Control of Microorganisms by Physical and Chemical Agents

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Introduction

- The ability to control microbial populations on inanimate objects, like eating utensils and surgical instruments, is of considerable practical importance.
- Sometimes it is necessary to eliminate all microorganisms from an object, whereas only partial destruction of the microbial population may be required in other situations.
- **Sterilization** [Latin *sterilis,* unable to produce offspring or barren] is the process by which all living cells, viable spores, viruses, and viroids are either destroyed or removed from an object or habitat.
- When sterilization is achieved by a chemical agent, the chemical is called a **sterilant**.
- **Disinfection** is the killing, inhibition, or removal of microorganisms that may cause disease.
- The primary goal is to destroy potential pathogens, but disinfection also substantially reduces the total microbial population.
- **Disinfectants** are agents, usually chemical, used to carry out disinfection and are normally used only on inanimate objects.
- **Sanitization** is closely related to disinfection.
- In sanitization, the microbial population is reduced to levels that are considered safe by public health standards.

…Introduction

- **Antisepsis** is the prevention of infection or sepsis and is accomplished with **antiseptics.**
- These are chemical agents applied to tissue to prevent infection by killing or inhibiting pathogen growth; they also reduce the total microbial population.
- Substances that kill organisms often have the suffix –cide.
- A disinfectant or antiseptic can be particularly effective against a specific group, in which case it may be called a **bactericide, fungicide, algicide,** or **viricide.**
- Other chemicals do not kill, but they do prevent growth. If these agents are removed, growth will resume.
- Their names end in -static —for example, **bacteriostatic** and **fungistatic.**

The Use of Physical Methods in Control: Heat

- Either **moist** or **dry heat** may be applied.
- Moist heat readily kills viruses, bacteria, and fungi.
- Exposure to boiling water for 10 minutes is sufficient to destroy vegetative cells and eucaryotic spores.
- Unfortunately the temperature of boiling water (100°C or 212°F) is not high enough to destroy bacterial endospores that may survive hours of boiling.
- Therefore boiling can be used for disinfection of drinking water and objects not harmed by water, but boiling does not sterilize.
- Initially effectiveness was expressed in terms of **thermal death point (TDP)**, the lowest temperature at which a microbial suspension is killed in 10 minutes.
- Because TDP implies that a certain temperature is immediately lethal despite the conditions, **thermal death time (TDT)** is now more commonly used.
- This is the shortest time needed to kill all organisms in a microbial suspension at a specific temperature and under defined conditions.
- However, such destruction is logarithmic, and it is theoretically not possible to “completely destroy” microorganisms in a sample, even with extended heating.
- Therefore an even more precise figure, the **decimal reduction time (*D*)** or ***D* value** has gained wide acceptance.

D-Value, z-Value and F-value

- The decimal reduction time is the time required to kill 90% of the microorganisms or spores in a sample at a specified temperature.
- In a semilogarithmic plot of the population remaining versus the time of heating, the *D* value is the time required for the line to drop by one log cycle or tenfold (Figure 7.1).
- *D* values are used to estimate the relative resistance of a microorganism to different temperatures through calculation of the ***z* value.**
- The *z* value is the increase in temperature required to reduce *D* to 1/10 its value or to reduce it by one log cycle when log *D* is plotted against temperature (**figure 7.2**).
- Another way to describe heating effectiveness is with the *F* value.
- The ***F* value** is the time in minutes at a specific temperature (usually 250°F or 121.1°C) needed to kill a population of cells or spores.

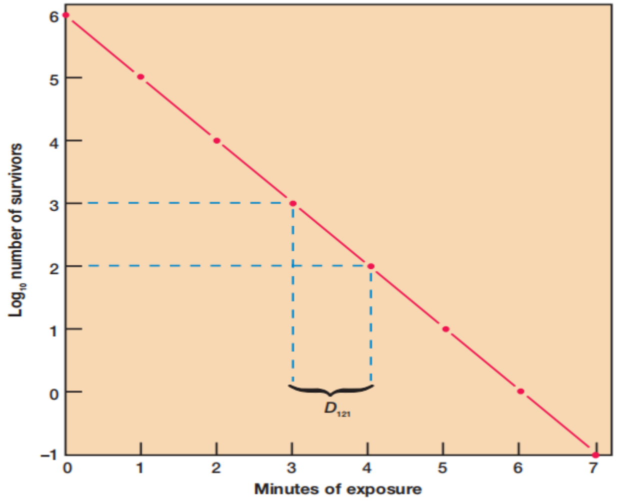

Figure 7.1 The Pattern of Microbial Death. An exponential plot of the survivors versus the minutes of exposure to heating at 121°C. In this example the D_{121} value is 1 minute. The data are from table 7.1.

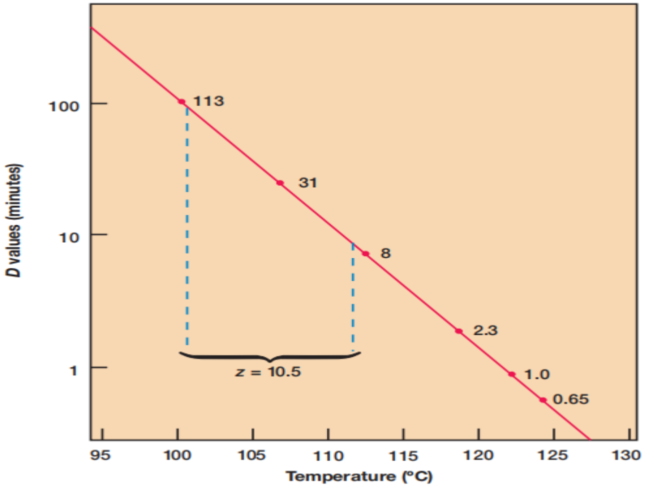

Figure 7.2 *z* Value Calculation. The *z* value used in calculation of time-temperature relationships for survival of a test microorganism, based on *D* value responses at various temperatures. The *z* value is the increase in temperature needed to reduce the decimal reduction time (*D*) to 10% of the original value. For this homogeneous sample of a test microorganism the *z* value is 10.5°. The *D* values are plotted on a logarithmic scale.

Moist Heat Sterilization

- Moist heat sterilization must be carried out at temperatures above 100°C in order to destroy bacterial endospores, and this requires the use of saturated steam under pressure.
- Steam sterilization is carried out with an **autoclave**, a device somewhat like a fancy pressure cooker.
- The development of the autoclave by Chamberland in 1884 tremendously stimulated the growth of microbiology.
- Water is boiled to produce steam, which is released through the jacket and into the autoclave’s chamber.
- The air initially present in the chamber is forced out until the chamber is filled with saturated steam and the outlets are closed.
- Hot, saturated steam continues to enter until the chamber reaches the desired temperature and pressure, usually 121°C and 15 pounds of pressure.
- At this temperature saturated steam destroys all vegetative cells and endospores in a small volume of liquid within 10 to 12 minutes.
- Treatment is continued for about 15 minutes to provide a margin of safety.
- Moist heat is thought to kill so effectively by degrading nucleic acids and by denaturing enzymes and other essential proteins.
- It also may disrupt cell membranes.

Pasteurization

- Many substances, such as milk, are treated with controlled heating at temperatures well below boiling, a process known as **pasteurization** in honor of its developer Louis Pasteur.
- Pasteur examined spoiled wine under the microscope and detected microorganisms that looked like the bacteria responsible for lactic acid and acetic acid fermentations.
- He then discovered that a brief heating at 55 to 60°C would destroy these microorganisms and preserve wine for long periods.
- In 1886 the German chemists V. H. and F. Soxhlet adapted the technique for preserving milk and reducing milk transmissible diseases.
- Milk pasteurization was introduced into the United States in 1889.
- Milk can be pasteurized in two ways. In the older method the milk is held at 63°C for 30 minutes.
- Large quantities of milk are now usually subjected to **flash pasteurization** or **high-temperature short-term (HTST) pasteurization**, which consists of quick heating to about 72°C for 15 seconds, then rapid cooling.
- The dairy industry also sometimes uses **ultrahigh-temperature (UHT) sterilization.** Milk and milk products are heated at 140 to 150°C for 1 to 3 seconds.
- UHT-processed milk does not require refrigeration and can be stored at room temperature for about 2 months without flavor changes.

Dry heat sterilization

- Many objects are best sterilized in the absence of water by **dry heat sterilization.**
- The items to be sterilized are placed in an oven at 160 to 170°C for 2 to 3 hours.
- Microbial death apparently results from the oxidation of cell constituents and denaturation of proteins.
- Although dry air heat is less effective than moist heat—*Clostridium botulinum* spores are killed in 5 minutes at 121°C by moist heat but only after 2 hours at 160°C with dry heat.
- Dry heat does not corrode glassware and metal instruments as moist heat does, and it can be used to sterilize powders, oils, and similar items.
- Most laboratories sterilize glass petri dishes and pipettes with dry heat.
- Despite these advantages, dry heat sterilization is slow and not suitable for heatsensitive materials like many plastic and rubber items.

Low Temperatures

- Often the most convenient control technique is to inhibit their growth and reproduction by the use of either freezing or refrigeration.
- This approach is particularly important in food microbiology.
- Freezing items at -20°C or lower stops microbial growth because of the low temperature and the absence of liquid water.
- Some microorganisms will be killed by ice crystal disruption of cell membranes, but freezing does not destroy contaminating microbes.
- Refrigeration greatly slows microbial growth and reproduction, but does not halt it completely.
- Fortunately most pathogens are mesophilic and do not grow well at temperatures around 4°C.
- Refrigerated items may be ruined by growth of psychrophilic and psychrotrophic microorganisms, particularly if water is present.
- Thus refrigeration is a good technique only for shorter-term storage of food and other items.

Filtration

- Filtration is an excellent way to reduce the microbial population in solutions of heat-sensitive material, and sometimes it can be used to sterilize solutions.
- There are two types of filters.
- **Depth filters:** consist of fibrous or granular materials that have been bonded into a thick layer filled with twisting channels of small diameter.
- The solution containing microorganisms is sucked through this layer under vacuum, and microbial cells are removed by physical screening or entrapment and also by adsorption to the surface of the filter material.
- Depth filters are made of diatomaceous earth (Berkefield filters), unglazed porcelain (Chamberlain filters), asbestos, or other similar materials.
- **Membrane filters:** These circular filters are porous membranes, a little over 0.1 mm thick, made of cellulose acetate, cellulose nitrate, polycarbonate, polyvinylidene fluoride, or other synthetic materials.
- Although a wide variety of pore sizes are available, membranes with pores about 0.2 m in diameter are used to remove most vegetative cells, but not viruses, from solutions ranging in volume from 1 ml to many liters.
- The solution is pulled or forced through the filter with a vacuum or with pressure from a syringe, peristaltic pump, or nitrogen gas bottle, and collected in previously sterilized containers.
- These filters are used to sterilize pharmaceuticals, ophthalmic solutions, culture media, oils, antibiotics, and other heat-sensitive solutions.

…Filtration

- Air also can be sterilized by filtration.
- Two common examples are surgical masks and cotton plugs on culture vessels that let air in but keep microorganisms out.
- **Laminar flow biological safety cabinets** employing **high-efficiency particulate air (HEPA) filters,** which remove 99.97% of 0.3 m particles, are one of the most important air filtration systems.
- Laminar flow biological safety cabinets force air through HEPA filters, then project a vertical curtain of sterile air across the cabinet opening.
- This protects a worker from microorganisms being handled within the cabinet and prevents contamination of the room.

Radiation

- **Ultraviolet (UV) radiation** around 260 nm is quite lethal but does not penetrate glass, dirt films, water, and other substances very effectively.
- UV lamps are sometimes placed on the ceilings of rooms or in biological safety cabinets to sterilize the air and any exposed surfaces.
- Because UV radiation burns the skin and damages eyes, people working in such areas must be certain the UV lamps are off when the areas are in use.
- **Ionizing radiation** is an excellent sterilizing agent and penetrates deep into objects.
- It will destroy bacterial endospores and vegetative cells, both procaryotic and eucaryotic; however, ionizing radiation is not always as effective against viruses.
- Gamma radiation from a cobalt 60 source is used in the cold sterilization of antibiotics, hormones, sutures, and plastic disposable supplies such as syringes.
- Gamma radiation has also been used to sterilize and“pasteurize” meat and other food.
- Both the Food and Drug Administration and the World Health Organization have approved food irradiation and declared it safe.

Questions

1. Define thermal death point (TDP), thermal death time (TDT), decimal reduction time (*D*) or *D* value, *z* value, and the *F* value.
2. Describe how an autoclave works. What conditions are required for sterilization by moist heat, and what three things must one do when operating an autoclave to help ensure success?
3. How are pasteurization, flash pasteurization, ultrahigh temperature sterilization, and dry heat sterilization carried out? Give some practical applications for each of these procedures.
4. How can low temperature be used to control microorganisms?
5. What are depth filters and membrane filters, and how are they used to sterilize liquids? Describe the operation of a biological safety cabinet.
6. Give the advantages and disadvantages of ultraviolet light and ionizing radiation as sterilizing agents. Provide a few examples of how each is used for this purpose.

The Use of Chemical Agents in Control

- Many different chemicals are available for use as disinfectants, and each has its own advantages and disadvantages.
- In selecting an agent, it is important to keep in mind the characteristics of a desirable disinfectant.
- Although the chemical must be toxic for infectious agents, it should not be toxic to people or corrosive for common materials.
- Although the chemical must be toxic for infectious agents, it should not be toxic to people or corrosive for common materials.
- The disinfectant should be stable upon storage, odorless or with a pleasant odor, soluble in water and lipids for penetration into microorganisms, and have a low surface tension so that it can enter cracks in surfaces.
- If possible the disinfectant should be relatively inexpensive.

Phenolics

- Phenol was the first widely used antiseptic and disinfectant.
- In 1867 Joseph Lister employed it to reduce the risk of infection during operations.
- Today phenol and phenolics (phenol derivatives) such as cresols, xylenols, and orthophenyl phenol are used as disinfectants in laboratories and hospitals.
- Phenolics act by denaturing proteins and disrupting cell membranes.
- They have some real advantages as disinfectants: phenolics are tuberculocidal, effective in the presence of organic material, and remain active on surfaces long after application.
- However, they do have a disagreeable odor and can cause skin irritation.

Table 7.5 Relative Efficacy of Commonly Used Disinfectants and Antiseptics

Class	Disinfectant	Antiseptic	Comment
Gas			
Ethylene oxide	3–4[a]	0[a]	Sporicidal; toxic; good penetration; requires relative humidity of 30% or more; microbicidal activity varies with apparatus used; absorbed by porous material; dry spores highly resistant; moisture must be present, and presoaking is most desirable
Liquid			
Glutaraldehyde, aqueous	3	0	Sporicidal; active solution unstable; toxic
Stabilized hydrogen peroxide	3	0	Sporicidal; use solution stable up to 6 weeks; toxic orally and to eyes; mildly skin toxic; little inactivated by organic matter
Formaldehyde + alcohol	3	0	Sporicidal; noxious fumes; toxic; volatile
Formaldehyde, aqueous	1–2	0	Sporicidal; noxious fumes; toxic
Phenolic compounds	3	0	Stable; corrosive; little inactivation by organic matter; irritates skin
Chlorine compounds	1–2	0	Fast action; inactivation by organic matter; corrosive; irritates skin
Alcohol	1	3	Rapidly microbicidal except for bacterial spores and some viruses; volatile; flammable; dries and irritates skin
Iodine + alcohol	0	4	Corrosive; very rapidly microbicidal; causes staining; irritates skin; flammable
Iodophors	1–2	3	Somewhat unstable; relatively bland; staining temporary; corrosive
Iodine, aqueous	0	2	Rapidly microbicidal; corrosive; stains fabrics; stains and irritates skin
Quaternary ammonium compounds	1	0	Bland; inactivated by soap and anionics; compounds absorbed by fabrics; old or dilute solution can support growth of gram-negative bacteria
Hexachlorophene	0	2	Bland; insoluble in water, soluble in alcohol; not inactivated by soap; weakly bactericidal
Chlorhexidine	0	3	Bland; soluble in water and alcohol; weakly bactericidal
Mercurial compounds	0	±	Bland; much inactivated by organic matter; weakly bactericidal

Alcohols and Halogens

- **Alcohols:** They are bactericidal and fungicidal but not sporicidal; some lipid-containing viruses are also destroyed.
- The two most popular alcohol germicides are ethanol and isopropanol, usually used in about 70 to 80% concentration.
- They act by denaturing proteins and possibly by dissolving membrane lipids.
- A 10 to 15 minute soaking is sufficient to disinfect thermometers and small instruments.
- **Halogens:** any of the five elements (fluorine, chlorine, bromine, iodine, and astatine) in group VIIA of the periodic table.
- The halogens iodine and chlorine are important antimicrobial agents.
- Iodine is used as a skin antiseptic and kills by oxidizing cell constituents and iodinating cell proteins. At higher concentrations, it may even kill some spores.
- Iodine has been complexed with an organic carrier to form an **iodophor.**
- Iodophors are water soluble, stable, and nonstaining, and release iodine slowly to minimize skin burns and irritation.
- They are used in hospitals for preoperative skin degerming and in hospitals and laboratories for disinfecting.

…Halogens

- Chlorine is the usual disinfectant for municipal water supplies and swimming pools and is also employed in the dairy and food industries.
- It may be applied as chlorine gas, sodium hypochlorite, or calcium hypochlorite, all of which yield hypochlorous acid (HClO) and then atomic oxygen.
- The result is oxidation of cellular materials and destruction of vegetative bacteria and fungi, although not spores.

$$Cl_2 + H_2O \longrightarrow HCl + HClO$$

$$Ca(OCl)_2 + 2H_2O \longrightarrow Ca(OH)_2 + 2HClO$$

$$HClO \longrightarrow HCl + O$$

- One potential problem is that chlorine reacts with organic compounds to form carcinogenic trihalomethanes, which must be monitored in drinking water.

Heavy Metals

- For many years the ions of heavy metals such as mercury, silver, arsenic, zinc, and copper were used as germicides.
- A 1% solution of silver nitrate is often added to the eyes of infants to prevent ophthalmic gonorrhea (in many hospitals, erythromycin is used instead of silver nitrate because it is effective against *Chlamydia* as well as *Neisseria*).
- Silver sulfadiazine is used on burns. Copper sulfate is an effective algicide in lakes and swimming pools.
- Heavy metals combine with proteins, often with their sulfhydryl groups, and inactivate them. They may also precipitate cell proteins.

Quaternary Ammonium Compounds

- **Detergents** are organic molecules that serve as wetting agents and emulsifiers because they have both polar hydrophilic and nonpolar hydrophobic ends.
- Although anionic detergents have some antimicrobial properties, only cationic detergents are effective disinfectants.
- The most popular of these disinfectants are quaternary ammonium compounds characterized by a positively charged quaternary nitrogen and a long hydrophobic aliphatic chain.
- They disrupt microbial membranes and may also denature proteins.
- Cationic detergents like benzalkonium chloride and cetylpyridiniumchloride kill most bacteria but not *M. tuberculosis* or endospores.
- Cationic detergents are often used as disinfectants for food utensils and small instruments and as skin antiseptics.

Aldehydes

- Both of the commonly used aldehydes, formaldehyde and glutaraldehyde, are highly reactive molecules that combine with nucleic acids and proteins and inactivate them, probably by crosslinking and alkylating molecules.
- They are sporicidal and can be used as chemical sterilants.
- Formaldehyde is usually dissolved in water or alcohol before use.
- A 2% buffered solution of glutaraldehyde is an effective disinfectant.
- It is less irritating than formaldehyde and is used to disinfect hospital and laboratory equipment.

Sterilizing Gases

- Many heat-sensitive items such as disposable plastic petri dishes and syringes, heart-lung machine components, sutures, and catheters are now sterilized with ethylene oxide gas.
- Ethylene oxide (EtO) is both microbicidal and sporicidal and kills by combining with cell proteins.
- It is a particularly effective sterilizing agent because it rapidly penetrates packing materials, even plastic wraps.
- Betapropiolactone (BPL) is occasionally employed as a sterilizing gas. In the liquid form it has been used to sterilize vaccinesand sera.
- BPL decomposes to an inactive form after several hours and is therefore not as difficult to eliminate as EtO.
- It also destroys microorganisms more readily than ethylene oxide but does not penetrate materials well and may be carcinogenic.
- For these reasons, BPL has not been used as extensively as EtO.
- Recently vapor-phase hydrogen peroxide has been used to decontaminate biological safety cabinets.

Questions

- Describe each of the following agents in terms of its chemical nature, mechanism of action, mode of application, common uses and effectiveness, and advantages and disadvantages:
  - phenolics,
  - alcohols,
  - halogens (iodine and chlorine),
  - heavy metals,
  - Quaternary ammonium compounds,
  - aldehydes,
  - ethylene oxide.