Chapter 7: Control of Microbial Growth

1. Physical Methods
2. Chemical methods
Important Terminology

<table>
<thead>
<tr>
<th>TABLE 7.1</th>
<th>Terminology Relating to the Control of Microbial Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Definition</strong></td>
<td><strong>Comments</strong></td>
</tr>
<tr>
<td><strong>Sterilization</strong></td>
<td>Destruction or removal of all forms of microbial life, including endospores.</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>
</tr>
<tr>
<td><strong>Disinfection</strong></td>
<td>Destruction of vegetative pathogens.</td>
</tr>
<tr>
<td><strong>Antisepsis</strong></td>
<td>Destruction of vegetative pathogens on living tissue.</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>
</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>
</tr>
</tbody>
</table>

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

Also, a microbicidal agent *kills* microbes whereas a microbistatic agent *inhibits* growth without killing...
Rate of Microbial Death

The rate at which a given microbe dies from treatment is constant, but the time required to kill ALL organisms present increases with population size or density.

**Graph:**
- **Axes:**
  - Y-axis: Logarithm of the number of cells surviving
  - X-axis: Time (minutes)
- **Lines:**
  - A, B, C: Different lines representing different organisms
- **Legend:**
  - High population load
  - Low population load

(b) The effect of high or low initial load of microbes. If the rate of killing is the same, it will take longer to kill all members of a larger population than a smaller one. This is true for both heat and chemical treatments.

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1. Physical Methods of Microbial Control
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 (higher pressure = higher temp.)

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

**TABLE 7.3**

<table>
<thead>
<tr>
<th>Pressure (psi in excess of atmospheric pressure)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>110</td>
</tr>
<tr>
<td>10</td>
<td>116</td>
</tr>
<tr>
<td>15</td>
<td>121</td>
</tr>
<tr>
<td>20</td>
<td>126</td>
</tr>
<tr>
<td>30</td>
<td>135</td>
</tr>
</tbody>
</table>

*At higher altitudes the atmospheric pressure is less, which must be taken into account in operation of an autoclave. For example, in order to reach sterilizing temperatures (121°C) in Denver, Colorado, whose altitude is 5280 feet (1600 meters), the pressure shown on the autoclave gauge would need to be higher than the 15 psi shown in the table.*
Verification of Target Temperature

“Indicators” are important to verify the necessary temperature was reached for the required time.
• different times (& temperatures) for different materials

<table>
<thead>
<tr>
<th>Container Size</th>
<th>Liquid Volume</th>
<th>Sterilization Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test tube: 18 x 150 mm</td>
<td>10 ml</td>
<td>15</td>
</tr>
<tr>
<td>Erlenmeyer flask: 125 ml</td>
<td>95 ml</td>
<td>15</td>
</tr>
<tr>
<td>Erlenmeyer flask: 2000 ml</td>
<td>1500 ml</td>
<td>30</td>
</tr>
<tr>
<td>Fermentation bottle: 9000 ml</td>
<td>6750 ml</td>
<td>70</td>
</tr>
</tbody>
</table>

*Sterilization times in the autoclave include the time for the contents of the containers to reach sterilization temperatures. For smaller containers, this is only 5 min or less, but for a 9000-ml bottle, it might be as much as 70 min. A container is usually not filled past 75% of its capacity.
Dry Heat

- requires higher temperatures, longer exposure time than moist heat
- leads to dessication (“drying out”) of materials, usually requires temperatures much higher than with moist heat

Pasteurization

A process of mild heating to eliminate spoilage, pathogenic organisms in milk, wine, beer…
- the more thermophilic organisms survive, however they generally don’t grow at food storage temperatures
- reduces spoilage without damaging the food product
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. ·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**
2. **Chemical Methods of Microbial Control**
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…)
- biguanides (chlorhexadine)
- peroxygens (hydrogen peroxide, ozone…)
- aldehydes (formaldehyde…)
- gaseous chemosterilizers (ethylene oxide…)
- “preservatives” (benzoic acid, sulfur dioxide…)
- heavy metals (silver, mercury, copper…)

Testing Chemical Disinfectants

Disc-diffusion tests
- paper discs soaked with test chemical are placed on a culture plate of target organism

Use-dilution tests
- dried (but viable) culture samples are immersed in chemical and then tested for viability (in growth medium)
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**

Biocidal Action of Various Concentrations of Ethanol in Aqueous Solution Against *Streptococcus pyogenes*

<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>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>60</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>50</td>
<td></td>
<td></td>
<td></td>
<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.
Halogen

Halogen 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 with an aqueous alcohol)
- they are oxidizing agents (remove e⁻), damage proteins

Biguananides

Biguananides such as chlorhexadine are effective skin antiseptics found in mouthwashes, surgical scrubs and acne medicines.
Peroxygens

Peroxygens such as hydrogen peroxide ($H_2O_2$) 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)

Preservatives

Chemicals added to foods to inhibit microbial growth and “preserve” food quality

- sorbic and benzoic acids (sorbate, benzoate)
- nitrates (contain $\text{NO}_3^-$) & nitrites (contain $\text{NO}_2^-$)
- sulfur dioxide (SO$_2$)
- inhibit enzymes, thought to be non-toxic for humans
Heavy Metals

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

- silver nitrate, copper sulfate, mercuric chloride (toxic)
- interact with & denature proteins to inhibit microbial growth

Surface-active Agents

Detergents (aka “surfactants”) that disrupt membranes

- detergents containing quaternary ammonium (NH$_4^+$) ions are the most effective and most widely used
- NOT effective toward endospores, Gram$^-$ & mycobacteria
Some Organisms are more Resistant than Others

### TABLE 7.7
The Effectiveness of Chemical Antimicrobials Against Endospores and Mycobacteria

<table>
<thead>
<tr>
<th>Chemical Agent</th>
<th>Endospores</th>
<th>Mycobacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mercury</td>
<td>No activity</td>
<td>No activity</td>
</tr>
<tr>
<td>Phenolics</td>
<td>Poor</td>
<td>Good</td>
</tr>
<tr>
<td>Bisphenols</td>
<td>No activity</td>
<td>No activity</td>
</tr>
<tr>
<td>Quaternary ammonium compounds</td>
<td>No activity</td>
<td>No activity</td>
</tr>
<tr>
<td>Chlorines</td>
<td>Fair</td>
<td>Fair</td>
</tr>
<tr>
<td>Iodine</td>
<td>Poor</td>
<td>Good</td>
</tr>
<tr>
<td>Alcohols</td>
<td>Poor</td>
<td>Good</td>
</tr>
<tr>
<td>Glutaraldehyde</td>
<td>Fair</td>
<td>Good</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>No activity</td>
<td>Fair</td>
</tr>
</tbody>
</table>

Some Organisms are more Resistant than Others

- Most Resistant
  - Prions
  - Endospores of bacteria
  - Mycobacteria
  - Cysts of protozoa
  - Vegetative protozoa
  - Gram-negative bacteria
  - Fungi, including most fungal spores
  - Viruses without envelopes
  - Gram-positive bacteria
  - Viruses with lipid envelopes

- Least Resistant

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Key Terms for Chapter 7

- sterilization, disinfection, antisepsis, degerming
- sanitization, microbicidal, microbistatic
- thermal death point, thermal death time
- autoclave, pasteurization
- ionizing vs nonionizing radiation
- disc-diffusion & use-dilution tests
- phenolics, aldehydes, peroxygens, halogens

Relevant Chapter Questions
rvw: 2, 3, 5-7, 9-13      MC: 1-5, 7-10