Chapter 7: Control of Microbial Growth

1. Physical Methods

2. Chemical methods

Important Terminology

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterilization</td>
<td>Destruction or removal of all forms of microbial life, including endospores. Usually done by steam under pressure or x-raying.</td>
</tr>
<tr>
<td>Commercial Sterilization</td>
<td>Sufficient heat treatment to kill endospores of Gram-negative bacteria in normal foods.</td>
</tr>
<tr>
<td>Disinfection</td>
<td>Destruction of vegetative pathogens; mostly uses of physical or chemical methods.</td>
</tr>
<tr>
<td>Antisepsis</td>
<td>Destruction of vegetative pathogens on living tissue; Treatment is delayed by chemical antimicrobial.</td>
</tr>
<tr>
<td>Degerming</td>
<td>Removal of microbes from a limited area, such as the skin or an injection site. Mostly a mechanical removal by an antiseptic solution.</td>
</tr>
<tr>
<td>Sanitization</td>
<td>Treatment intended to lower microbial counts so as to make drinking water 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.

Graphs showing the rate of microbial death with different susceptibilities to a given treatment and the effect of high or low initial load of microbes. The graphs illustrate that high initial loads lead to a longer period of time to achieve a given reduction in microbial numbers compared to low initial loads.
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

<table>
<thead>
<tr>
<th>Thermal Death Point (TDP)</th>
<th>Thermal Death Time (TDT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>• lowest temperature at which ALL organisms killed in 10'</td>
<td>• time required to kill ALL organisms at a given temp</td>
</tr>
</tbody>
</table>
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

**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>Sterilization Vessel Volume (mL)</th>
<th>Sterilization Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mL</td>
<td>10 mL</td>
<td>15</td>
</tr>
<tr>
<td>15 mL</td>
<td>15 mL</td>
<td>30</td>
</tr>
<tr>
<td>50 mL</td>
<td>50 mL</td>
<td>60</td>
</tr>
<tr>
<td>100 mL</td>
<td>100 mL</td>
<td>120</td>
</tr>
</tbody>
</table>

*Stereilization times are in the container include the time for the ascent of the container to reach sterilization temperature in smaller containers, this is only 5 min or less, but in a 5L bottle, takes approx 1-2hrs. 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 (CH₃-CH₂OH) and isopropanol (CH₃-CHOH-CH₃) 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>
<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>10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>20</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>30</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>
<tr>
<td>50</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

NOTE: A plus sign indicates no bacterial growth, a plus sign indicates visible growth, a blank space indicates growth (highlighted area represents control). Ethanol at 30% is effective against B. subtilis spores.

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

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

Peroxygens
Peroxygens such as hydrogen peroxide (H₂O₂) and ozone (O₃) damage macromolecules via –OH radicals
- overwhelm the protective enzymes of aerobic organisms
- effective for treating open wounds
- peroxyacetic acid can even kill endospores

\[
\text{acetic acid} + \text{hydrogen peroxide} \rightarrow \text{peroxyacetic acid} + \text{water}
\]

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)

Preservatives
Chemicals added to foods to inhibit microbial growth and “preserve” food quality
- sorbic and benzoic acids (sorbate, benzoate)
- nitrates (contain NO₃⁻) & nitrites (contain NO₂⁻)
- sulfur dioxide (SO₂)
- 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 (NH₄⁺) ions are the most effective and most widely used
- NOT effective toward endospores, Gram- & mycobacteria

Some Organisms are more Resistant than Others

<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>Mercury</td>
<td>Poor</td>
<td>Good</td>
</tr>
<tr>
<td>Bifidobacteria</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>Chlorine</td>
<td>Fair</td>
<td>Fair</td>
</tr>
<tr>
<td>Iodine</td>
<td>Poor</td>
<td>Good</td>
</tr>
<tr>
<td>Alcohol</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>

Copyright © 2007 Pearson Education, inc. publishing as Benjamin Cummings
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