading rates, oxygen supply, and control and operation of the final settling tank. This tank has two functions: clarification and thickening. For routine operation, sludge settleability is determined by use of the sludge volume index (SVI) (Bitton, 2005). SVI is determined by the following formula:

$$
SVI = \frac{V \cdot 1000}{MLSS}
$$
 (Eq. 24.4)

where $V =$ volume of settled sludge after 30 minutes (ml/l).

 The microbial biomass produced in the aeration tank must settle properly from suspension so that it may be wasted or returned to the aeration tank. Good settling occurs when the sludge microorganisms are in the endogenous phase, which occurs when carbon and energy sources are limited, and the microbial specific growth rate is local (Bitton, 2005). A mean cell residence time of three to four days is necessary for effective settling (Metcalf and Eddy, 2003). Poor settling may also be caused by sudden changes in temperature, pH, absence of nutrients, and presence of toxic metals and organics. A common problem in the activated sludge process is filamentous bulking, which consists of slow settling and poor compaction of solids in the clarifier. Filamentous bulking is usually caused by the excessive growth of filamentous microorganisms. The filaments produced by these bacteria interfere with sludge settling and compaction. A high SVI (>150 ml/g) indicates bulking conditions. Filamentous bacteria are able to predominate under conditions of low dissolved oxygen, low F/M, low nutrients, and high sulfide levels. Filamentous bacteria can be controlled by treating the return sludge with chlorine or hydrogen peroxide to kill filamentous microorganisms selectively.

FIGURE 24.10 Denitrification systems: (A) single-sludge system, (B) multisludge system. Modified from Curds and Hawkes, 1983.

FIGURE 24.11 Denitrification system: Bardenpho process.

24.2.2.3 Nitrogen Removal by the Activated Sludge Process

 Activated sludge processes can be modified for nitrogen removal to encourage nitrification followed by denitrification. The establishment of a nitrifying population in activated sludge depends on the wastage rate of the sludge, and therefore on the BOD load, MLSS, and retention time. The growth rate of nitrifying bacteria (μ_n) must be higher than the growth rate (μ_h) of heterotrophs in the system. In reality, the growth rate of nitrifiers is lower than that of heterotrophs in sewage; therefore, a long sludge age is necessary for the conversion of ammonia to nitrate. Nitrification is expected at a sludge age greater than four days (Bitton, 2005).

 Nitrification must be followed by denitrification to remove nitrogen from wastewater. The conventional activated sludge system can be modified to encourage denitrification. Three such processes are as follows:

Single sludge system (Fig. 24.10A). This system comprises a series of aerobic and anaerobic tanks in lieu of a single aeration tank.

- \bullet Multisludge system (Fig. 24.10B). Carbonaceous oxidation, nitrification, and denitrification are carried out in three separate systems. Methanol or settled sewage serves as the source of carbon for denitrifiers.
- Bardenpho process (Fig. 24.11). The process consists of two aerobic and two anoxic tanks followed by a sludge settling tank. Tank 1 is anoxic and is used for denitrification, with wastewater used as a carbon source. Tank 2 is an aerobic tank utilized for both carbonaceous oxidation and nitrification. The mixed liquor from this tank, which contains nitrate, is returned to tank 1. The anoxic tank 3 removes the nitrate remaining in the effluent by denitrification. Finally, tank 4 is an aerobic tank used to strip the nitrogen gas that results from denitrification, thus improving mixed-liquor settling.

24.2.2.4 Phosphorus Removal by Activated Sludge Process

 Phosphorus can also be reduced by the activity of microorganisms in modified activated sludge processes. The process

FIGURE 24.12 Microbiology of the A/O process. From Pepper, Gerba, and Brusseau, 2006.

depends on the uptake of phosphorus by the microbes during the aerobic stage and subsequent release during the anaerobic stage. Two of several systems in use are as follows:

- A/O (anaerobic/oxic) process. The A/O process consists of a modified activated sludge system that includes an anaerobic zone (detention time 0.5–1 h) upstream of the conventional aeration tank (detention time 1–3 h). Figure 24.12 illustrates the microbiology of the A/O process. During the anaerobic phase, inorganic phosphorus is released from the cells as a result of polyphosphate hydrolysis. The energy liberated is used for the uptake of BOD from wastewater. Removal efficiency is high when the BOD/ phosphorus ratio exceeds 10 (Metcalf and Eddy, 2003). During the aerobic phase, soluble phosphorus is taken up by bacteria, which synthesize polyphosphates using the energy released from BOD oxidation.
- Bardenpho process. This system also removes nitrogen as well as phosphorus by a nitrification–denitrification process (Fig. 24.11).

24.2.3 Tertiary Treatment

Tertiary treatment of effluent involves a series of additional steps after secondary treatment to further reduce organics, turbidity, nitrogen, phosphorus, metals, and pathogens. Most processes involve some type of physicochemical treatment such as coagulation, filtration, activated carbon adsorption of organics, reverse osmosis, and additional disinfection. Tertiary treatment of wastewater is practiced for additional protection of wildlife after discharge into rivers or lakes. Even more commonly, it is performed when the wastewater is to be reused for irrigation (e.g., food crops, golf courses), for recreational purposes (e.g., lakes, estuaries), or for drinking water.

24.2.4 Removal of Pathogens by Sewage Treatment Processes

 There have been a number of reviews on the removal of pathogenic microorganisms by activated sludge and other wastewater treatment processes (Leong, 1983). This information suggests that significant removal especially of enteric bacterial pathogens can be achieved by these processes (Table 24.5). However, disinfection and/or advanced tertiary treatment are necessary for many reuse applications to ensure pathogen reduction. Current issues related to pathogen reduction are treatment plant reliability, removal of new and emerging enteric pathogens of concern, and the ability of new technologies to effect pathogen reduction. Wide variation in pathogen removal can result in significant numbers of pathogens passing through a process for various time periods. The issue of reliability is of major importance if the reclaimed water is intended for recreational or potable reuse, where short-term exposures to high levels of pathogens could result in significant risk to the exposed population.

 Compared with other biological treatment methods (i.e., trickling filters), activated sludge is relatively efficient in reducing the numbers of pathogens in raw wastewater. Both sedimentation and aeration play a role in pathogen reduction. Primary sedimentation is more effective for the removal of the larger pathogens such as helminth eggs, but solid-associated bacteria and even viruses are also removed. During aeration, pathogens are inactivated by antagonistic microorganisms and by environmental factors such as temperature. The greatest removal probably occurs by adsorption or entrapment of the organisms within the biological floc that forms. The ability of activated sludge to remove viruses is related to the ability to remove solids. This is because viruses tend to be solid associated, and are subject to removal along with the floc. Activated sludge typically removes 90% of the enteric bacteria and 90–99% of the enteroviruses and rotaviruses (Rao *et al.*, 1986). Ninety percent of *Giardia* and *Cryptosporidium* can also be removed (Rose and Carnahan, 1992), being largely concentrated in the sludge. Because of their large size, helminth eggs are effectively removed by sedimentation and are rarely found in sewage effluent in the United States, although they may be detected in the sludge. However, although the removal of the enteric pathogens may seem large, it is important to remember that initial concentrations are also large (i.e., the concentration of all enteric viruses in 1 liter of raw sewage may be as high as 100,000 in some parts of the world).

a *Primary sedimentation and disinfection.*

b_{Primary sedimentation, trickling filter or activated sludge, and disinfection.}

Primary sedimentation, trickling filter or activated sludge, disinfection, coagulation, filtration, and disinfection.

^d*Filtration only.*

TABLE 24.6 Average Removal of Pathogen and Indicator Microorganisms in a Wastewater Treatment Plant, St. Petersburg, Florida

a Escherichia coli *host ATCC 15597.*

 Tertiary treatment processes involving physicochemical processes can be effective in further reducing the concentration of pathogens and enhancing the effectiveness of disinfection processes by the removal of soluble and particulate organic matter (Table 24.6). Filtration is probably the most common tertiary treatment process. Mixed-media filtration is most effective in the reduction of protozoan parasites. Usually, greater removal of *Giardia cysts* occurs than of *Cryptosporidium* oocysts because of the larger size of the cysts (Rose and Carnahan, 1992). Removal of enteroviruses and indicator bacteria is usually 90% or less. Addition of coagulant can increase the removal of poliovirus to 99% (U.S. EPA, 1992a).

 Coagulation, particularly with lime, can result in significant reductions of pathogens. The alkaline conditions (pH 11–12) that can be achieved with lime can result in significant inactivation of enteric viruses. To achieve removals of 90% or greater, the pH should be maintained above 11 for at least an hour (Leong, 1983). Inactivation of the viruses occurs by denaturation of the viral protein coat. The use of iron and aluminum salts for coagulation can also result in 90% or greater reductions in enteric viruses. The degree of effectiveness of these processes, as in other solids separating processes, is highly dependent on the hydraulic design and, in particular, coagulation and flocculation. The degree of removal observed in bench-scale tests may not approach those seen in full-scale plants, where the process is more dynamic.

 Reverse osmosis and ultrafiltration are also believed to result in significant reductions in enteric pathogens, although few studies have been done in full-scale facilities. Removal of enteric viruses in excess of 99.9% can be achieved (Leong, 1983).

24.2.5 Removal of Organics and Inorganics by Sewage Treatment Processes

 In addition to nutrients such as nitrogen and phosphorus, and microbial pathogens, there are other constituents within sewage that need to be kept at low concentrations. These include inorganics, exemplified by metals, and organic priority pollutants. Metals and organics are normally associated with the solid fraction of sewage, and neither are significantly removed by sewage treatment. However, when point source control mechanisms are implemented to prevent industrial discharges, the concentration of metals and organics within sewage can be significantly reduced. In particular, over the past 15 years in the United States this has resulted in decreased metal concentrations. More recently there has been concern over the presence of pharmaceuticals such as endocrine disruptors in sewage.

24.3 OXIDATION PONDS

 The next two sections discuss several alternatives to largescale modern wastewater treatment process discussed in Section 24.2. The first of these are sewage lagoons, which are often referred to as oxidation or stabilization ponds. These are the oldest of the wastewater treatment systems. Usually no more than a hectare in area and just a few meters deep, oxidation ponds are natural "stew pots," where wastewater is detained while organic matter is degraded (Fig. 24.13). A period of time ranging from one to four weeks (and sometimes longer) is necessary to complete the decomposition of organic matter. Light, heat, and settling of the solids can also effectively reduce the number of pathogens present in the wastewater.

 The following four categories of oxidation ponds are often used in series:

- Aerobic ponds (Fig. 24.14A), which are naturally mixed, must be shallow (up to 1.5 m) because they depend on penetration of light to stimulate algal growth that promotes subsequent oxygen generation. The detention time of wastewater is generally 3 to 5 days.
- Anaerobic ponds (Fig. $24.14B$) may be 1 to 10 m deep, and require a relatively long detention time of 20–50 days. These ponds, which do not require expensive

FIGURE 24.13 An oxidation pond. Typically these are only 1–2 meters deep, and small in area. From Pepper, Gerba, and Brusseau, 2006.

mechanical aeration, generate small amounts of sludge. Often, anaerobic ponds serve as a pretreatment step for high-BOD organic wastes rich in protein and fat (e.g., meat wastes) with a heavy concentration of suspended solids.

- Facultative ponds (Fig. 24.15) are most common for domestic waste treatment. Waste treatment is provided by both aerobic and anaerobic processes. These ponds range in depth from 1 to 2.5 m and are subdivided in three layers: an upper aerated zone, a middle facultative zone, and a lower anaerobic zone. The detention time varies between five and 30 days.
- Aerated lagoons or ponds (Fig. 24.16), which are mechanically aerated, may be 1–2 m deep and have a detention time of less than 10 days. In general, treatment depends on the aeration time and temperature, as well as the type of wastewater. For example, at 20°C an aeration period of five days results in 85% BOD removal.

 Because sewage lagoons require a minimum of technology and are relatively low in cost, they are most common in developing countries and in small communities in the United States, where land is available at reasonable prices. However, biodegradable organic matter and turbidity are not as effectively reduced as during activated sludge treatment.

 Given sufficient retention times, oxidation ponds can cause significant reductions in the concentrations of enteric pathogens, especially helminth eggs. For this reason, they have been promoted widely in the developing world as a low-cost method of pathogen reduction for wastewater reuse for irrigation. However, a major drawback of ponds is the potential for short-circuiting because of thermal gradients even in multipond systems designed for long retention times (i.e., 90 days). Even though the amount of shortcircuiting may be small, detectable levels of pathogens can often be found in the effluent from oxidation ponds.

FIGURE 24.14 Pond profiles: (A) aerobic waste pond profile, and (B) anaerobic waste pond profile.

FIGURE 24.15 Microbiology of facultative ponds. Modified from Bitton, 2005.

 Inactivation and/or removal of pathogens in oxidation ponds is controlled by a number of factors, including temperature, sunlight, pH, bacteriophage, predation by other microorganisms, and adsorption to or entrapment by settleable solids. Indicator bacteria and pathogenic bacteria may be reduced by 90–99% or more, depending on retention times.

24.4 SEPTIC TANKS

 Until the middle of the twentieth century in the United States, many rural families and quite a few residents of towns and small cities depended on pit toilets or "outhouses" for waste disposal. In rural areas of developing countries these are still used. These pit toilets, however, often allowed untreated wastes to seep into the groundwater, allowing pathogens to contaminate drinking water supplies. This risk to public health led to the development of septic tanks and properly constructed drain fields. Primarily, septic tanks serve as repositories where solids

are separated from incoming wastewater and biological digestion of the waste organic matter can take place under anaerobic conditions. In 1997, 25% of the homes in the United States depended on septic tanks. Approximately 33% of all new homes constructed use septic tanks. Most septic tanks are located in the eastern United States (Fig. 24.17). In a typical septic tank system (Fig. 24.18), the wastewater and sewage enter a tank made of concrete, metal, or fiberglass. There, grease and oils rise to the top as scum, and solids settle to the bottom. The wastewater and sewage then undergo anaerobic bacterial decomposition, resulting in the production of a sludge. The wastewater usually remains in the septic tank for just 24–72 h, after which it is channeled out to a drain field. This drain field or leach field is composed of small perforated pipes that are embedded in gravel below the surface of the soil.

FIGURE 24.18 Septic tank (on-site treatment system). Source: U.S. **FIGURE 24.16** An aerated lagoon. EPA, 2002. From Pepper, Gerba, and Brusseau, 2006.

FIGURE 24.17 Percentage of U.S. residents utilizing septic tanks for onsite wastewater treatment. Source: U.S. Census Bureau, 1990. From Pepper, Gerba, and Brusseau, 2006.

Periodically, the residual sludge in the septic tank, known as septage, is pumped out into a tank truck and taken to a treatment plant for disposal.

 Although the concentration of contaminants in septic tank septage is typically much greater than that found in domestic wastewater (Table 24.7), septic tanks can be an

 From Pepper, Gerba, and Brusseau, 2006.

a Primarily iron (Fe), zinc (Zn), and aluminum (Al).

effective method of waste disposal where land is available and population densities are not too high. Thus, they are widely used in rural and suburban areas. As suburban population densities increase, however, groundwater and surface water pollution may arise, indicating a need to shift to a commercial municipal sewage system. (In fact, private septic systems are sometimes banned in many suburban areas.) Moreover, septic tanks are not appropriate for every area of the country. They do not work well, for example, in cold, rainy climates, where the drain field may be too wet for proper evaporation, or in areas where the water table is shallow. High densities of septic tanks can also be responsible for nitrate contamination of groundwater. Finally, most of the waterborne disease outbreaks associated with groundwater in the United States are thought to result from contamination by septic tanks.

24.5 LAND APPLICATION OF WASTEWATER

 Although treated domestic wastewater is usually discharged into bodies of water, it may also be disposed of via land application for crop irrigation or as a means of additional treatment and disposal. The three basic methods used in the application of sewage effluents to land include low-rate irrigation, overland flow, and high-rate infiltration. Characteristics of each of these are listed in Table 24.8 . The choice of a given method depends on the conditions prevailing at the site under consideration (loading rates, methods of irrigation, crops, and expected treatment).

TABLE 24.8 General Characteristics of the Three Methods Used for Land Application of Sewage Effluent

 From Pepper, Gerba, and Brusseau, 2006*.*

(B) Overland Flow

(C) High-Rate Infiltration

With low-rate irrigation (Fig. 24.19A), sewage effluents are applied by sprinkling or by surface application at a rate of 1.5 to 10 cm per week. Two-thirds of the water is taken up by crops or lost by evaporation, and the remainder percolates through the soil matrix. The system must be designed to maximize denitrification in order to avoid pollution of groundwater by nitrates. Phosphorus is immobilized within the soil matrix by fixation or precipitation. The irrigation method is used primarily by small communities and requires large areas, generally on the order of 5–6 hectares per 1000 people.

In the overland flow method (Fig. 24.19B), wastewater effluents are allowed to flow for a distance of 50–100 m along a 2–8% vegetated slope and are collected in a ditch. The loading rate of wastewater ranges from 5 to 14 cm a week. Only about 10% of the water percolates through the soil, compared with 60% that runs off into the ditch. The remainder is lost as evapotranspiration. This system requires clay soils with low permeability and infiltration.

 High-rate infiltration treatment is also known as soil aquifer treatment (SAT) or rapid infiltration extraction (RIX) (Fig. 24.19C). The primary objective of SAT is the treatment of wastewater at loading rates exceeding 50 cm per week. The treated water, most of which has percolated through coarse-textured soil, is used for groundwater recharge, or may be recovered for irrigation. This system requires less land than irrigation or overland flow methods. Drying periods are often necessary to aerate the soil system and avoid problems due to clogging. The selection of a site for land application is based on many factors including soil types, drainage and depth, distance to groundwater, groundwater movement, slope, underground formations, and degree of isolation of the site from the public.

 Inherent in land application of wastewater are the risks of transmission of enteric waterborne pathogens. The degree of risk is associated with the concentration of pathogens in the wastewater and the degree of contact with humans. Land application of wastewater is usually considered an intentional form of reuse and is regulated by most states. Because of limited water resources in the western United States, reuse is considered essential. Usually, stricter treatment and microbial standards must be met before land application. The highest degree of treatment is required when wastewater will be used for food crop irrigation, with lesser treatment for landscape irrigation or fiber crops. For example, the state of California requires no disinfection of wastewater for irrigation and no limits on coliform bacteria. However, if the reclaimed wastewater is used for surface irrigation of food crops and open landscaped areas, chemical coagulation (to precipitate suspended matter), followed by filtration and disinfection to reduce the coliform concentration to 2.2/100 ml is required. In some cities excess effluent is disposed of in riverbeds that are normally dry. Such disposal can create riparian areas (Fig. 24.20).

 Because high-rate infiltration may be practiced to recharge aquifers, additional treatments of secondary wastewater may be required. However, as some removal of pathogens can be expected, treatment requirements may be less. The degree of treatment needed may be influenced by the amount or time it takes the reclaimed water to travel from the infiltration site to the point of extraction, and the depth of the unsaturated zone. The greatest concern has been with the transport of viruses, which, because of their small size, have the greatest chance of traveling large distances within the subsurface. Factors that influence the transport of viruses are discussed in Chapter 19. Generally, several

FIGURE 24.20 Effluent outfall of the Roger Road Wastewater Treatment Plant in Tucson, Arizona. Here, extensive growth of vegetation due to the effluent produces a riparian habitat. From Pepper, Gerba, and Brusseau, 2006.

meters of moderately fine-textured, continuous soil is necessary for virus reductions of 99.9% or more (Yates, 1994).

24.6 WETLANDS AND AQUACULTURE SYSTEMS

 Wetlands, which are typically less than 1 m in depth, are areas that support aquatic vegetation and foster the growth of emergent plants such as cattails, bulrushes, reeds, sedges, and trees. They also provide important wetland habitat for many animal species. Wetland areas have been receiving increasing attention as a means of additional treatment for secondary effluents. The vegetation provides surfaces for the attachment of bacteria and aids in the filtration and removal of such wastewater contaminants as biological oxygen and excess carbon. Factors involved in the reduction of wastewater contaminants are shown in Table 24.9 . Although both natural and constructed wetlands have been used for wastewater treatment, recent work has focused on constructed wetlands because of regulatory requirements. Two types of constructed wetland systems are in general use: (1) free water surface (FWS) systems; and (2) subsurface flow systems (SFS). An FWS wetland is similar to a natural marsh because the water surface is exposed to the atmosphere. Floating and submerged plants, such as those shown in Figure 24.21A, may be present. SFS consist of channels or trenches with relatively impermeable bottoms filled with sand or rock media to support emergent vegetation.

 During wetland treatment, the wastewater is usable. It can, for instance, be used to grow aquatic plants such as water hyacinths (Fig. 24.21B) and/or to raise fish for human consumption. The growth of such aquatic plants provides not only additional treatment for the water but also a food source for fish and other animals. Such aquaculture **TABLE 24.9** Principal Removal and Transformation Mechanisms in Constructed Wetlands involved in Contaminant Reduction

From Pepper, Gerba, and Brusseau, 2006.

FIGURE 24.21 (A) Common aquatic plants used in constructed wetlands. (B) An artificial wetland system in San Diego, California, utilizing water hyacinths.

systems, however, tend to require a great deal of land area. Moreover, the health risk associated with the production of aquatic animals for human consumption in this manner must be better defined.

 There has been increasing interest in the use of natural systems for the treatment of municipal wastewater as a form of tertiary treatment (Kadlec and Knight, 1996). Artificial or constructed wetlands have a higher degree of biological activity than most ecosystems; thus transformation of pollutants into harmless by-products or essential nutrients for plant growth can take place at a rate that is useful for the treatment of municipal wastewater (Case Study 24.1). Most artificial wetlands in the United States use reeds or bulrushes, although floating aquatic plants such as water hyacinths and duckweed have also been used. To reduce potential problems with flying insects,

Case Study 24.1 Sweetwater Wetlands Infiltration–Extraction Facility in Tucson, Arizona

 Tucson, Arizona, is located in the Sonoran Desert in the southwestern United States. Because of limited water supplies reclamation of wastewater is critical. To meet water needs in the region, a system was built to provide tertiary effluents derived from an activated sludge/trickling filter system of sufficient quality to be used for landscape irrigation. The system is composed of several components that allows for various treatments and storage of tertiary effluent (Figure 24.23). A tertiary treatment plant filters the secondary effluent (to reduce turbidity and microorganisms) and provides additional disinfection. The backwash from the filters is then discharged into an artificial wetland for treatment. When the water exits the wetland it is discharged into infiltration basins, where it is further treated. In times of low reclaimed water demand (winter) the tertiary effluent may be discharged directly into the infiltration basins. The subsurface aquifer is then used as a storage facility, the water being pumped to the surface (extraction) when needed during periods of peak demand.

 The multiple barriers of conventional and natural technologies are designed to enhance the removal of chemical and microbial contaminants. Filtration of the secondary wastewater during tertiary treatment allows for reduction of the larger protozoan parasites (which are more resistant to disinfection than enteric bacteria and viruses) and more effective disinfection. In the wetlands, protozoan parasites settle out and bacteria and viruses are reduced by inactivation by sunlight (UV light) and microbial antagonism. Infiltration of the water through the soil results in further removal of pathogens by filtration and adsorption to soil particles (especially viruses).

FIGURE 24.22 Aerial view of Sweetwater Recharge Facilities. Numbered blue areas are infiltration basins. Photo courtesy of the Water Reuse Association.

subsurface flow wetlands have also been built (Fig. 24.24). In these types of wetlands all of the flow of the wastewater is below the surface of a gravel bed containing plants tolerant of water-saturated soils. Most of the existing information on the performance of these wetlands concerns coliform and fecal coliform bacteria. Kadlec and Knight (1996) have summarized the existing literature on this topic. They point out that natural sources of indicators in treatment wetlands never reach zero because wetlands are open to wildlife. Reductions in fecal coliforms are generally greater than 99%, but there is a great deal of variation, probably depending on the season, type of wetland, numbers and type of wildlife, and retention time in the wetland. Volume-based and area-based bacterial die-off models have been used to estimate bacterial die-off in surface flow wetlands (Kadlec and Knight, 1996).

 In one study of a mixed-species surface flow wetland with a detention time of approximately four days several other types of microorganisms were examined. Results showed that *Cryptosporidium* was reduced by 53%, Giardia by 58%, and enteric viruses by 98% (Karpiscak *et al.*, 1996).

FIGURE 24.23 Sweetwater artificial wetlands in Tucson, Arizona.

FIGURE 24.24 Sweetwater site, Tucson, Arizona. This is an example of a subsurface flow wetland used to treat secondary treated wastewater.

24.7 SLUDGE PROCESSING

 Primary, secondary, and even tertiary sludges generated during wastewater treatment are a major by-product of the treatment process. These sludges, in turn, are usually subjected to a variety of treatments. Raw sludge is sometimes subjected to screening to remove coarse materials including grit that cannot be broken down biologically. Thickening is usually done to increase the solids content of the sludge. This can be achieved via centrifugation, which increases the solids content to approximately 12%. Dewatering can further concentrate the solids content to 20–40%. This is normally achieved via filtration or by the use of drying beds. Conditioning enhances the separation of solids from the liquid phase. This is usually accomplished by the addition of inorganic salts such as alum, lime, ferrous or ferric salts, or synthetic organic polymers known as polyelectrolytes. All of these processes reduce the water content of the sludge, which ultimately reduces transportation costs to the final disposal and/or utilization site.

 Finally, stabilization technologies are available, reducing both the solids content of the sludge and inactivating pathogenic microbes present in the sludge.

Information Box 24.1 Advantages and Disadvantages of Anaerobic Digestion

Advantages

- No oxygen requirement, which reduces cost
- Reduced mass of biosolids due to low energy yields of anaerobic metabolism (see also Chapter 3)
- Methane produced, which can be used to generate electricity
- Enhanced degradation of xenobiotic compounds

Disadvantages

- Slower than aerobic digestion
- More sensitive to toxics

Adapted from Bitton, 2005.

24.7.1 Stabilization Technologies

24.7.1.1 Aerobic Digestion

 Aerobic digestion consists of adding air or oxygen to sludge in a 4- to 8-foot-deep open tank. The oxygen concentration within the tank must be maintained above 1 mg/l to avoid the production of foul odors. The mean residence time in the tank is 12–60 days, depending on the tank temperature. During this process, microbes aerobically degrade organic substrate, reducing the volatile solids content of the sludge by 40–50% (U.S. EPA, 1992b). Digestion temperatures are frequently moderate or mesophilic (30–40°C). By increasing the oxygen content, thermophilic digestion can be induced $(>60^{\circ}C)$. By increasing the temperature and the retention time, the degree of pathogen inactivation can be enhanced. Pathogen concentrations ultimately determine the treatment level of the product. Class B biosolids can contain many human pathogens (see Section 24.8.1). Class A biosolids, which result from more stringent and enhanced treatment, contain very low or nondetectable levels of pathogens. The degree of treatment, Class A versus Class B, has important implications on the reuse potential of the material for land application (see Section 24.8). Aerobic digestion generally results in the production of Class B biosolids.

24.7.1.2 Anaerobic Digestion

 Anaerobic microbial digestion occurs under low redox conditions, with low oxygen concentrations. Carbon dioxide is a major terminal electron acceptor used (Chapter 3) and results in the conversion of organic substrate to methane and carbon dioxide. This process reduces the volatile solids by 35–60% (Bitton, 2005), and results in the production of Class B biosolids. The advantages and disadvantages of anaerobic digestion relative to aerobic digestion are shown in Information Box 24.1.

FIGURE 24.25 Scrap timber and wood products that are frequently used as a bulking agent in biosolid composting. From Pepper, Gerba, and Brusseau, 2006.

24.7.2 Sludge Processing to Produce Class A Biosolids

 Class B biosolids that arise following digestion can be further treated to Class A levels prior to land application (see Section 24.8). The three most important technologies to achieve this goal are composting, lime treatment, and heat treatment.

24.7.2.1 Composting

Composting consists of mixing sludge with a bulking agent that normally has a high C:N ratio (Fig. 24.25). This is necessary because of the low C:N ratio of the sludge. The mixtures are normally kept moist but aerobic. These conditions result in very high microbial activity and the generation of heat that increases the temperature of the composting material. Factors affecting the composting process are shown in Information Box 24.2. There are three main types of composting systems:

- The aerated static pile process typically consists of mixing dewatered digested sludge with wood chips (Fig. 24.26). Aeration of the pile is normally provided by blowers during a 21-day composting period. During this active composting period, temperatures increase to the mesophilic range (20–40°C) where microbial degradation occurs via bacteria and fungi. Temperatures subsequently increase (to 40–80°C), with microbial populations dominated by thermophilic (heat-tolerant) and spore-forming organisms. These high temperatures inactivate pathogenic microorganisms and frequently result in a Class A biosolid product. Subsequently, the compost is cured for at least 30 days, during which time temperatures within the pile decrease to ambient levels.
- The windrow process is similar to the static pile process except that, instead of a pile, the sludge and bulking agent are laid out in long rows of dimensions $2 \text{ m} \times 3 \text{ m} \times 80 \text{ m}$ (Fig. 24.27). Aeration for windrows is provided by turning the windrows several times

Information Box 24.2 Factors Affecting Efficient Composting

 Temperature. Adequate aeration and moisture must be maintained to ensure temperatures reach 60°C, to inactivate microbial pathogens.

 Aeration. Air must be provided via blowers or by turning.

Moisture. Conditions must be neither too moist, which promotes anaerobic activity, nor too dry, which limits microbial activity.

C:N ratio. The C:N ratio of the substrate should be maintained around 25:1, to ensure adequate but not excessive amounts of nitrogen for the microbes.

Surface area of bulking agent. Shredded material should be used to increase substrate surface area for microbial metabolism.

Source: Pepper, Gerba, and Brusseau, 2006.

FIGURE 24.26 The wood bulking agent for composting. The wood is shredded to increase the surface area of the bulking agent for composting. From Pepper, Gerba, and Brusseau, 2006.

FIGURE 24.27 Biosolid composting via the windrow process. Here three windrows are illustrated. From Pepper, Gerba, and Brusseau, 2006.

a week. Once again, if the composting process is efficient, Class A biosolids are produced.

In enclosed systems the composting is conducted in steel vessels 10–15 m high by 3–4 m diameter. For this type of composting, aeration via blowers and temperature of the composting are carefully controlled. This results in a high quality Class A compost, with

little or no odor problems. However, costs of enclosed systems are higher.

24.7.2.2 Lime and Heat Treatment

Lime stabilization involves the addition of lime as $Ca(OH)_{2}$ or CaO, such that the pH of digested sludge is equal to or greater than 12 for at least 2 h. Liming is very effective at inactivating bacterial and viral pathogens, but less so for parasites (Bitton, 2005). Lime stabilization also reduces odors and can result in a Class A biosolid product.

 Heat treatment involves heating sludge under pressure to temperatures up to 260°C for 30 minutes. This process kills microbial pathogens and parasites, and also further dewaters the sludge.

24.8 LAND APPLICATION OF BIOSOLIDS AND ANIMAL WASTES: AN HISTORICAL PERSPECTIVE AND CURRENT OUTLOOK

 Use of animal wastes and manures as a fertilizer source for agricultural crop production has been practiced since the days of the Roman Empire. During the twentieth century in both the United States and Europe, small agricultural farms frequently consisted of both crop and animal production. Consequently, animal wastes were naturally land applied to enhance crop production. Although fossil fuel–based fertilizers replaced much of the use of manures following World War II, the practice continues today, particularly in developing countries.

 In the United States, land application of municipal wastewater and biosolids has been practiced for its beneficial effects and for disposal purposes since the advent of modern wastewater treatment about 100 years ago (see Information Box 24.3). In England in the 1850s, " sewage farms" were established to dispose of untreated sewage. By 1875 about 50 farms were utilizing land treatment in England, as were many others close to other major cities in Europe. In the United States, sewage farms were established by about 1900. At this same time, primary sedimentation and secondary biological treatment were introduced as a rudimentary form of wastewater treatment, and land application of sludges began. It is interesting to note that prior to wastewater treatment, "sludge" per se did not exist. Municipal sludge in Ohio was used as a fertilizer as early as 1907. Early on land application was carried out with little regard to potential pollution effects.

 Since the early 1970s, more emphasis has been placed on applying sludge to cropland at rates to supply adequate nutrients for crop growth (Hinesly *et al.*, 1972). In the 1970s and 1980s many studies were undertaken to investigate the potential benefits and hazards of land application, in both the United States and Europe. Ultimately in 1993, federal regulations were established via the Part

Information Box 24.3 Definitions of Sewage Sludge and Biosolids

 Sewage sludge. The solid, semisolid, or liquid residue generated during the treatment of domestic sewage in a treatment works.

Biosolids. EPA: The primarily organic solid product yielded by municipal wastewater treatment processes that can be beneficially recycled (whether or not they are currently being recycled).

National Research Council (2002): Sewage sludge that has been treated to meet the land-application standards in the Part 503 rule or any other equivalent land-application standards or practices.

503 Sludge Rule. This document—"The Standards for the Use or Disposal of Sewage Sludge" (EPA, 1993; 1994) was designed to "adequately protect human health and the environment from any reasonably anticipated adverse effect of pollutants." As part of these regulations, two classes of treatment were defined as Class A and Class B biosolids, with different restrictions for land applications, based on the level of treatment.

 Land application increased when restrictions were placed on ocean dumping. By the year 2000, 60% of all biosolids were land applied in the United States. Currently most U.S. land application utilizes Class B biosolids; however, due to public concerns over potential hazards, in some areas land application of Class B biosolids has been banned.

 Biosolids are applied to agricultural and nonagricultural lands as soil amendment because they can improve the chemical and physical properties of soils, and because they contain nutrients for plant growth. Land application on agricultural land is utilized to grow food crops such as corn or wheat, and nonfood crops such as cotton. Nonagricultural land application includes forests, rangelands, public parks, golf courses, and cemeteries. Biosolids are also used to revegetate severely disturbed lands such as mine tailings or strip mine areas.

24.8.1 Class A Versus Class B Biosolids

 Biosolids are divided into two classes on the basis of pathogen content: Class A and Class B (Information Box 24.4). Class A biosolids are treated to reduce the presence of pathogens to below detectable levels and can be used without any pathogen-related restrictions at the application site. Class A biosolids can also be bagged and sold to the public. Class B biosolids are also treated to reduce pathogens but still contain detectable levels of them. Class B biosolids have site restrictions to minimize the potential for human exposure until environmental factors such as heat,

Information Box 24.4 Part 503 Pathogen Density Limits

Standard Density Limits (Dry Weight)

 Pathogen or Indicator Class A Salmonella $<$ 3 MPN/4 g total solids or Fecal coliforms <1000 MPN/g and Enteric viruses \leq 1 PFU/4 g total solids and Viable helminth ova $\lt 1/4$ g total solids *Class B* Fecal coliform density <2,000,000 MPN/g total solids Adapted from U.S. EPA (2000).

 Information Box 24.5 Land Application Methods

From Pepper, Gerba, and Brusseau, 2006.

sunlight, or desiccation have further reduced pathogen numbers. Class B biosolids cannot be sold or given away in bags or other containers or used at sites with public use.

24.9 METHODS OF LAND APPLICATION OF BIOSOLIDS

 The method of land application of biosolids essentially depends on the percent solids contained within them, which determines whether the biosolids are liquid in nature or a "cake" (Information Box 24.5). Figures. 24.28, 5.12, 5.14, and 5.15 illustrate all methods of land application, which can be grouped into two categories:

- Injection. Liquid biosolids are injected to a soil depth of 6–9 inches. Injection vehicles simultaneously disc the field. Injection processes reduce odors, and bioaerosols, as well as the risk of runoff to surface waters.
- Surface application. Liquid or cake biosolids are surface applied and subsequently tilled into the soil (Fig. 24.29).

24.10 PATHOGENS OF CONCERN IN CLASS B BIOSOLIDS

 Contaminants of concern related to land application of Class B biosolids include microbes, metals, organics, and

FIGURE 24.28 Land application of liquid biosolids via a spray applicator. Courtesy I. L. Pepper.

FIGURE 24.29 An agricultural field in Tucson, Arizona, that has just received an application of biosolid material. (A) Immediately after land application. (B) The biosolids are incorporated via a tractor. From Pepper, Gerba, and Brusseau, 2006.

pharmaceuticals. The occurrence incidence, significance, and characteristics of enteric organisms are discussed in Chapter 22. Data on the incidence of many of these pathogens in Class B biosolids is lacking.

TABLE 24.10 Comparison of Antibiotic Resistant Bacteria in Environmental and Food Samples

Modified from Brooks et al., 2007

^aAmpicillin (32 μ g ml $^{-1}$), cephalothin (32 μ g ml $^{-1}$), ciprofloxacin (4 μ g ml $^{-1}$), and tetracycline (16 μ g ml $^{-1}$).

24.10.1 Other Biological Concerns with Biosolids

24.10.1.1 Antibiotic Resistant Bacteria

 Bacteria are procaryotic organisms with the ability to metabolize and replicate very quickly. They are also very adaptable genetically. When confronted with an antibiotic, there need only be one bacterial cell with a genetic or mutational change, which confers resistance to that antibiotic, that subsequently allows for the proliferation of antibiotic resistant bacteria. Thus the more that antibiotics are used, the greater the likelihood of antibiotic resistant strains developing. The greatest concern with antibiotic resistance is the potential for human pathogenic strains to become resistant to overused antibiotics, which subsequently cannot contain the infectious agent. The widespread sometimes indiscriminant use of antibiotics has raised the question: "Can antibiotic resistance genes be transferred from nonpathogenic bacteria to human pathogenic strains in the environment?"

 Brooks *et al.* (2006) evaluated the incidence of antibiotic resistant bacteria (ARB) in biosolids and a variety of other environmental samples and foodstuffs. Table 24.10 shows that Class B biosolids did not contain unusually high numbers of ARB; in fact, the relative incidence was less than that found in pristine soil. Interestingly, ARB concentrations were also lower than those found in common foodstuffs such as lettuce. Therefore, food itself could be an important route of exposure to ARB. Gene transfer events in soil are thought to be relatively infrequent without selective pressure (Neilson *et al.*, 1994), which reduces the risk of antibiotic resistance gene transfer to human pathogenic bacteria. Finally, note that soil itself is the original source of human antibiotics.

24.10.1.2 Endotoxin

 Endotoxin, or lipopolysaccharide (LPS) derived from the cell wall of gram-negative bacteria, is a highly immunogenic molecule present ubiquitously in the environment (Fig. 5.1) (Michel, 2003). Although most surfaces contain some traces of dust-associated endotoxin, it is primarily of concern as an aerosol, since most human endotoxin ailments are pulmonary associated (Sharif *et al.*, 2004). Exposures to aerosolized endotoxin have been studied regarding occupational exposures to cotton dust, composting plants, and feed houses (Castellan et al., 1987). Exposures to levels of endotoxin as low as 0.2 endotoxin unit (EU) per cubic meter derived from poultry dust have been found to cause acute pulmonary ailments such as decreases in forced expiratory volume (Donham et al., 2000). Chronic effects such as asthma and chronic bronchitis have been found to be due to exposures of endotoxin from cotton dust as little as 10 $EU/m³$ on a daily basis (Olenchock, 2001).

 Endotoxin concentrations in a variety of environmental samples were investigated by Brooks *et al.* (2006), who

TABLE 24.11 Aerosolized Endotoxin Concentrations Detected Downwind of Biosolids Operations, a Wastewater Treatment Plant Aeration Basin, and a Tractor Operation

^aEU/m³, endotoxin units per cubic meter.

^b *NA, not applicable.*

showed that the endotoxin level in Class B biosolids is similar in magnitude to that of other wastes including animal manures and compost. For example, swine barns were found to have mean concentrations of endotoxin of 4385 $EU/m³$ (Duchaine *et al.*, 2001), while composting plants ranged from 10 to 400 EU/m^3 (Clark *et al.*, 1983). Since the relevance of endotoxin to human health is via inhalation, the potential for aerosolization of endotoxin during land application of biosolids has also recently caused concern. One study shows that endotoxin values measured during biosolids application are comparable to those found in untreated agricultural soils (Table 24.11). Therefore, aerosolization of soil particles can result in endotoxin aerosolization, regardless of whether biosolids are involved (Brooks *et al.*, 2006). This is not surprising since bacterial concentrations in soil routinely exceed $10⁸$ per gram, with a majority of bacteria being gram negative. Soil particles containing sorbed microbes can be aerosolized and hence act as a source of endotoxin.

 What levels of endotoxin are considered safe? It has been suggested that no more than a maximum air concentration of 1000 EU/m³ should be considered safe until additional studies have been conducted (Rylander et al., 1983). Most of the samples from wastewater treatment plants, land application of biosolids, and composting sites contain endotoxin levels less than this maximum concentration. Further, it can be noted that despite the presence

of endotoxin within these operations, there is no evidence linking residential impact to these operations. This is likely because beyond these site boundaries endotoxin levels drop quickly to background concentrations.

24.10.1.3 Prions

 Concern about prions has arisen with the advent of prion animal diseases such as bovine spongiform encephalopathy (BSE) in the United Kingdom and other parts of Europe. The BSE prions concentrate in an animal's brain and spinal cord, but they have been detected only in sheep blood at low concentrations. Animal manure would have no or low concentrations of BSE prions except possibly for wastes from slaughterhouses (Ward *et al.*, 1984). However, the presence of prions in such wastes is uncertain (EPA, 2001). Prions are generally transmitted from animal to animal (cow to cow, sheep to sheep). The risk of prion transmission to biosolids from animals is low but can increase with the presence of small amounts of neural tissues or placenta coming from slaughterhouses. At present, there has been little evidence of prion-contaminated manures in the United States.

 Prions are very difficult to inactivate and require rigorous treatment (Godfree, 2001). The higher the solids content of the waste, the more rigorous the treatment required (EPA, 2001). Prions are resistant to high temperatures; scrapie prions are inactivated at temperatures of 100°C or above. At 121°C, only 0.01% of the prions were resistant to thermal inactivation (Rohwer, 1984). Kirchmayr *et al.* (2006) demonstrated that prions in spiked anaerobic sludge survived incubation at mesophilic (35°C) temperatures but were reduced at thermophilic (55°C) temperatures. When added to soil, prions bind strongly (Rigou et al., 2006).

24.10.2 Risks from Pathogens in Biosolids

 What are the risks from pathogens in biosolids? This is not an easy question to answer partly because there are many different types of pathogens and partly because each environment that biosolids are incorporated into is different. That said, Class B biosolids routinely contain human pathogens (principal pathogens of concern in biosolids are identified in Chapter 26). The pathogens found in a particular source of biosolids reflect the incidence of disease in the community from which the biosolids are derived. (Pepper *et al*., 2006)

 Little is currently known about emerging pathogens such as the SARS virus (causing severe acute respiratory syndrome) in biosolids. However, regardless of the pathogen of concern, the major routes of potential human exposure to pathogens in biosolids remain the same, specifically via air, soil, and water. Exposure can also occur via vectors, such as flies, and to prevent this, so-called vector-attraction reduction requirements are enforced (NRC, 2002). These involve specific biosolid treatment and rapid incorporation of land applied biosolids $(< 6 h$).

 Human exposure to pathogens via air results from the formation of aerosolized biological particles that are referred to as bioaerosols. Until relatively recently, little was known of the risk of infection from bioaerosols generated during land application of biosolids, and this topic was utilized by environmental activists to challenge the efficacy of land application. National studies across the United States, however, have demonstrated that the risk is lower than previously thought (Brooks *et al.*, 2005) (see also Chapter 5).

 In principle, pathogens originally present in biosolids applied to land can contaminate surface or groundwater. However, most soils limit the movement of microbes to groundwater. Normally, significant migration will only occur in coarse textured soils or karst topography, with a shallow depth to groundwater. Viruses have the greater possibility to migrate through soil; however, they have been found to tightly bind to biosolids, and little leaching appears to occur. No direct cause and effect has been identified in surface or groundwater near land where biosolids has been applied. Pathogen survival in and transport through soil are considered together in this section. Human pathogens that are routinely found in domestic sewage sludge include viruses, bacteria, protozoan parasites, and helminths. Of those pathogens, viruses are the smallest

and least complex, generally have a short survival period in soil, and have the greatest potential for transport in soil. Survival of viruses has been shown to be temperaturedependent and decreases as temperature increases. Soil type affects virus survival, with longer survival occurring on clay loam biosolids-amended soils than on sandy loam biosolids-amended soil. Rapid loss of soil moisture also limits virus survival.

 Like virus survival, bacterial survival in soil is affected by temperature, pH, and moisture. Soil nutrient availability also plays a role for bacteria, as do a neutral soil pH and soil at field capacity. Of the pathogenic bacteria, *Salmonella* and *Escherichia coli* can survive for a long time in biosolids-amended soil—up to 16 months for *Salmonella* . In contrast, *Shigella* has a shorter survival time than either *Salmonella* or *E. coli* . Studies on indicator organisms have shown that total and fecal coliforms as well as fecal streptococci can all survive for weeks to several months, depending on soil moisture and temperature conditions (Pepper *et al.*, 1993).

 Regrowth, an increase in numbers of pathogens, is also important when evaluating the survival of pathogenic and indicator bacteria in soil and biosolids compost. *Salmonella* , *E. coli* , and fecal coliforms are all capable of regrowth. Following land application of biosolids, regrowth of actual pathogens is negligible (Zaleski et al., 2005a). However, regrowth of *Salmonella* can occur in Class A biosolids if they are stored prior to land application and exposed to reinoculation via bird or other animal excrement (Zaleski et al., 2005b). Regrowth has also occurred after composting processes. Regrowth of fecal coliforms is more common than pathogens and has been documented following land application of Class B biosolids (Pepper *et al.*, 1993). Regrowth of *Salmonella* in Class A biosolids has been shown to occur under anaerobic conditions (Castro-del Campo *et al.*, 2007).

 The protozoan parasites often associated with biosolids include *Giardia* and *Cryptosporidium* spp. However, little research has been conducted on the survival of these parasites in biosolids-amended soil. Helminths are perhaps the most persistent of enteric pathogens. *Ascaris* eggs can survive several years in soils.

 The transport of microorganisms through soils or the vadose zone is affected by a complex array of abiotic and biotic factors, including adhesion processes, filtration effects, physiological state of the cells, soil characteristics, water flow rates, predation, and intrinsic mobility of the cells, as well as the presence of biosolids. For viruses, the potential for transport is large, although viruses can adsorb to soil colloidal particles and to the biosolids themselves, thus limiting transport. Virus sorption is controlled by the soil pH. Most viruses are negatively charged (isoelectric point 3–6), so that at a neutral soil pH soil sorption is reduced, whereas at more acidic soil pH values the viruses are positively charged, increasing sorption.

 The larger size of bacteria means that soil acts as a filter, limiting bacterial transport. Soil would also limit the transport of the even larger protozoa and helminths. However, microorganisms may be transported through soil cracks and macrochannels via preferential flow.

 Pathogen survival and transport in soil should be evaluated from a public health perspective. Pathogens are routinely present in Class B biosolids and are capable of surviving for days, weeks, or even months, depending on the organism and environment. Therefore, site restrictions with duration based on subsequent land use are necessary following land application. For many soils, contamination of aquifers due to vertical migration of pathogens from land-applied biosolids is unlikely because of the sorption of viruses and the soil filtration potential for larger pathogens. In coarse textured, sandy soil or high-permeability karst topography, however, groundwater contamination events are possible.

24.11 PATHOGENS IN ANIMAL MANURES

 Animal wastes predominantly include manures from cows, pigs, and chickens. Animal wastes are pollutants of increasing concern both to the public and to regulatory bodies because they have the potential to contaminate both surface and groundwater. Animal agricultural wastes can be divided into two production types: range and pasture production, and confined or concentrated animal production.

 In range and pasture systems, the concentration of wastes is generally much more diffuse or dispersed than it is when large numbers of animals are confined to relatively small areas. Range and pasture systems have two principal effects on surface water quality: (1) increased turbidity through the movement of soil particles into streams, rivers, and lakes; and (2) increased fecal coliform counts in areas of heavy animal use.

 In the past, animals were concentrated only intermittently. The period of confinement was a transitory phase followed by a return to pasture, after such management activities as milking or shearing. However, animal production is occurring in increasingly controlled environments owing to the success of efforts to raise productivity and diminish climatic, feeding, and mortality variables. Larger numbers of animals are being raised in concentrated animal feeding operations or CAFOs principally, feedlots, dairies, swine operations, poultry houses, and intensive aquaculture. The number of CAFOs more than doubled from 1982 to 1997, increasing from 5,000 to 11,200. Almost every county in the United States has a CAFO with more than 10,000 animals. This shift in production methods has changed the age-old method of reincorporation of animal wastes as manure on the farm where it was produced. Specialization has largely divorced animal production from the production of crops: a concentrated animal facility may be located far from crop production, and the same family (or the same corporation) may not pursue the two types of production. The production of large numbers of animals on a small land base has resulted in the stockpiling of wastes at specific locations, the construction of large wastestorage ponds, and oftentimes, waste applications to land in excess of agronomic crop needs.

 More than 150 microbial pathogens have been identified from all animal species that can be transmitted to man (Gerba and Smith, 2005). Pathogens can be transmitted from animals to humans when manure is used as a fertilizer for food crops eaten raw and by storm water runoff from manured areas or by percolation to groundwater. Pathogens commonly associated with produce and surface water contamination include *Escherichia coli* O157:H7, *Campylobacter, Salmonella, Listeria monocytogenes* , and *Cryptosporidium parvum* . Manure should be composted to effectively eliminate pathogens and applied appropriately to minimize contamination.

QUESTIONS AND PROBLEMS

- **1.** What are the three major steps in modern wastewater treatment?
- **2.** Why is it important to reduce the amount of biodegradable organic matter and nutrients during sewage treatment?
- **3.** When would tertiary treatment of wastewater be necessary?
- **4.** What are some types of tertiary treatment?
- **5.** What are the processes involved in the removal of heavy metals from wastewater during treatment by artificial wetlands.
- **6.** What are the three types of land application of wastewater? Which one is most likely to contaminate the groundwater with enteric viruses? Why? What factors determine how far viruses will be transported in groundwater? How does nitrogen removal occur? How does Phosphorus removal occur?
- **7.** What are the major contaminants in groundwater associated with the use of on-site treatment systems?
- **8.** What factors may determine the concentration of enteric pathogens in domestic raw sewage?
- **9.** Five milliliters of a wastewater sample is added to dilution water in a 300-ml BOD bottle. If the following results are obtained, what is the BOD after 3 days and 5 days?

- **10.** What is the difference between Class A and Class B biosolids? Name three processes that can be used to produce Class A biosolids.
- **11.** List some advantages and disadvantages of the wetland treatment of sewage.
- **12.** What is the major mechanism of pathogen removal during activated sludge treatment?
- **13.** What treatment processes would you need to obtain an 8-log₁₀ reduction of enteric viruses? *Giardia*?
- **14.** How effective do you think sunlight is in killing *Cryptosporidium* oocysts? Enteric viruses?

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