Chapter 9: Control of Microbial Growth

1. Physical Methods
2. Chemical methods

Important Terminology (pg. 263-264)

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterilization</td>
<td>Destruction or removal of all forms of microbial life, including endospores.</td>
<td>Usually done by heat, dry heat, or chemical means such as ethylene oxide.</td>
</tr>
<tr>
<td>Commercial Sterilization</td>
<td>Efficient means to kill endospores of Clostridium botulinum.</td>
<td>Also called endospore-cidal or lethal sterilization. Usually done by heat, dry heat, or chemical means such as ethylene oxide.</td>
</tr>
<tr>
<td>Disinfection</td>
<td>Destruction of vegetative pathogens.</td>
<td>Also called vegetative-cidal or lethal disinfection. Usually done by heat, dry heat, or chemical means such as ethylene oxide.</td>
</tr>
<tr>
<td>Antisepsis</td>
<td>Destruction of vegetative pathogens.</td>
<td>Also called vegetative-cidal or lethal antisepsis. Usually done by heat, dry heat, or chemical means such as ethylene oxide.</td>
</tr>
<tr>
<td>Degerming</td>
<td>Removal of microbes from a limited area, such as the skin around an injection site.</td>
<td>Also called mechanical removal by an alcohol-soaked needle.</td>
</tr>
<tr>
<td>Sanitization</td>
<td>Treatment intended to lower microbial counts on eating and drinking utensils to safe public health levels.</td>
<td>Also called high-temperature washing or by dipping into a chemical disinfectant.</td>
</tr>
</tbody>
</table>

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

Also, a **microbicidal** agent **kills** microbes whereas a **microbicidal** agent **inhibits** growth without killing.

Rates of Microbial Death (pg. 264)

- **Constant percentage of the extant population is killed each minute.**
- **Rate of death is constant, but the time required to kill ALL organisms depends on population density.**

- **90% die in 1 min.**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Number of living microbes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>$10^9$</td>
</tr>
<tr>
<td>1</td>
<td>$10^7$</td>
</tr>
<tr>
<td>2</td>
<td>$10^5$</td>
</tr>
<tr>
<td>3</td>
<td>$10^3$</td>
</tr>
<tr>
<td>4</td>
<td>$10^1$</td>
</tr>
<tr>
<td>5</td>
<td>$10^0$</td>
</tr>
<tr>
<td>6</td>
<td>$10^{-1}$</td>
</tr>
<tr>
<td>7</td>
<td>$10^{-3}$</td>
</tr>
<tr>
<td>8</td>
<td>$10^{-5}$</td>
</tr>
<tr>
<td>9</td>
<td>$10^{-7}$</td>
</tr>
<tr>
<td>10</td>
<td>$10^{-9}$</td>
</tr>
</tbody>
</table>

1 min is 60 seconds.
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

Red medium means spores were killed; autoclaved objects are sterile
Yellow medium means spores were viable; autoclaved objects not sterile

Pasteurization

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

<table>
<thead>
<tr>
<th>Process</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Historical (batch) pasteurization</td>
<td>62°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>

- thermostophilic organisms survive, however they don’t cause spoilage or disease (why not?)
Low Temperatures
Low temperatures can be microbicidal and/or microbiostatic:
• refrigeration is microbiostatic by simply slowing down or eliminating microbial growth, it does NOT kill
• freezing can be microbicidal due to the formation of ice crystals, though some organisms can survive freezing

Dessication
The elimination of moisture by dessication is a microbiostatic 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. \( \cdot OH \) 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 (quaternary ammonium ions or “quats”)
- peroxygens (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)

### 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” (I$\textsubscript{2}$ mixed as a tincture with an aqueous alcohol)
- halogens are thought to be oxidizing agents (remove e-) that damage and denature proteins

### Peroxygens

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

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

### 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 (NH₄⁺) 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
<table>
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<td>• sanitization, microbicidal, microbistatic</td>
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<tr>
<td>• thermal death point, thermal death time</td>
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<tr>
<td>• autoclave, pasteurization</td>
</tr>
<tr>
<td>• ionizing vs nonionizing radiation</td>
</tr>
<tr>
<td>• phenolics, aldehydes, peroxygens, halogens surfactants, “quats”</td>
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Relevant Chapter Questions
MC: 1-6, 8-12, 14, 16-9   SA: 1-8, 10-13