

Chapter 13 Control of Microbial Growth

Free Online Textbook*:
Microbiology by OpenStax

<http://cnx.org/content/col12087/1.4>

(*the online version of the book is routinely being updated.)

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Definitions

- **suffixes — cide and — cidal:**
 - DESTRUCTION of a type of microbe.
 - Examples:
 - Bactericide
 - Fungicide
 - Virucide
 - “Germicide”: **ethylene oxide, propylene oxide, aldehyde.**
- **suffixes — stasis and — static:**
 - INHIBITION but not complete destruction of a type of microbe.
 - Examples:
 - Bacteriostatic
 - Fungistatic
 - Virustatic
 - “Germistatic”: **refrigeration, freezing.**

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Definitions

- **Antisepsis:**
 - REDUCTION in the number of microorganisms and viruses, particularly potential pathogens, on living tissues.
 - Examples:
 - **Iodine. Alcohol.**
 - Antiseptics are frequently “Disinfectants” whose strength has been reduced to make them safe for living tissues.
- **Aseptic:**
 - refers to an environment or procedure FREE of pathogenic contaminants.
 - Examples:
 - **preparation of a surgical field.**
 - **handwashing (3 minute surgical scrub version).**
 - **flame sterilization of laboratory equipment.**

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Definitions

- **Degerming:**

- **physical removal** of microbes by some sort of **mechanical** means.
- Examples:
 - **the scrubbing part of Handwashing**
 - Alcohol **Swabbing** at the site of an injection.

- **Pasteurization:**

- use of **HEAT** to destroy pathogens and reduce the number of spoilage microbes in foods and beverages.
- heat treatment of **milk** and **fruit juice** is hot enough to *destroy most microbes but minimize the alteration of taste and destruction of nutrients.*

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Definitions

- **Sanitation:**

- removal of pathogens from objects to meet PUBLIC HEALTH standards. (same as “disinfection”, just different location)
- Examples:
 - washing dishes in **scalding water** in restaurants.
- **standards** of sanitation **vary greatly** among governmental jurisdictions.

- **Disinfection:**

- destruction of MOST microbes and viruses on NONLIVING tissue at HOME. (same as “sanitation”, just different location).
- Examples:
 - **phenolics**
 - **alcohols**
 - **aldehydes**
 - **soaps**

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Definitions

- **Sterilization:**

- destruction of ALL microbes, viruses and bacterial endospores in or on an object.
- Example:
 - preparation of **microbial culture media**
 - preparation of **canned food**
- typically achieved by:
 - **steam under pressure**
 - **incineration**
 - **ethylene oxide gas**
- term does not apply to prions (infectious proteins), because standard sterilization techniques do not destroy them.

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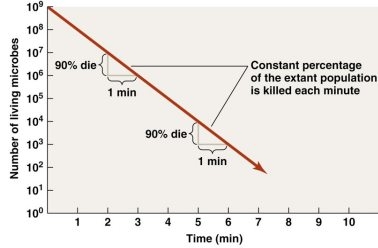
Definitions

- **Microbial Death:**

- the permanent loss of reproductive ability under ideal environmental conditions.

- **Microbial Death Rate:**

- a way to evaluate the efficacy of an antimicrobial agent.
- is constant over time for a particular microbe under a particular set of conditions.



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Action of Antimicrobial Agents

- **Alteration of cell walls & membranes:**

- cell wall maintains integrity of cell.
 - when damaged, the cell bursts because of osmotic effects.
- cytoplasmic membrane controls passage of chemicals into and out of the cell.
 - when damaged, the cellular contents leak out.
- in enveloped viruses, the envelope is a membrane made of proteins and phospholipids with the function of attaching a virus to its target cell.
 - when damaged, this interrupts viral replication.
 - thus, non-enveloped viruses have a greater tolerance of harsh environmental conditions and antimicrobial agents.

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Action of Antimicrobial Agents

- **damage to proteins & nucleic acids:**

- protein function depends on its 3-D shape.
 - extreme heat or certain chemicals can **denature** proteins.
- chemicals, radiation, and heat can alter or destroy nucleic acids:
 - produces fatal mutants.
 - halts protein synthesis through action on RNA.

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The Selection of Microbial Control Agents

- Ideally, agents should be:
 - **inexpensive**
 - **fast-acting**
 - **stable during storage**
 - **capable of controlling microbial growth while being harmless to humans, animals, and objects.**
- Factors affecting the efficacy of antimicrobial methods:
 - site to be treated:
 - harsh chemicals and extreme heat cannot be used on humans, animals and fragile objects.
 - microbial control based on the site of a medical procedure.
 - penetrating versus non-penetrating medical instruments and supplies.

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Relative Susceptibilities of Microbes to Antimicrobial Agents

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

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Physical Methods of Microbial Control: Moist Heat

- **Moist Heat:**
 - used to disinfect, sanitize, and sterilize.
 - denatures proteins & destroys cytoplasmic membranes.
 - more effect than dry heat.
 - methods of microbial control using moist heat:
 - **boiling**
 - **autoclaving**
 - **pasteurization**
 - **ultrahigh-temperature sterilization**

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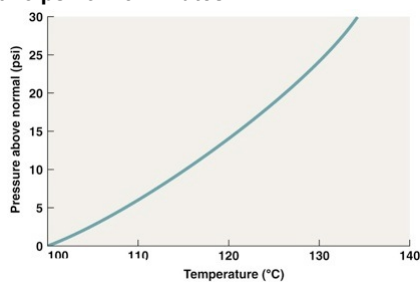
Moist Heat Method: Boiling

- kills vegetative cells of:
 - bacteria
 - fungi
 - protozoan trophozoites
 - most viruses
- Boiling Time is critical:
 - different elevations (altitudes) require different boiling times.
- Things that can survive boiling:
 - endospores
 - protozoan cysts
 - some viruses

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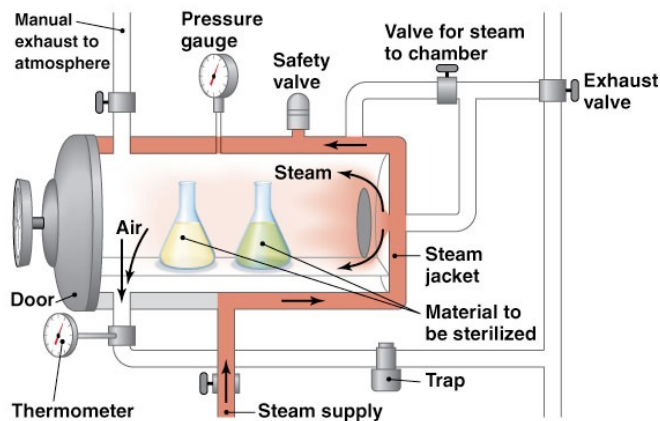
Moist Heat Method: Autoclaving

- pressure applied to boiling water prevents steam from escaping
- boiling temperature increases as pressure increases.
- large single volumes & solids take longer to autoclave.
 - 1 liter in 1 container takes longer than 1 liter in a bunch of vials.
- autoclave conditions:
 - **121°C at 15 psi for 15 minutes.**



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Moist Heat Method: Autoclaving



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Moist Heat Method: Pasteurization

- used for milk, ice cream, yogurt, and fruit juice.
- NOT a method of sterilization.
 - heat-tolerant microbes can survive!!!
- Pasteurization of milk:
 - Historical (**Batch**) Method: 60°C for 30 minutes.
 - **Flash** Method: 72°C for 15 minutes.
 - Ultrahigh-temperature pasteurization: 135°C for 1 second.
 - Ultrahigh-temperature sterilization: 140°C for 1-3 seconds.
 - treated liquids can be stored at room temperature.

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Physical Methods of Microbial Control: Dry Heat

- **Dry Heat:**
 - used for materials that cannot be sterilized with moist heat.
 - denatures proteins and oxidizes metabolic and structural chemicals.
 - requires higher temperatures and longer heat times than moist heat methods.
 - **incineration** is the ultimate means of sterilization.
 - heating loops in the bunsen burner incinerates microbes.

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Physical Methods of Microbial Control: Refrigeration & Freezing

- decreases microbial metabolism, growth & reproduction.
 - chemical reactions occur more slowly at low temperatures.
 - liquid water not available for many needed reactions.
- **Psychrophilic** microbes can still grow.
- **Refrigeration** halts the growth of most pathogens.
- **Slow Freezing** is more effective at inhibiting microbial metabolism than quick freezing because the ice crystals have time to form and puncture microbial cell membranes
- organisms vary in susceptibility to freezing.

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Physical Methods of Microbial Control: Desiccation

• Desiccation:

- is **drying**, and has been used for thousands of years to preserve foods such as fruit, peas, beans, grain, nuts and yeast.
- it works by removing the liquid water needed for metabolism.
- can inhibit the spread of most pathogens.
- some molds can still grow, which need little water.



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Physical Methods of Microbial Control: Lyophilization

• Lyophilization:

- is a technique **combining freezing & drying**, to preserve microbes and other cells for years.
 - this process starts with a instant freeze of a culture using liquid nitrogen or frozen carbon dioxide (dry ice)
 - then a vacuum removes frozen water through a process called sublimation, in which the water is transformed directly from a solid to a gas (skipping the liquid state).
- this process prevents the formation of the ice crystals that can damage cell membranes and kill cells.

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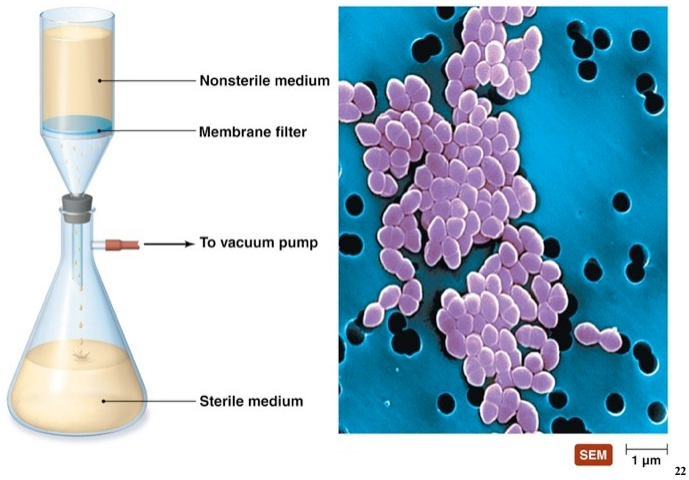
Physical Methods of Microbial Control: Filtration

• Filtration:

- is the passage of a liquid or gas through a sieve designed to trap particles — in this case, really small stuff such as cells, bacteria, spores and viruses.
- a vacuum can be used to pull the fluids through the filter.
- in the late 1800s, filters were able to trap cells, but the pores were still too large to trap viruses, and at the time they were named "**filterable viruses**", and today we just call them **viruses**.
- today, we have filters with pores small enough to trap viruses as well.
- **good method for sterilizing heat sensitive materials:**
 - eye meds, antibiotics, vaccines, liquid vitamins, enzymes and culture media.

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Physical Methods of Microbial Control: Filtration



Physical Methods of Microbial Control: Osmotic Pressure

• Osmotic Pressure:

- high concentrations of salt or sugar in foods to inhibit growth.
- cells in **hypertonic solutions** of salts or sugar will lose water to that solution.
- fungi have a greater ability than bacteria to survive hypertonic environments.

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Physical Methods of Microbial Control: Radiation

• Ionizing Radiation:

- wavelengths are shorter than 1 nm.
- ejects electrons from atoms to create ions.
- ions will do the following:
 - disrupt hydrogen bonding
 - oxidize double covalent bonds
 - create hydroxide ions which will denature other molecules such as DNA.
- Electron Beams:
 - effective at killing but do not penetrate well.
 - used to sterilize spices, meats, Sutures, Gloves, Syringes
- Gamma Rays:
 - penetrate well but require hours to kill microbes.
 - used to sterilize spices, meats, fresh fruit and vegetables.

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Physical Methods of Microbial Control: Radiation

- **Non-Ionizing Radiation:**
 - wavelengths are greater than 1 nm.
 - excites electrons, causing them to make new covalent bonds.
 - affects the 3-D structure of proteins and nucleic acids.
 - UV light at 260 nm causes pyrimidine dimers in DNA
 - UV light does not penetrate well.
 - UV light used to disinfecting:
 - air
 - transparent fluids
 - surfaces of objects such as barber's shears and operating tables.
 - sewage water (cuts down on the amount of chlorine needed).

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Physical Methods of Microbial Control: Radiation



The circular **radura symbol** is used in the United States to label irradiated foods.



Non-irradiated

Irradiated

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Chemical Methods of Microbial Control: Phenol & Phenolics

- **Phenol & Phenolics:**
 - intermediate to low level disinfectants.
 - denatures proteins & disrupts cell membranes.
 - effective in the presence of organic matter.
 - remain active for prolonged time.
 - commonly used in:
 - health care settings
 - labs
 - home
 - have a **disagreeable odor** & possible side effects
 - **rashes**
 - Example:
 - active ingredient in **Lysol**

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Chemical Methods of Microbial Control: Alcohols

- **Alcohols:**

- intermediate level disinfectants
- denatures proteins & disrupts cytoplasmic membranes.
- more effective than soap in removing bacteria from hands !!!
- Rubbing Alcohol (Isopropanol) is slightly superior to Drinking Alcohol (Ethanol).
- 100% alcohol is not effective as the denaturation of proteins requires water — this is why you see isopropanol sold in 70% and 90% concentrations.
- may evaporate before antimicrobial action takes place.
- Example:
 - swabbing of skin with 70% ethanol prior to needle injection.

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Chemical Methods of Microbial Control: Halogens

- **Halogens:**

- intermediate level antimicrobial chemicals
- hypothesized to damage enzymes via oxidation or by denaturation.
- Examples:
 - **iodine tablets** to purify water
 - some protozoan cysts can still survive (you take your chances)!
 - **Betadine** (an iodophor)
 - **Chlorine** treatments to public drinking water and pools
 - **Bleach**
 - chloramines (like chlorine but lasts longer)
 - bromine disinfection of hot tubs, pools, cooling towers

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Chemical Methods of Microbial Control: Halogens



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Chemical Methods of Microbial Control: Oxidizing Agents

• Oxidizing Agents:

- high level disinfectants & antiseptics.
- kills by oxidation of microbial enzymes.
- Examples:
 - Peroxides such as **Hydrogen Peroxide** which can disinfect and sterilize surfaces but is not useful for treating open wounds because of its catalase activity that will also damage your cells.
 - **Ozone (O₃)** is a reactive form of oxygen that is generated when Oxygen (O₂) is subjected to an electrical discharge — is noted as that “fresh smell” after a thunderstorm. It is more expensive and more effective than chlorine.
 - **Peracetic Acid** is used to sterilize food processors and leaves no toxic residue... it is capable of dissolving some metals.

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Chemical Methods of Microbial Control: Surfactants

• Surfactants:

- are “surface active” chemicals which reduce the surface tension of solvents such as water by *decreasing the attraction among the individual molecules.*
- Examples:
 - **Soaps:**
 - have hydrophilic & hydrophobic ends.
 - good mechanical degerming agents but NOT antimicrobial.
 - if a soap is “*advertised as antiseptic*” it is because it typically contains other antimicrobial chemicals.
 - **Detergents:**
 - are positively charged organic surfactants.
 - **Quaternary Ammonia Compounds (Quats):**
 - low level disinfectants
 - ideal for many medical and industrial applications
 - Example: **Cepacol mouthwash**

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Chemical Methods of Microbial Control: Heavy Metals

• Heavy Metals:

- low level bacteriostatic & fungistatic agents.
- heavy metal ions will denature proteins.
- Examples:
 - 1% **silver nitrate** eye drops used to prevent blindness caused by *N. gonorrhoeae*.
 - Was commonly applied (and in some places still is) to a newborns eyes just after a vaginal birth if the mom has the infection.
 - **Thimerosal** (contains mercury) used to preserve vaccines.
 - was falsely linked as a cause of Autism.
 - controversy resulted in removing thimerosal from all childhood vaccinations, but they are still used in some adult vaccinations.
 - **Copper** to control algal growth
 - it interferes with chlorophyll.

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Chemical Methods of Microbial Control: Aldehydes

• Aldehydes:

- compounds containing terminal —CHO groups.
- cross-link functional groups to denature proteins and inactivate nucleic acids.
- Examples:
 - **Glutaraldehyde:** 10 minutes will disinfect but 10 hours will sterilize. 2% solutions used to wipe down hospital rooms.
 - **Formalin** used in embalming and disinfection of rooms and instruments. It is very irritating to the mucous membranes and carcinogenic.

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Chemical Methods of Microbial Control: Gaseous Agents

• Gaseous Agents:

- denatures proteins and DNA by cross-linking functional groups.
- gases such as **ethylene oxide** are used in closed chambers to sterilize items.
- used in hospitals & dental offices to sterilize:
 - sutures
 - plastic lab materials
 - mattresses & pillows
 - electronic equipment
- disadvantages:
 - extremely poisonous & hazardous to people
 - highly explosive
 - potentially carcinogenic

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