9 Controlling Microbial Growth in the Environment

CHAPTER SUMMARY

Basic Principles of Microbial Control (pp. 262–264)

Precise terminology is important when discussing microbial control in the environment, as is an understanding of the concept of microbial death rate and the action of antimicrobial agents.

Terminology of Microbial Control

Many terms of microbial control are familiar to the general public but often misused. Precise definitions are as follows:

In its strictest sense, **sterilization** refers to the removal or destruction of all microbes, including viruses and bacterial endospores, in or on an object. (The term does not apply to prions.) In practical terms, sterilization techniques eradicate harmful microbes, but some innocuous microbes may still be present. The term **aseptic** describes an environment or procedure that is free of contamination by pathogens.

Disinfection refers to the use of physical or chemical agents known as *disinfectants* to inhibit or destroy microorganisms, especially pathogens. It does not guarantee elimination of all pathogens, and is used only when discussing treatment of inanimate objects. When a chemical is used on skin or other tissue, the process is called **antisepsis** and the agent is an **antiseptic**. **Degerming** is the removal of microbes from a surface by scrubbing, whether that surface is human skin or a table top.

Sanitization is the process of disinfecting plates and utensils used by the public to reduce the number of pathogenic microbes to meet acceptable public health standards. Dishes are disinfected in a dishwasher at home, but are sanitized in a dishwasher in a restaurant.

Pasteurization is the use of heat to kill pathogens and reduce the number of spoilage microorganisms in food and beverages. Milk, fruit juices, wine, and beer are commonly pasteurized.

Agents or techniques that inhibit the growth of microbes without necessarily killing them are indicated by the suffix *-stasis* or *-static*. For example, refrigeration is *bacteriostatic*. By contrast, words ending in *-cide* or *-cidal* refer to agents or methods that destroy or permanently inactivate a particular type of microbe. For example, *fungicides* kill fungal hyphae, spores, and yeasts.

Microbial Death Rates

Scientists define **microbial death** as the permanent loss of reproductive ability under ideal environmental conditions. One technique for evaluating the efficacy of an antimicrobial agent is to calculate the **microbial death rate**, which is usually found to be constant over time for any particular microorganism under a particular set of conditions. When the microbial death rate is plotted on a semilogarithmic graph, this constant death rate produces a straight line.

Action of Antimicrobial Agents

The modes of action of antimicrobial agents fall into two basic categories: those that disrupt the integrity of cells by adversely altering their cell walls or cytoplasmic membranes, and those that interrupt cellular metabolism and reproduction by interfering with the structures of proteins and nucleic acids.

The Selection of Microbial Control Methods (pp. 264–266)

A perfect antimicrobial method or agent would be inexpensive, fast-acting, stable during storage, harmless to humans, and effective against all types of microbes. Since such ideals do not exist, scientists consider several factors when evaluating methods and agents.

Factors Affecting the Efficacy of Antimicrobial Methods

One factor affecting the choice of antimicrobial is the site to be treated. For example, harsh chemicals or intense heat cannot be used on human tissues. Another factor is the relative susceptibility of the microorganisms. Generally, scientists and medical personnel select a method to kill the hardiest microorganisms present, assuming that more fragile microbes will be killed as well. The most resistant microbes are bacterial endospores, species of *Mycobacterium*, and cysts of protozoa. The third factor affecting the efficacy of antimicrobials is the environmental conditions under which it is used, such as temperature and pH. For example, since chemicals react faster at higher temperatures, warm disinfectants generally work better than cool ones.

Methods for Evaluating Disinfectants and Antiseptics

Scientists have developed several methods to measure the efficacy of antimicrobial agents:

Phenol is an antiseptic used during surgery in the late 1800s. Since then, scientists have evaluated the efficacy of various disinfectants and antiseptics by calculating a ratio that compares the agent's ability to control microbes to that of phenol. This ratio is referred to as the **phenol coefficient**. A phenol coefficient greater than 1.0 indicates that an agent is more effective than phenol.

In the **use-dilution test**, a researcher dips several metal cylinders into broth cultures of bacteria, briefly dries them, then immerses each into a different dilution of the disinfectants being evaluated. After 10 minutes, the cylinders are removed and incubated. The most effective agent is the one that entirely prevents microbial growth at the highest dilution.

In-use tests provide accurate determination of an agent's efficacy under real-life conditions, such as when swabs are taken from objects in a hospital emergency department.

Physical Methods of Microbial Control (pp. 267–276)

Physical methods of microbial control include exposure of the microbes to extremes of heat and cold, desiccation, filtration, osmotic pressure, and radiation.

Heat-Related Methods

Heat is one of the older and more common means of microbial control. High temperatures denature proteins, interfere with the integrity of cytoplasmic membranes and cell walls, and disrupt the function and structure of nucleic acids. Microorganisms vary in their susceptibility to heat. The **thermal death point** is the lowest temperature that kills all cells in a broth in 10 minutes, while **thermal death time** is the time it takes to completely sterilize a particular volume of liquid at a set temperature. **Decimal reduction time (D)** is the time required to destroy 90% of the microbes in a sample.

Moist heat is more effective than dry heat because water is a better conductor of heat than air. Boiling kills the vegetative cells of bacteria and fungi, the trophozoites of protozoa, and most viruses within 10 minutes at sea level. It is not effective when true sterilization is required. In such cases, autoclaving is required. An **autoclave** is a device consisting of a pressure chamber, pipes, valves, and gauges, that uses steam heat under pressure to sterilize chemicals and objects that can tolerate moist heat.

Pasteurization, a method of heating foods to kill pathogens and control spoilage organisms without altering the quality of the food, can be achieved by several methods: the historical (batch) method at 63°C for 30 minutes, flash pasteurization at 72°C for 15 seconds, and ultrahigh-temperature pasteurization at 134°C for 1 second.

For substances such as powders and oils that cannot be sterilized by moist heat, sterilization can be achieved by the use of dry heat at much higher temperatures for longer times. Complete incineration is the ultimate means of sterilization.

Refrigeration and Freezing

Refrigeration between 0°C and 7°C halts the growth of most pathogens, which are predominantly mesophiles. Slow freezing at temperatures below 0°C is effective in inhibiting microbial metabolism; however, many vegetative bacterial cells, bacterial endospores, and viruses can survive subfreezing temperatures for years.

Desiccation and Lyophilization

Desiccation, or drying, has been used for thousands of years to preserve such foods as fruits, peas, and yeast. It inhibits microbial growth because metabolism requires liquid water. **Lyophilization**, or freeze-drying, preserves microbes and other cells for many years. In this process, scientists freeze a culture in liquid nitrogen or frozen carbon dioxide, then remove the water via a vacuum. Lyophilization prevents the formation of large damaging ice crystals, leaving enough viable cells to enable the culture to be reconstituted many years later.

Filtration

When used as a method of microbial control, **filtration** is the passage of air or a liquid through a material that traps and removes microbes. Some membrane filters manufactured of nitrocellulose or plastic have pores small enough to trap the smallest viruses and even some large protein molecules. *HEPA (high-efficiency particulate air)* filters remove microbes and particles from air.

Osmotic Pressure

High concentrations of salt or sugar inhibit microbial growth by **osmotic pressure**, drawing out of cells the water they need to carry out their metabolic functions. Honey, jams, salted fish, and pickles are examples of foods preserved by osmotic pressure. Fungi have a greater tolerance for hypertonic environments than bacteria, which explains why refrigerated jams may grow mold.

Radiation

There are two types of **radiation**: *Particulate radiation* consists of high-speed subatomic particles freed from their atoms, whereas *electromagnetic radiation* is atomic energy without mass traveling at the speed of light. **Ionizing radiation** is electromagnetic radiation with wavelengths shorter than 1 nm, such as electron beams, gamma rays, and X-rays. It creates ions that produce effects leading to the denaturation of important molecules and cell death. **Nonionizing radiation,** such as ultraviolet light, visible light, infrared light, and radio waves, has wavelengths longer than 1 nm. Of these types, only ultraviolet light has sufficient energy to be a practical antimicrobial agent. It causes pyrimidine dimers, which can kill affected cells.

Chemical Methods of Microbial Control (pp. 276–281)

Eight major categories of antimicrobial chemicals are used as antiseptics and disinfectants.

Phenol and Phenolics

Phenolics are compounds derived from phenol molecules that have been chemically modified by the addition of halogens or organic functional groups such as chlorine. They are intermediate- to low-level disinfectants that denature proteins and disrupt cell membranes in a wide variety of pathogens.

Alcohols

Alcohols such as isopropanol (rubbing alcohol) denature proteins and disrupt cell membranes; they are used either as 70–90% aqueous solutions or in a *tincture*, which is a combination of an alcohol and another antimicrobial agent. Alcohols are bactericidal, fungicidal, and virucidal against enveloped viruses; however, they are not effective against fungal spores or bacterial endospores. They are considered intermediate-level disinfectants.

Halogens

Halogens are the four very reactive, nonmetallic chemical elements: iodine, chlorine, bromine, and fluorine. Halogens are used as intermediate-level disinfectants and antiseptics to kill microbes in water or on medical instruments or skin. Although their exact mode of action is unknown, they are believed to denature enzymes. Iodine is used medically, whereas chlorine is more commonly used by municipalities to treat drinking water supplies, wastewater, and swimming pools. In hot tubs, bromine is more effective than chlorine, because it evaporates more slowly at high temperatures.

Oxidizing Agents

Oxidizing agents such as hydrogen peroxide, ozone, and peracetic acid are high-level disinfectants and antiseptics that release oxygen radicals, which are toxic to many microbes, especially anaerobes. Hydrogen peroxide can disinfect and even sterilize surfaces, but it is not useful in the treatment of open wounds, because it is quickly neutralized by the catalase enzyme released from damaged human cells. Some Canadian and European municipalities use ozone rather than chlorine to treat their drinking water because ozone is more effective as an antimicrobial and does not produce carcinogenic by-products. Peracetic acid is an extremely effective sporicide used to sterilize equipment.

Surfactants

Surfactants are "surface active" chemicals. They include *soaps*, molecules that have both hydrophobic ends which act primarily to break up oils during degerming, and negatively-charged hydrophilic ends that attract water. *Detergents* are positively-charged organic surfactants such as **quaternary ammonium compounds** (**quats**), which disrupt cellular membranes. However, quats are considered low-level disinfectants because they are not effective against mycobacteria, endospores, or nonenveloped viruses, and some pathogens actually thrive in them.

Heavy Metals

Heavy metal ions such as arsenic, silver, mercury, copper, and zinc are low-level disinfectants that denature proteins. For most applications, they have been superceded by less-toxic alternatives, but silver still plays an antimicrobial role in some surgical dressings, burn creams, and catheters.

Aldehydes

Aldehydes are compounds containing terminal —CHO groups. Classified as high-level disinfectants, they cross-link organic functional groups in proteins and DNA. A 2% solution of glutaraldehyde and a 37% aqueous solution of formaldehyde (called *formalin*) are used to disinfect or sterilize medical or dental equipment and in embalming fluid.

Gaseous Agents

Many items, such as plastic laboratory ware, artificial heart valves, mattresses, and dried foods, cannot be sterilized easily with heat or water-soluble chemicals, nor is irradiation always practical. However, such items can be sterilized within a closed chamber containing highly reactive gases such as *ethylene oxide*, *propylene oxide*, and *beta-propiolactone*, which denature proteins and DNA by cross-linking organic functional groups, thereby killing everything they contact without harming inanimate objects. These gases are explosive and potentially carcinogenic.

Antimicrobials

Antimicrobials include *antibiotics*, which are produced naturally by microorganisms, *semisynthetics*, which are chemically modified antibiotics, and *synthetics*, which are wholly synthetic antimicrobial drugs. These compounds are typically used to treat disease, but can also function as intermediate-level disinfectants.

Development of Resistant Microbes

There is little evidence that the extensive use of antimicrobial chemicals in household cleansers and personal care products enhances human health; however, it does promote the development of strains of microbes resistant to antimicrobial chemicals. This is because, when susceptible cells die, they reduce competition for resources, allowing any remaining resistant cells to proliferate. Many experts therefore recommend limiting the use of such chemicals to food handling and health care involving high-risk patients and newborns.

KEY THEMES

As we have seen so far in our studies, microbes are very durable organisms, capable of surviving almost everywhere. In nature, this is fine, but this hardiness becomes extremely problematic when we try to confront those microbes that are capable of making us sick. As you study this chapter, focus on this key observation:

- *Microbial control is one of the fundamental drives of microbiology:* By intensive study, we have learned much about microorganisms, their structure, and how they survive. While this understanding helps us to better understand the biological world, it also provides us with the tools we need to develop physical and chemical methods of controlling their growth around us, on us, and inside of us.

QUESTIONS FOR FURTHER REVIEW

Answers to these questions can be found in the answer section at the back of this study guide. Refer to the answers only after you have attempted to solve the questions on your own.

Multiple Choice

1. Which of the following would not be destroyed by sterilization?
   a. Bacteria
   b. Prions
   c. Viruses
   d. All could be destroyed by sterilization
2. Which microbial control method below could be used directly on a hospital patient?
   a. Degerming
   b. Sanitization
   c. Antisepsis
   d. Both a and c
3. Assume that a chemical control agent causes missense mutations within the bacterial population it is used against. The agent is:
   a. Bacteriocidal
   b. Bacteriostatic
   c. Either bacteriocidal or bacteriostatic
   d. Neither bacteriocidal nor bacteriostatic
4. Which of the following evaluation techniques is no longer used to gauge the effectiveness of antiseptics?
   a. Phenol coefficient
   b. Use-dilution test
   c. In-use test
   d. None of the above
5. Which test below is actually used to determine the effectiveness of chemicals in the environment?
   a. Phenol coefficient
   b. Use-dilution test
   c. In-use test
   d. None of the above
6. Endospores are least likely to survive which heating method?
   a. Boiling
   b. Autoclaving
   c. Pasteurization
   d. Endospores can routinely survive all of these methods

7. Moist and dry heat generally work to kill microbes by:
   a. Physically destroying/denaturing cellular proteins
   b. Physically incinerating the cells
   c. Physically denaturing nucleic acids
   d. Inhibiting cellular processes by allosterically modifying proteins
8. Which method below would allow for the most effective preservation of viable microbial samples over long periods of storage?
   a. Slow-freezing
   b. Dessication
   c. Lyophilization
   d. Refrigeration
9. Assume you needed to remove all of the microbes from a very large volume of liquid. Which method below would be the most effective and efficient in doing this?
   a. Autoclaving
   b. Filtration
   c. Radiation
   d. Pasteurization
10. Which of the following physical methods of microbial control is not used in the food industry?
    a. Pressure cooking
    b. Pasteurization
    c. Osmotic pressure
    d. Nonionizing radiation
11. Chemical methods of microbial control would be least effective against which microbial entity?
    a. Enveloped virus
    b. Fungal cell
    c. Bacterial endospore
    d. Nonenveloped virus
12. Which chemical control agent listed below works against the broadest spectrum of microbes?
    a. Phenols
    b. Alcohols
    c. Halogens
    d. Oxidizing agents
13. Soaps, by themselves, are examples of:
    a. Surfactants
    b. Degerming agents
    c. Antiseptics
    d. Both a and b
14. Which chemical control agent listed below has the highest disinfectant classification?
    a. Heavy metals
    b. Halogens
    c. Aldehydes
    d. Phenolics
15. Which chemical control agent listed below is not an antiseptic?
    a. Ethylene oxide
    b. Detergent
    c. Rubbing alcohol
    d. Iodine

Fill in the Blanks

1. Bleach is an example of a chemical ____________________.
2. If an antibiotic kills a bacterial pathogen, it is said to be ______________;

   if it only inhibits the growth of the bacterial pathogen, it is said to be

   ____________________.

3. Temperature generally ____________________ (increases/decreases) the effectiveness of antimicrobial agents.
4. ____________________ is the lowest temperature that will kill all of the cells (sterilize) in a broth culture in 10 minutes.
5. ____________________ heat is more effective in killing microbes than ____________________ heat.
6. ____________________ and ____________________ bacteria are capable of surviving pasteurization, but they are generally not pathogenic and so don't make us sick even if they remain in food or beverages.
7. The two types of radiation that can be used to control microbial populations are ____________________ and ____________________.
8. Triclosan is a ____________________ found in many consumer products such as hand soaps.
9. Iodine is used two ways medically, either as a ____________________ or as an ____________________.
10. ____________________ are antimicrobial chemicals that are made naturally by certain microorganisms.

Short-Answer Questions for Thought and Review

1. List the following microbial control techniques in order of effectiveness with the least effective at removing microbes listed first, and the most effective listed last: pasteurization, degerming, sterilization, disinfection, sanitization.

2. Summarize the two modes of action that chemical and physical microbial control agents/processes use against cells. What, in general, happens in each and why is this a problem for the microbes?

3. What properties do chemical control agents with high activities have that make them effective?

Critical Thinking

1. A nursing home wishes to test various disinfectants to find the ones that are most effective. Which evaluation method do you think would be most valuable in identifying the disinfectant that works best against the microorganisms in the nursing home, and why?
2. Which methods of microbial control inhibit microbial growth by effectively removing water from the microbes? Describe specifically how each method works.
3. Though there is no firm proof yet that antimicrobial soaps are leading to increases in resistance among microbes, the CDC still recommends limiting the use of these agents. Why?

Concept Building Questions

1. Figure 9.2 presents the relative susceptibilities of microbes to antimicrobial drugs. Explain, in terms of what you have learned about structure in Chapter 3, what the fundamental structural differences are between the more susceptible microbes and the least susceptible microbes. What specifically must a high level germicide do to kill the most resistant microbes?
2. Based on what you have learned in previous chapters about chemical bonding, microbial structure, function, metabolism, and genetics, list some possible mechanisms by which a bacterial cell could become resistant to a chemical such as triclosan. Give an example from each topic listed in this question.