

Chapter 11. Physical and Chemical Agents for Microbial Control

- **Principles of microbial control**
- **Chemical antimicrobial agents**
- **Physical antimicrobial agents**

➤ **Why do we need to control microorganisms?**

- Prevention of infections and spread of **disease** caused by pathogens (viruses, bacteria, fungi, spores, eggs, cysts)
 - **Bacterial endospores** are most resistant to destruction. If you kill them you can assume all others have been destroyed
- Prevention of **food spoilage**
- Prevention of deterioration of manufactured goods, building materials, odors

Microbial control includes:

1. **Killing** microorganisms
2. Physically **removing** microorganisms
3. **Inhibiting** microbial growth or metabolism

Terminology

Disinfection

- Destruction of pathogenic, nonsporulating microorganisms or their toxins
 - **Disinfectant** Agent that disinfects **objects**
 - Dishes
 - Counter tops
 - Lavatories
 - **Antiseptic** Agent that disinfects **living tissue**
 - Wounds
 - Skin prior to injections or incisions

Sterilization

- Killing or removal of **all microorganisms** (including spores and viruses)
 - Surgical instruments, syringes, dental equipment
 - Injected drugs, intravenous fluids
 - Catheters and surgical implants

Viable Alive, Capable of living, germinating or reproducing under favorable conditions (cells, spores and viruses)

Nonviable Dead (cells and spores)
Unable to infect cells (viruses)

Bacteriostatic Effect of an agent that inhibits bacterial growth
Organism is not killed –remains viable

Bactericidal Effect of an agent that kills bacteria

Bactericide Agent that kills bacteria

Sporocide Agent that kills spores (bacterial endospores and fungal spores)

Fungicide Agent that kills fungi

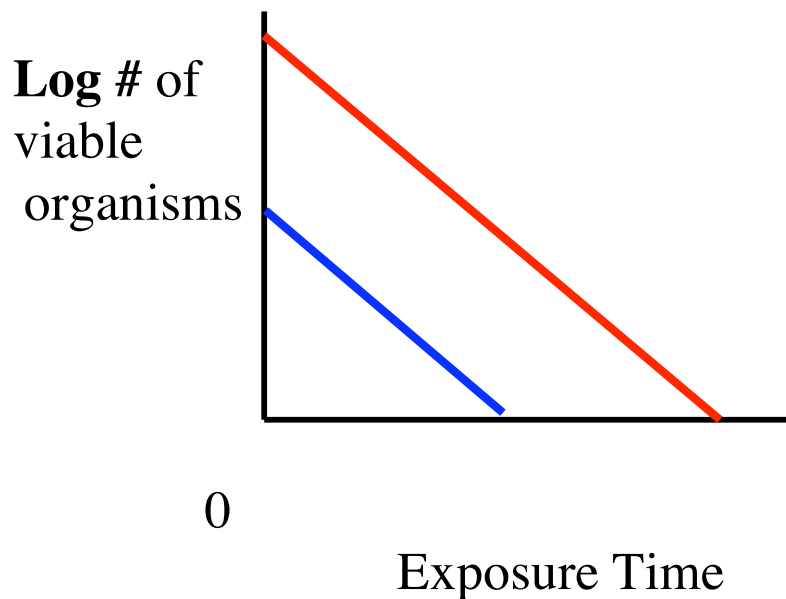
Viricide Agent that destroys viruses

Factors that affect rate of microbial death

1. Degree of contamination (microbial load)

- Time needed to sterilize something depends on the number of organisms present

Ex. Sterilization of surgical instruments with heat

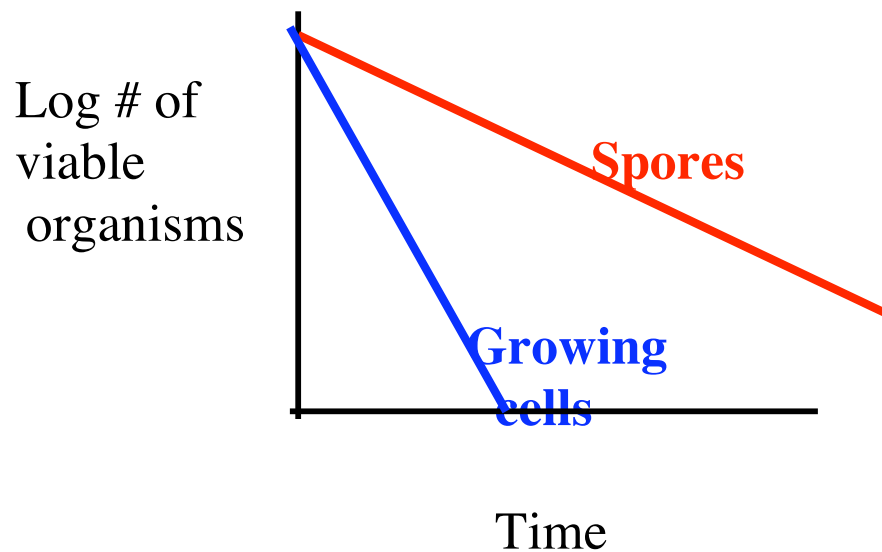


- More organisms = longer time needed to achieve sterility

2. Factors affecting susceptibility of microorganism to antimicrobial agents

- **Phase of growth**

- **Rapidly dividing cells** (vegetative cells) are easier to kill
- **Spores and inactive cells** are more resistant



- **Species**

- Some organisms are resistant to particular killing agents

Ex. *Deinococcus radiodurans* is resistant to radiation

3. **Temperature and pH**

Ex. Killing effect of **ethanol** may be greater **at high temperature or at high or low pH**

4. **Concentration** of antimicrobial agent

5. Presence of material that **interferes with the action** of the agent

Ex. blood, saliva, oil, dirt and debris

Figure 11.2

Primary Targets of Antimicrobial Agents

1. **Proteins** Disruption of structure (**denaturation**)
Chemical modification of amino acids

Ex. Enzymes and structural proteins

2. **Lipids** Disruption of **membranes**

Ex. Phospholipid bilayer

3. **Nucleic acids** Denaturation and chemical modification

Ex. DNA and RNA

Chemical Antimicrobial Agents

- 1. Soaps and detergents**
- 2. Acids and bases** (Ex. acetic acid, ammonia)
- 3. Halogens** (Ex. chlorine and iodine)
- 4. Alcohols** (Ex. ethanol and isopropanol --rubbing alcohol)
- 5. Phenols** (Ex. *ortho*-Phenylphenol --Lysol)
- 6. Oxidizing agents** (Ex. Hydrogen peroxide)
- 7. Alkylating agents** (Ex. Ethylene oxide --a gas)
- 8. Heavy metals** (Ex. mercury, silver, zinc)
- 9. Natural plant products** (Ex. clove and thyme oil)

1. Soaps and detergents

- Physically remove organisms from:
Skin, food utensils, clothing, bedding
- Kills some microorganisms
Ex. *Streptococcus* and flu virus

Soap



- Carboxyl group is hydrophilic and long carbon chain is hydrophobic
- Solubilizes hydrophobic oil, dirt, dried blood, etc. containing microbes so they can be washed away

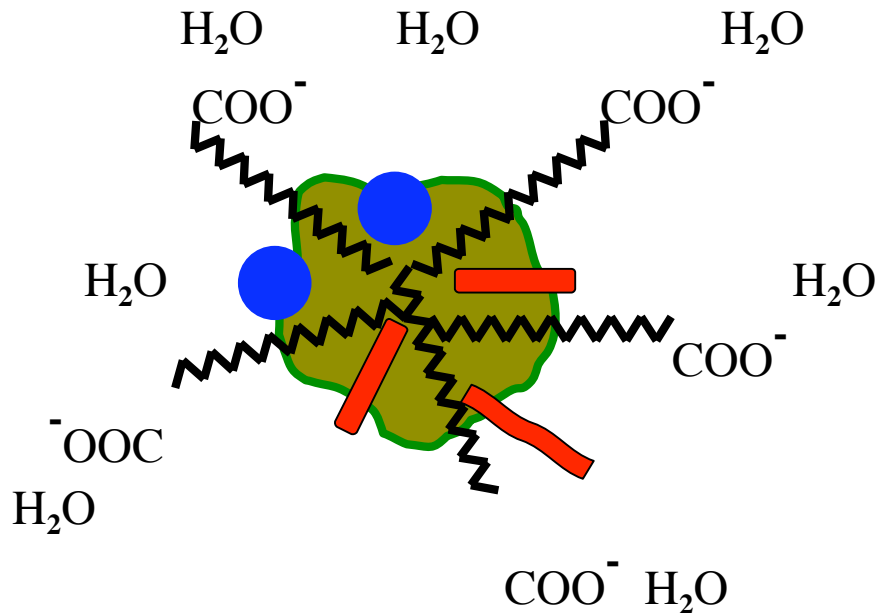


Figure 11.3

2. Acids and bases

- Low or high pH kills or inhibits growth of microorganism

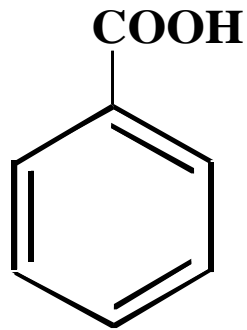
Acids:

Lactic, Acetic, Citric, Benzoic acid → Food preservatives

Bases:

Trisodium phosphate → Kills *Salmonella* on chicken
Carcasses

Calcium hydroxide → Treatment for periodontitis



Benzoic acid

3. Halogens (Chlorine and Iodine)

Chlorine bleach

→ **Disinfectant** for food utensils, dairy equipment, clothing, bedding

Iodine

→ Skin **antiseptic**. Wounds, surgery, injections,
venipuncture, treatment of drinking water

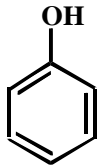
4. Alcohols

Ethanol and isopropanol (rubbing alcohol)

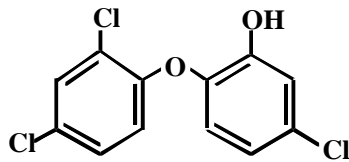
→ Skin prior antiseptic

5. Phenols

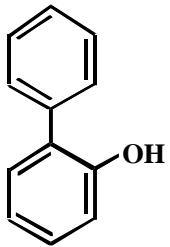
- Disrupt cell membranes, denature proteins
 - Hexachlorophene causes brain damage in infants
 - *ortho*-Phenylphenol (in Lysol)
Hospitals and bathrooms
 - Triclosan
Toothpaste, mouthwash, antimicrobial soaps



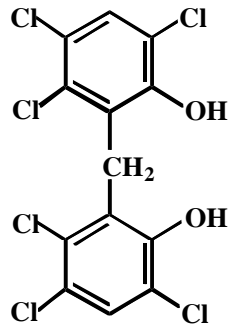
Phenol



Triclosan



Ortho-phenylphenol



Hexachlorophene

6. Oxidizing agents

- Hydrogen peroxide, H_2O_2

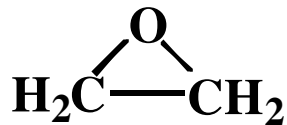
Good penetration of puncture wounds

7. Alkylating agents

- React chemically with proteins and nucleic acids

-Formaldehyde CH_2O

Inactivates cells, viruses and protein toxins



-Ethylene oxide (gas)

Good penetration

Uses low temperatures, good for sterilizing things that are heat-sensitive

Needs special equipment, highly toxic to humans

8. Heavy metals

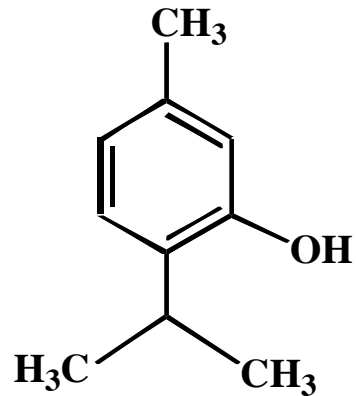
-Mercury, silver, gold, copper, arsenic, zinc

-Inactivate proteins (toxic to animals and humans)

Figure 11.4

9. Other chemical agents

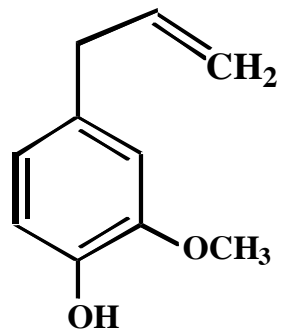
- Natural plant products



-Thymol from thyme (a phenol)

Topical antiseptic

Internally as antihelmintic (parasitic worms)



-Eugenol from cloves (a phenol)

Dental antiseptic and analgesic

- Sulfites (SO_3^{2-}) and nitrites (NO_2^{2-})
 - Food preservatives (vegetables, wine, meat)

Physical Antimicrobial Agents and Processes

<u>Agent</u>	<u>Mechanisms of action</u>
1. Heat	Denatures proteins and nucleic acids, Disrupts membranes
2. Refrigeration	Slows growth and metabolism
3. Drying	Lack of water hinders metabolism and growth.
4. Irradiation	Promotes chemical reactions that damage proteins, nucleic acids, membranes and other organic compounds
5. Filtration	Physically removes organisms from gases and liquids

Heat Sterilization

1. Dry heat

- Oven: 171°C --1 hr, 160°C -- 2 hr, or 121°C --16 hr
Metal, glass, powders and oils
- Flame
Inoculating loops and mouth of culture tubes

2. Moist heat (steam, boiling water)

- Good penetration
- Pressure increases boiling point of water above 100°C
- Autoclave Uses steam and high temperature
15 lb/in², 121°C, 15-20 min
Kills cells, spores and viruses
- Sterilization indicators (autoclave and ethylene oxide)
Ensure proper sterilization conditions were achieved
Indicator tape (chemical indicator)
Endospore strip (biological indicator)

Table 11.3

Bacterial endospores most resistant – usually require
temperatures above boiling

Table 11.4

Figure 11.5

Pasteurization

- Invented by Louis Pasteur to preserve wine
- Uses temperature below 100°C
- Foods are heated to kill pathogens and reduce the number of spoilage organisms
Temperature is not high enough to “cook” the food
Preserves natural taste and texture
- Kills pathogens in heat sensitive food like milk, cheese and beer
 - Listeria*
 - Salmonella*
 - Mycobacterium tuberculosis* in raw milk killed by:
 - Flash method 71.6°C for 15 sec
 - Holding method 62.9°C for 30 min
- Not sterilization. Nonpathogenic organisms and spores may still be alive

Refrigeration

- Prevents spoilage of foods
- Slows metabolism and growth
- Doesn't kill microorganisms
- Refrigeration at 5°C
- Freezing at -20°C or lower

Drying

- Prevents spoilage
- Removes water needed by cells for metabolism
- Fruit, vegetables, grain, meat, fish

Freeze-drying (lyophilization)

- Food is frozen, then placed under a vacuum
 - Water evaporates, food stays cold until dry
 - Preserves color and flavor
 - Instant coffee, fruit and other foods

Radiation

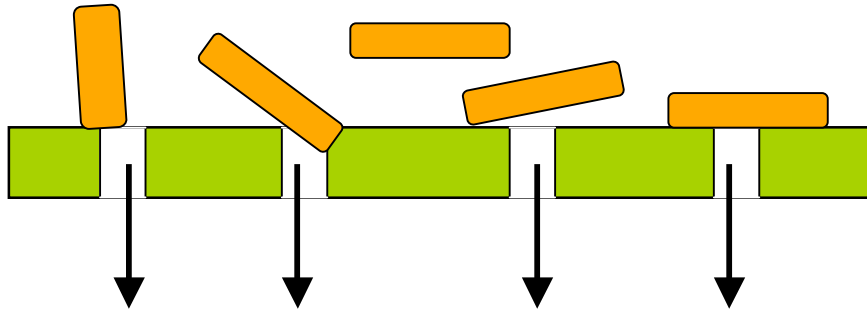
- Ultraviolet light
 - Damages nucleic acids
 - Doesn't penetrate glass, clothing, paper
 - Effective against airborne pathogens in hospitals and animal holding facilities
 - Reduces number of organisms on surfaces
 - Ex. in operating rooms
- Ionizing radiation: x-rays, gamma rays
 - Sterilizes foods like milk
 - Kills pathogens in meat
 - Ex. Salmonella and Campylobacter* on chicken
- Microwave radiation
 - Raises the temperature of things that contain water
 - Endospores are resistant as they contain little water

Figure 11.9

Figure 11.6

Filtration

- Removes microorganisms by passage of solution or gas through a filter

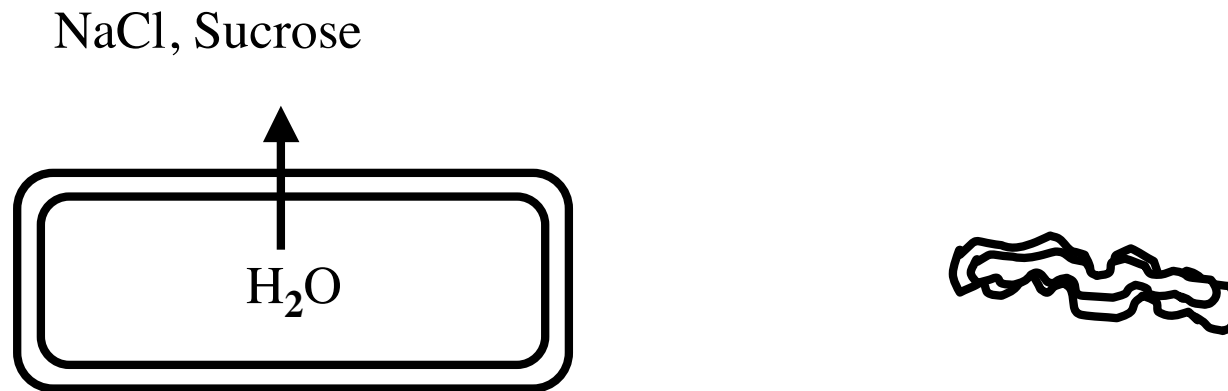


- Like pasteurization, good for heat-sensitive materials
Ex.
Injected drugs, antibiotics, vaccines, vitamins, IV fluids
Some beer is filtered rather than pasteurized to preserve flavor
- Membrane filters can have various pore sizes that remove different sizes of microorganisms
 - 0.02 μm \rightarrow viruses
 - 0.2 μm \rightarrow bacteria, spores, cysts, eggs
 - Useful for treatment of drinking water
- High-efficiency particulate air filters (HEPA) are used in ventilation systems in hospitals

Figure 11.1

Osmotic pressure

- **Osmosis** Movement of water molecules through a semi-permeable membrane that blocks movement of salts and other dissolved substances
Ex. cell membranes
- High concentrations of salt or sugar preserve food
Ex. Salted meat, jelly
- Water move out of cell by osmosis



Dehydrates cell's cytoplasm (like drying); stops metabolism

Figure 11.1