Chapter 9: Control of Microbial Growth

1. Physical Methods

2. Chemical methods
# Important Terminology (pg. 263-264)

**TABLE 7.1** Terminology Relating to the Control of Microbial Growth

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sterilization</strong></td>
<td>Destruction or removal of all forms of microbial life, including endospores.</td>
<td>Usually done by steam under pressure or a sterilizing gas such as ethylene oxide.</td>
</tr>
<tr>
<td><strong>Commercial Sterilization</strong></td>
<td>Sufficient heat treatment to kill endospores of <em>Clostridium botulinum</em> in canned food.</td>
<td>More-resistant endospores of thermophilic bacteria may survive, but they will not germinate and grow under normal storage conditions.</td>
</tr>
<tr>
<td><strong>Disinfection</strong></td>
<td>Destruction of vegetative pathogens.</td>
<td>May make use of physical or chemical methods.</td>
</tr>
<tr>
<td><strong>Antisepsis</strong></td>
<td>Destruction of vegetative pathogens on living tissue.</td>
<td>Treatment is almost always by chemical antimicrobials.</td>
</tr>
<tr>
<td><strong>Degerming</strong></td>
<td>Removal of microbes from a limited area, such as the skin around an injection site.</td>
<td>Mostly a mechanical removal by an alcohol-soaked swab.</td>
</tr>
<tr>
<td><strong>Sanitization</strong></td>
<td>Treatment intended to lower microbial counts on eating and drinking utensils to safe public health levels.</td>
<td>May be done with high-temperature washing or by dipping into a chemical disinfectant.</td>
</tr>
</tbody>
</table>

 sterilization > commercial sterilization > disinfection = antisepsis > degerming > sanitization

Also, a microbi**cidal** agent *kills* microbes whereas a microbi**static** agent *inhibits* growth without killing
Rates of Microbial Death (pg. 264)

Constant percentage of the extant population is killed each minute.

- 90% die in 1 minute.
- Rate of death is constant, but the time required to kill ALL organisms depends on population density.
1. Physical Methods of Microbial Control

Chapter Reading – pp. 268-275
Physical Methods to Control Growth

1) Temperature
   - high or low temperatures that limit microbial growth

2) Filtration
   - physical removal of microorganisms

3) Dessication
   - removal of water

4) Osmotic Pressure
   - high concentrations of solutes (salts, sugars)

5) Radiation
   - high energy emissions that cause molecular damage
Treatment with Heat

Heat denatures proteins & other macromolecules at a rate that depends on 3 factors.

1) temperature

2) amount of moisture
   • water is much more effective at transferring heat than dry air, causing proteins to denature & coagulate

3) length of exposure
   • larger microbial populations and larger materials require longer exposure times

Thermal Death Point (TDP)
• lowest temperature at which ALL organisms killed in 10’

Thermal Death Time (TDT)
• time required to kill ALL organisms at a given temp.
Sterilization by Autoclaving

Autoclaves are chambers of high pressure steam used for sterilization

- method of choice for heat-tolerant, small-size material
- inexpensive to use, non-toxic

higher pressures = higher temperatures (w/o loss of moisture)
Verification of Target Temperature

“Indicators” are important to verify the necessary temperature was reached for the required time:

- test vials of endospores
- “autoclave tape” with indicator chemicals that change color

After autoclaving, flexible vial is squeezed to break ampule and release medium onto spore strip.

Yellow medium means spores are viable; autoclaved objects not sterile

Red medium means spores were killed; autoclaved objects are sterile
Pasteurization

A process of mild heating to eliminate spoilage, pathogens without damaging the food product (e.g., food, wine, beer).

**TABLE 9.2** Moist Heat Treatments of Milk

<table>
<thead>
<tr>
<th>Process</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Historical (batch) pasteurization</td>
<td>63°C for 30 minutes</td>
</tr>
<tr>
<td>Flash pasteurization</td>
<td>72°C for 15 seconds</td>
</tr>
<tr>
<td>Ultra-high-temperature pasteurization</td>
<td>135°C for 1 second</td>
</tr>
<tr>
<td>Ultra-high-temperature sterilization</td>
<td>140°C for 1–3 seconds</td>
</tr>
</tbody>
</table>

- thermophilic organisms survive, however they don’t cause spoilage or disease
Low Temperatures

Low temperatures can be microbicidal and/or microbistatic:

- refrigeration is *microbistatic* by simply slowing down or eliminating microbial growth, it does NOT *kill*

- freezing *can* be microbicidal due to the formation of ice crystals, though many organisms can survive freezing

Dessication

The elimination of moisture by dessication is a microbistatic treatment.

- microbes cannot metabolize & grow but are typically NOT killed and thus can grow if moisture is restored
Filtration

Filters with pore sizes smaller than microbial cells (0.2 μm) can effectively sterilize liquids

- vacuum pressure pulls liquid through filter
- receptacle to capture filtrate must be sterile
- more costly than heat sterilization
- best method for the sterilization of liquids that cannot tolerate high temperatures
Treatment with Radiation

High energy **electromagnetic** radiation
  - short wavelength UV, x-rays, gamma rays

High energy **particle** radiation
  - e.g., electron beams
Ionizing vs Nonionizing Radiation

Ionizing radiation

- has high enough energy to cause the removal of electrons from atoms
  - x-rays, gamma rays, electron beams
- results in free radicals (usu. \( \cdot \text{OH} \) from water)

Nonionizing radiation

- energy is too low to remove electrons but can cause other types of damage:
  - e.g., UV radiation which causes specific DNA damage

**Both types of radiation can be used to sterilize**
Physical Barriers

Microbial contamination can be minimized with the use of:

- safety cabinets
- HEPA filters
- lab coats, gloves, surgical masks
2. Chemical Methods of Microbial Control

Chapter Reading – pp. 265-267, 275-281
Effectiveness of Chemicals

Chemicals rarely achieve sterility (usually disinfection, antisepsis) & their effects can be quite variable:

• effectiveness varies depending on the organism
• may not make contact with all organisms present
  • e.g., dense microbial populations or biofilms
• can be inhibited by various organic molecules
  • e.g., lipids and proteins that may bind to it

The choice of chemical agent depends on:
• target organism(s)
• degree of microbial control needed
• material to be treated (e.g., countertop, human skin)
Types of Chemical Disinfectants

- phenol-based compounds (aka “phenolics”)
- alcohols (ethanol, isopropanol…)
- halogens (chlorine, iodine…)
- surfactants (quarternary ammonium ions or “quats”)
- peroxoxygenes (hydrogen peroxide, ozone…)
- aldehydes (formaldehyde…)
- gaseous chemosterilizers (ethylene oxide…)
- “preservatives” (benzoic acid, sulfur dioxide…)
- heavy metals (silver, mercury, copper…)


Phenol-based Compounds

Phenol was one of the first chemical disinfectants

- damages microbial plasma membranes
- can be irritating to human tissues

**especially effective against the mycobacteria and their lipid-rich cell walls**

Many derivatives of phenol have been developed that are less irritating but as effective:

- O-phenylphenol or cresol (used in “Lysol”)
- bisphenols (used in antibacterial soaps, kitchenware)
Alcohols

Ethanol (CH$_3$-CH$_2$OH) and isopropanol (CH$_3$-CHOH-CH$_3$) are most commonly used.

- denature proteins, disrupt membrane lipids
- effective against most fungi & bacteria, NOT endospores and viruses w/o envelopes
- NOT very effective on open wounds (poor contact)
- MOST effective when mixed with water (necessary for denaturation to occur)

### TABLE 7.6

<table>
<thead>
<tr>
<th>Concentration of Ethanol (%)</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>95</td>
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<tr>
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<td>60</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>50</td>
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<td>−</td>
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<td>+</td>
<td>+</td>
</tr>
<tr>
<td>40</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

NOTE: A minus sign indicates no biocidal action (bacterial growth); a plus sign indicates biocidal action (no bacterial growth). The highlighted area represents bacteria killed by biocidal action.
Halogens

Halogens are the “salt-forming” elements (F, Cl, Br, I) w/7 valence electrons (group VIIA of the periodic table).

Many compounds that contain chlorine or iodine are effective disinfectants:

- “bleach” (sodium hypochlorite: NaOCl)
- “iodine” (I₂ mixed as a tincture with an aqueous alcohol)
- halogens are thought to be oxidizing agents (remove e⁻) damage and denature proteins
Peroxygens

Peroxygens such as hydrogen peroxide ($\text{H}_2\text{O}_2$) and ozone ($\text{O}_3$) damage macromolecules via $\text{–OH}$ radicals

- overwhelm the protective enzymes of aerobic organisms
- effective for treating open wounds
- peroxyacetic acid can even kill endospores

$$\text{CH}_3\text{COOH} + \text{HO–OH} \rightleftharpoons \text{CH}_3\text{C(O)}\text{OOH} + \text{H}_2\text{O}$$

acetic acid  hydrogen peroxide  peroxyacetic acid  water

Aldehydes (-HC=O)

Formaldehyde & glutaraldehyde crosslink and inactivate proteins (to sterilize) however they are irritants and thus not used as antiseptics (good for embalming!).
Gaseous Chemosterilizers

Gaseous chemicals used to *sterilize* in a closed chamber (usually *ethylene oxide* or *chlorine dioxide*):

- denatures proteins, requires >4 hrs to sterilize
- can be toxic to humans (carcinogenic)
Heavy Metals

Compounds that contain metals such as silver (Ag), mercury (Hg) & copper (Cu):

- interact with & denature proteins
to inhibit microbial growth

Surfactants

Detergents that disrupt membranes

- detergents containing quaternary ammonium (NH$_4^+$) ions or "quats" are the most effective and most widely used

*NOT effective against endospores & mycobacteria
Resistance Rankings

Most resistant

- Prions
- Bacterial endospores
- Mycobacteria
- Cysts of protozoa
- Active-stage protozoa (trophozoites)
- Most Gram-negative bacteria
- Fungi
- Nonenveloped viruses
- Most Gram-positive bacteria
- Enveloped viruses

Most susceptible
Key Terms for Chapter 9

- sterilization, disinfection, antisepsis, degemming
- sanitization, microbicidal, microbistatic
- thermal death point, thermal death time
- autoclave, pasteurization
- ionizing vs nonionizing radiation
- phenolics, aldehydes, peroxygens, halogens
- surfactants, “quats”

Relevant Chapter Questions
MC: 1-6, 8-12, 14, 16-9  SA: 1-8, 10-13