A study material for M.Sc. Biochemistry (Semester: IV) Students
on the topic (EC-1; Unit II)

Control of Microbial Growth

The Sterilization Methods

Vyomesh Vibhaw
Assistant Professor (Part Time)
Department of Biochemistry
Patna University
Mob. No.:- +91-9708381107, +91-8825217209
E. Mail: vyomesh.vibhaw@gmail.com

Physical and chemical control of microbes

Frequently Used Terminology

Sterilization is the process by which all living cells, viable spores, viruses, and viroids are either destroyed or removed from an object or habitat.

Disinfection is the killing, inhibition, or removal of microorganisms that may cause disease. The primary goal is to destroy potential pathogens, but disinfection also substantially reduces the total microbial population.

Sanitization is the microbial population is reduced to levels that are considered safe by public health standards.

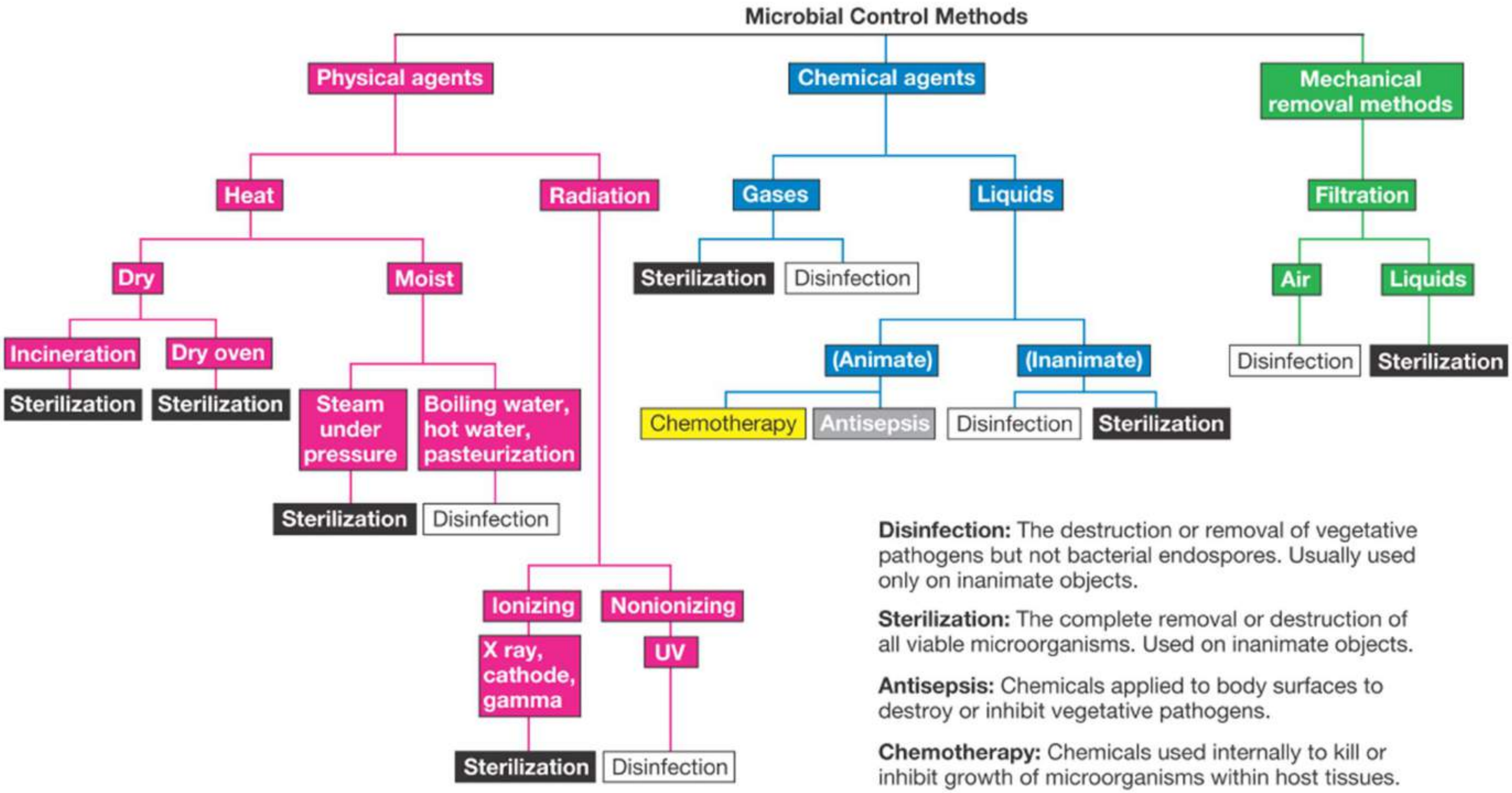
Antisepsis is the prevention of infection or sepsis and is accomplished with antiseptics. These are chemical agents applied to tissue to prevent infection by killing or inhibiting pathogen growth; they also reduce the total microbial population. Because they must not destroy too much host tissue, antiseptics are generally not as toxic as disinfectants.

Chemotherapy: chemicals used internally to kill or inhibit growth of microorganism within host tissue.

Conditions Influencing Antimicrobial Activity

- Several critical factors play key roles in determining the effectiveness of an antimicrobial agent, including:
  - Population size
  - Types of organisms
  - Concentration of the antimicrobial agent
  - Duration of exposure
  - Temperature
  - pH
  - Organic matter
  - Biofilm formation

Microbial Control Methods



Disinfection: The destruction or removal of vegetative pathogens but not bacterial endospores. Usually used only on inanimate objects.

Sterilization: The complete removal or destruction of all viable microorganisms. Used on inanimate objects.

Antisepsis: Chemicals applied to body surfaces to destroy or inhibit vegetative pathogens.

Chemotherapy: Chemicals used internally to kill or inhibit growth of microorganisms within host tissues.

Physical Methods

- Moist Heat
- Dry Heat
- Low Temperatures
- Filtration
- Radiation

Physical Methods: Moist Heat

- Mechanism of killing is a combinantion of protein/nucleic acid denaturation and membrane disruption
- Effectiveness Heavily dependent on type of cells present as well as environmental conditions (type of medium or substrate)
- Bacterial spores much more difficult to kill than vegetative cells

Physical Methods: Moist Heat

- Measurements of killing by moist heat
  - Thermal death point (TDP): Lowest temperature at which a microbial suspension is killed in 10 minutes; Because TDP implies that a certain temperature is immediately lethal despite the conditions.
  - Thermal death time (TDT): Shortest time needed to kill all organisms in a suspension at a specified temperature under specific conditions.
  - Decimal reduction time (*D* value): The time required to reduced a population of microbes by 90% (a 10-fold, or one decimal, reduction) at a specified temperature and specified conditions.
  - *z* value: The change in temperature, in ºC, necessary to cause a tenfold change in the *D* value of an organism under specified conditions.
  - *F* value: The time in minutes at a specific temperature (usually 121.1°C or 250 °F) needed to kill a population of cells or spores.

Physical Methods: Moist Heat

- Calculations using *D* and *z* values
  - Given: For *Clostridium botulinum* spores suspended in phosphate buffer, D_{121} = 0.204 min
  - How long would it take to reduce a population of *C. botulinum* spores in phosphate buffer from 10^{12} spores to 10^{0} spores (1 spore) at 121°C?
    Answer: Since 10^{12} to 10^{0} is 12 decimal reductions, then the time required is 12 x 0.204 min = 2.45 min

Physical Methods: Moist Heat

- Methods of Moist Heat
  - Boiling at 100°C
    - Effective against most vegetative cells; ineffective against spores; unsuitable for heat sensitive chemicals & many foods
  - Autoclaving/pressure canning
    - Temperatures above 100°C achieved by steam pressure
    - Most procedures use 121.1°C, achieved at approx. 15 psi pressure, with 15 - 30 min autoclave time to ensure sterilization
    - Sterilization in autoclave in biomedical or clinical laboratory must by periodically validated by testing with spores of *Clostridium* or *Bacillus stearothermophilus*

Fig. 25.2. A schematic diagram of laboratory autoclave.

Physical Methods: Moist Heat

- Methods of Moist Heat
  - Pasteurization
    - Used to reduce microbial numbers in milk and other beverages while retaining flavor and food quality of the beverage
    - Retards spoilage but does not sterilize
    - Traditional treatment of milk, 63°C for 30 min
    - Flash pasteurization (high-temperature short term pasteurization); quick heating to about 72°C for 15 sec, then rapid cooling

Physical Methods: Moist Heat

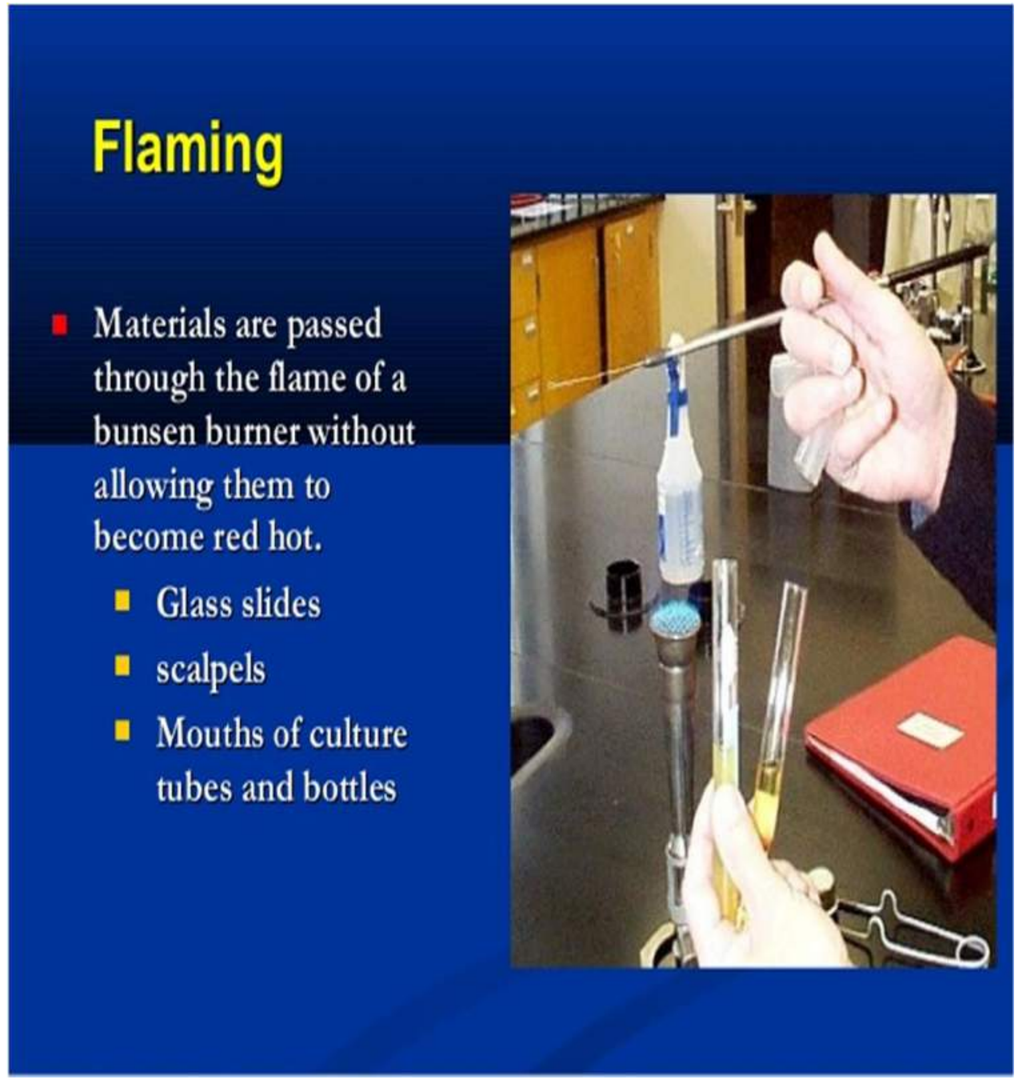
- Methods of Moist Heat
  - Ultrahigh-temperature (UHT) sterilization
    - Milk and similar products heated to 140 - 150°C for 1 - 3 sec
    - Very quickly sterilizes the milk while keeping its flavor & quality
    - Used to produce the packaged "shelf milk" that does not require refrigeration

Products That Can Be Pasteurized

Foods	Liquids
Butter	Milk
Cheese	Honey
Cream Cheese	Vinegar
Sour Cream	Fruit Juices
Yogurt	Cider
Ice Cream	Lemon Juice
Nuts	
Sauerkraut	
Eggs	
Lobster meat	
Crab meat	

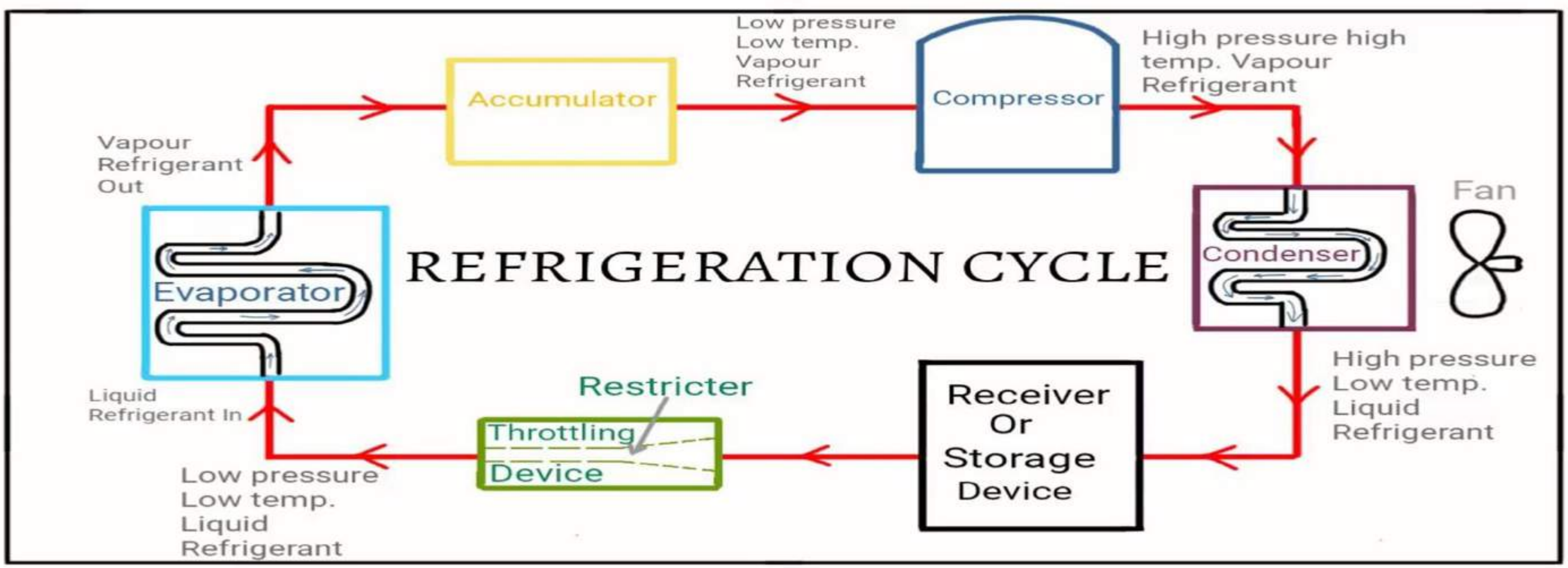
Physical Methods: Dry Heat

- Incineration
  - Burner flames
  - Electric loop incinerators
  - Air incinerators used with ferementers; generally operated at 500°C
- Oven sterilization
  - Used for dry glassware & heat-resistant metal equipment
  - Typically 2 hr at 160°C is required to kill bacterial spores by dry heat



Physical Methods: Low Temperatures

- Refrigerator:
  - around 4°C
  - inhibits growth of mesophiles or thermophiles; psychrophiles will grow
- Freezer:
  - “ordinary” freezer around -10 to -20°C
  - “ultracold” laboratory freezer typically -80°C
  - Generally inhibits all growth; many bacteria and other microbes may survive freezing temperatures

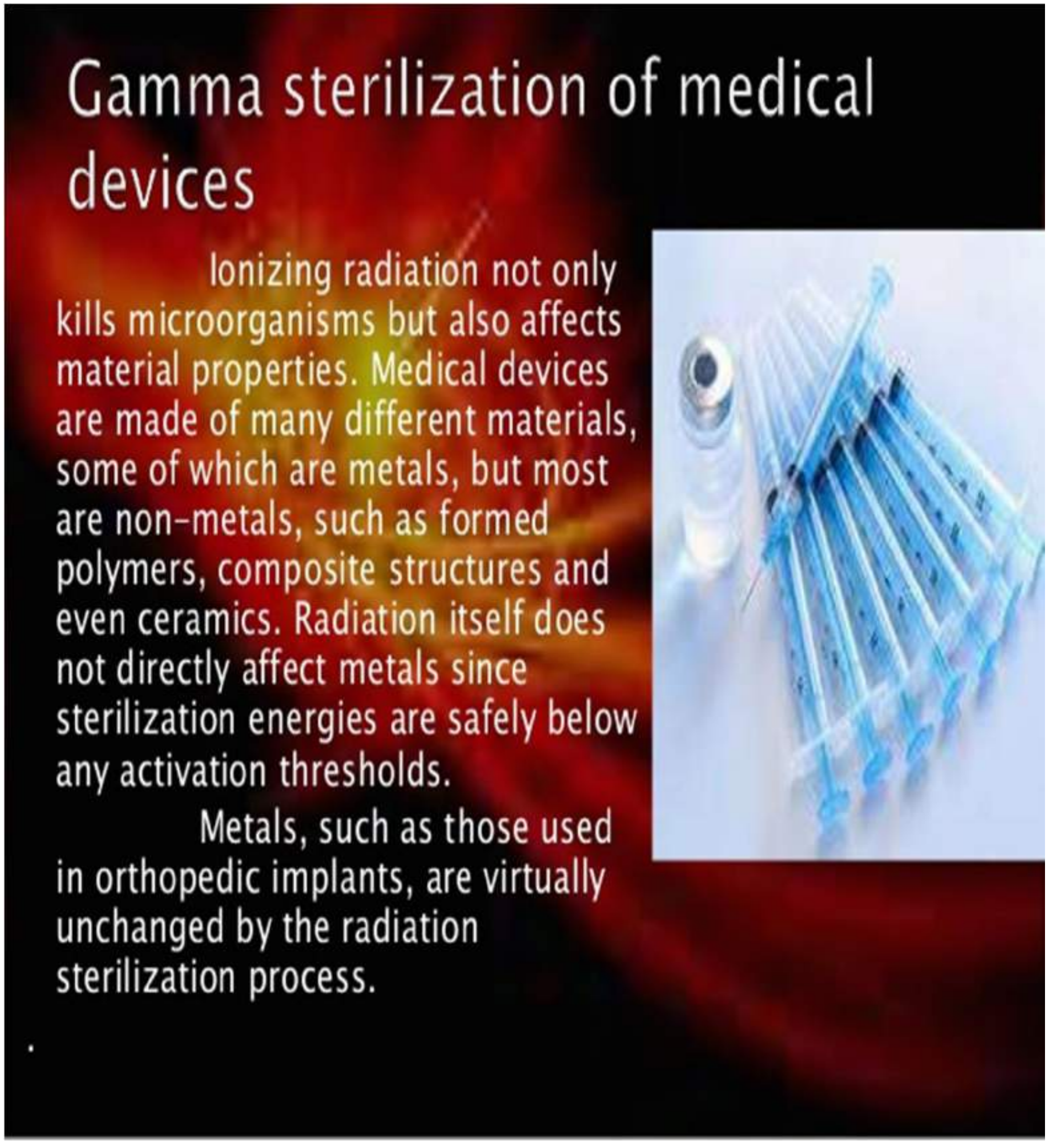


Physical Methods: Radiation

- Ultraviolet Radiation
  - DNA absorbs ultraviolet radiation at 260 nm wavelength
  - This causes damage to DNA in the form of thymine dimer mutations
  - Useful for continuous disinfection of work surfaces, e.g. in biological safety cabinets

Physical Methods: Radiation

- Ionizing Radiation
  - Gamma radiation produced by Cobalt-60 source
  - Powerful sterilizing agent; penetrates and damages both DNA and protein; effective against both vegetative cells and spores
  - Often used for sterilizing disposable plastic labware, e.g. petri dishes; as well as antibiotics, hormones, sutures, and other heat-sensitive materials
  - Also can be used for sterilization of food; has been approved but has not been widely adopted by the food industry



Chemical Agents

- Phenolics
- Alcohols
- Halogens
- Heavy metals
- Quaternary Ammonium Compounds
- Aldehydes
- Sterilizing Gases
- Evaluating Effectiveness of Chemical Agents

Chemical Agents: Phenolics

- Aromatic organic compounds with attached -OH
- Denature protein & disrupt membranes
- Phenol derivatives: orthocresol, orthophenylphenol, hexachlorophene
- Commonly used as disinfectants (e.g. “Lysol”); are tuberculocidal, effective in presence of organic matter, remain on surfaces long after application, disagreeable odor & skin irritation
- Hexachlorophene has been one of the most popular antiseptics because once applied it persists on the skin and reduces skin bacteria for long periods. However, it can cause brain damage and is now used in hospital nurseries only in response to a staphylococcal outbreak.

Chemical Agents: Alcohols

- Ethanol; isopropanol; used at concentrations between 70 – 80%
- Denature proteins; disrupt membranes by dissolving membrane lipids
- Kills vegetative cells of bacteria & fungi but not spores
- Used in disinfecting surfaces; thermometers; “ethanol-flaming” technique used to sterilize glass plate spreaders or dissecting instruments at the lab bench

Chemical Agents: Halogens

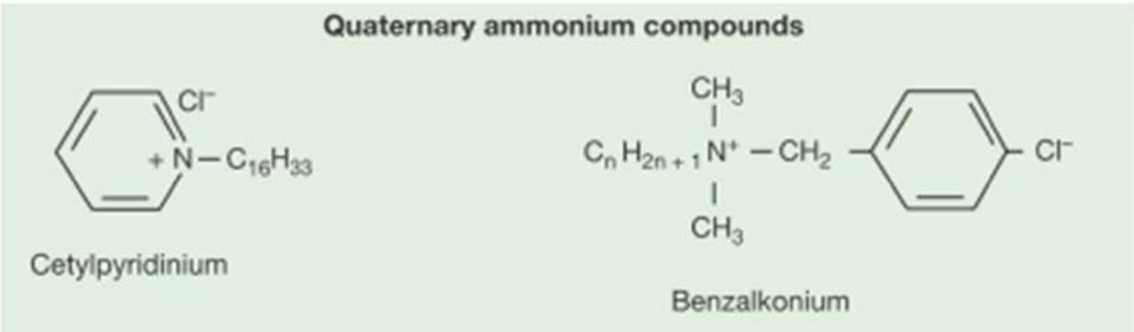
- Act as oxidizing agents; oxidize proteins & other cellular components
- The halogens iodine and chlorine are important antimicrobial agents.
- Chlorine compounds
  - Used in disinfecting municiple water supplies (as sodium hypochlorite, calcium hypochlorite, or chlorine gas)
  - Sodium Hypochlorite (Chlorine Bleach) used at 10 - 20% dilution as benchtop disinfectant
  - Halazone tablets (parasulfone dichloroamidobenzoic acid) used by campers to disinfect water for drinking
- Iodine Compounds
  - Tincture of iodine (2% or more iodine in a water-ethanol solution of potassium iodide.)
  - Iodophors: Iodine complexed to an organic carrier; e.g. Wescodyne, Betadyne. Iodophors are water soluble, stable, and nonstaining, and release iodine slowly to minimize skin burns and irritation.
  - Used as antiseptics for cleansing skin surfaces and wounds

Chemical Agents: Heavy Metals

- Mercury, silver, zinc, arsenic, copper ions
- Form precipitates with cell proteins
- At one time were frequently used medically as antiseptics but much of their use has been replaced by less toxic alternatives
- Examples: 1% silver nitrate was used as opthalmic drops in newborn infants to prevent gonorrhea; has been replaced by erythromycin or other antibiotics;
- copper sulfate used as algicide in swimming pools

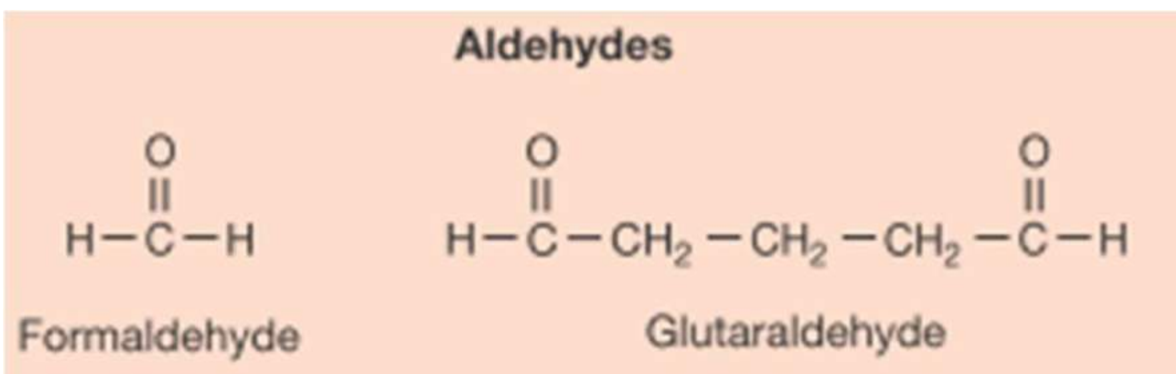
Chemical Agents: Quaternary Ammonium Compounds

- Quaternary ammonium compounds are detergents that have antimicrobial activity and are effective disinfectants.
- Amphipathic molecules (having both polar hydrophilic and no polar hydrophobic components) that act as emulsifying agents
- The hydrophilic portion of a quaternary ammonium compound is a positively charged quaternary nitrogen; thus quaternary ammonium compounds are cationic detergents.
- Denature proteins and disrupt membranes
- Used as disinfectants and skin antiseptics
- Examples: cetylpyridinium chloride, benzalkonium chloride



Chemical Agents: Aldehydes

- Formaldehyde and gluteraldehyde
- React chemically with nucleic acid and protein, inactivating them
- Aqueous solutions can be used as disinfectants

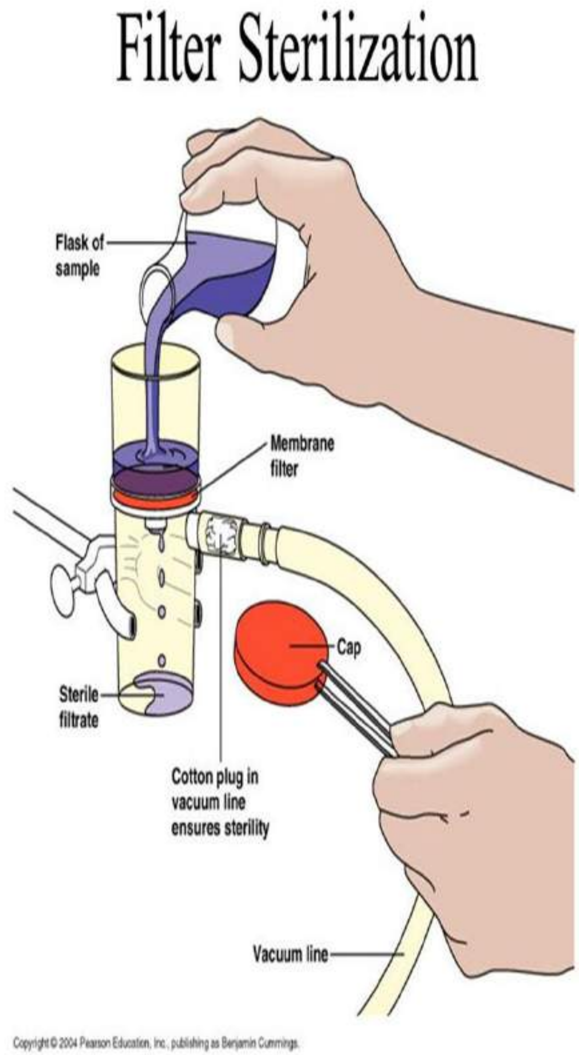


Chemical Agents: Sterilizing Gases

- Ethylene oxide (EtO)
  - Used to sterilize heat-sensitive equipment and plasticware
  - It is both microbicidal and sporicidal and kills by combining with cell proteins.
  - Sterilization is carried out in a special ethylene oxide sterilizer, very much resembling an autoclave in appearance, that controls the EtO concentration, temperature, and humidity.
  - Explosive; supplied as a 10 – 20% mixture with either CO_2 or dichlorofluoromethane
  - The ethylene oxide concentration, humidity, and temperature influence the rate of sterilization. A clean object can be sterilized if treated for 5 to 8 hours at 38°C or 3 to 4 hours at 54°C when the relative humidity is maintained at 40 to 50% and the EtO concentration at 700 mg/liter.

Mechanical Methods: Filtration

- Used for physically removing microbes and dust particles from solutions and gasses often used to sterilize heat-sensitive solutions or to provide a sterilized air flow
- Depth filters: it consist of fibrous or granular materials that have been bonded into a thick layer filled with twisting channels of small diameter. The solution containing microorganisms is sucked through this layer under vacuum, and microbial cells are removed by physical screening or entrapment and also by adsorption to the surface of the filter material. Depth filters are made of diatomaceous earth (Berkefield filters), unglazed porcelain (Chamberlain filters), asbestos, or other similar materials.
- Membrane filters: eg. Nitrocellulose, nylon polyvinylidene difluoride
- HEPA filters: High efficiency particulate air filters used in laminar flow biological safety cabinets. It ia a type of Depth filter.



Acknowledgement and Suggested Readings:

1. Microbiology, An Introduction; Tortora, Funke and Case; Pearson Publication
2. Microbiology; Prescott, Harley and Klein; The MacGraw-Hill Companies
3. Microbiology: Principles and Explorations; Jacquelyn G Black; John Wiley and Sons Inc.
4. Brock Biology of Microorganisms; Madigan, Martinko, Stahl and Clark; Benjamin Cummings (Pearson Publication)

Thanks