VIVEKANANDA COLLEGE
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NAAC ACCREDITED 'A' GRADE

Topic	: Bacterial Growth Regulation and Control
Course Title	: Basic Microbiology and Microbial Genetics
Paper	: CC 10
Unit	: II
Semester	: 4
Name of the Teacher	: Dr. Kakali Roy
Name of the Department	: Biochemistry

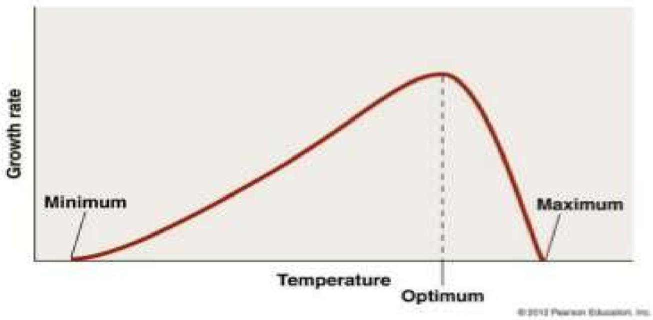
Physical factors influencing growth

Temperature

- Temperature influences rate of chemical reactions and protein structure integrity -----rate of enzyme activity
- At **low temperature**, every 10ºC rise in temp. **metabolic activity rises --growth** occurs
- At **high temp**. bacteria killed by **denaturing enzymes**, inhibiting transport carrier molecules or change in membrane integrity
- Each bacteria shows a characteristic temperature dependence

Effect of temperature

- **Minimum growth temperature** - microbe is able to conduct metabolism
- **Maximum growth temperature** – microbe continues to metabolize
- **Optimum growth temperature** – highest growth rate



- **Growth rate plotted against temperature**

22°C 30°C 37°C

- **Growth of *Escherichia coli* on nutrient agar at three different temperature**

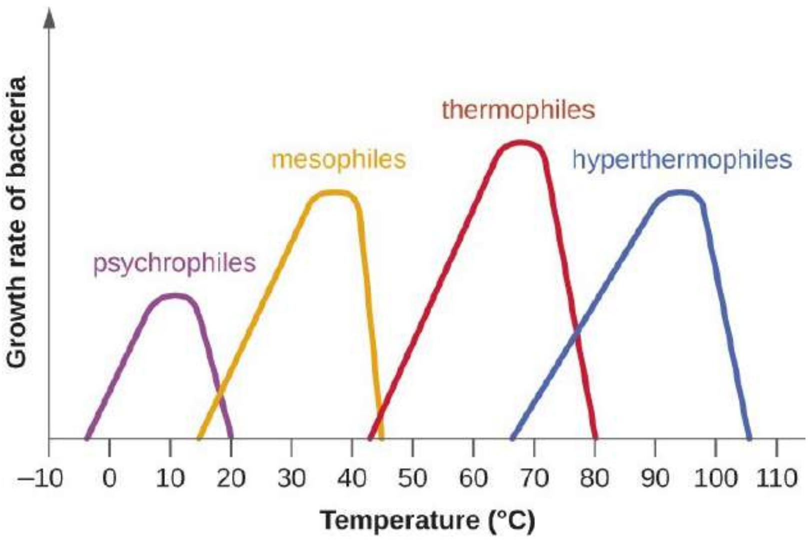
Factors that Affect Microbial Growth

Temperature –

- Affects proteins and lipid membranes
  - If too low, membranes become rigid and fragile
  - If too high, membranes become too fluid

- **Categories based on Optimum Temperature**
  - **Psychrophile** – optimum below 15°C
  - **Mesophile** – optimum between 20°C – 40°C
  - **Thermophile** – optimum higher than 45°C
  - **Hyperthermophiles** – optimum above 80°C

Classification	Minimum temp.	Optimum temp.	Maximum temp.
Extreme psychrophile	–10	10~15	20
Psychrophile	–10	20~30	42
Mesophile	5	28~43	52
Thermophile	30	50~65	70
Extreme thermophile	65	80~90	100



Examples :

Psychrophile : *Flavobacterium sp*.

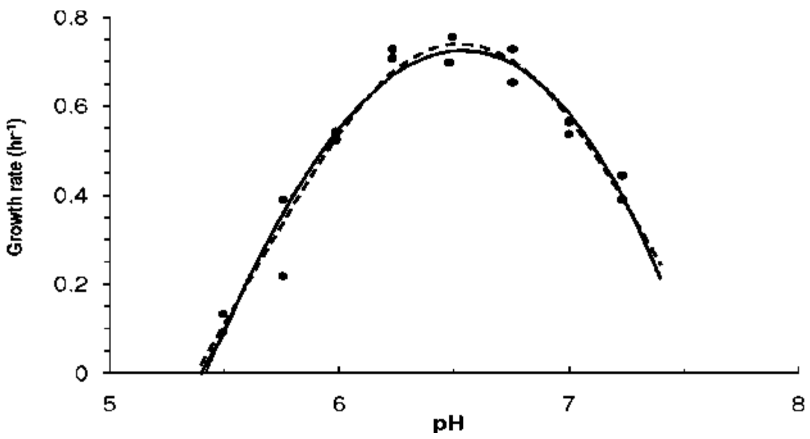
Mesophile : *Escherichia coli*

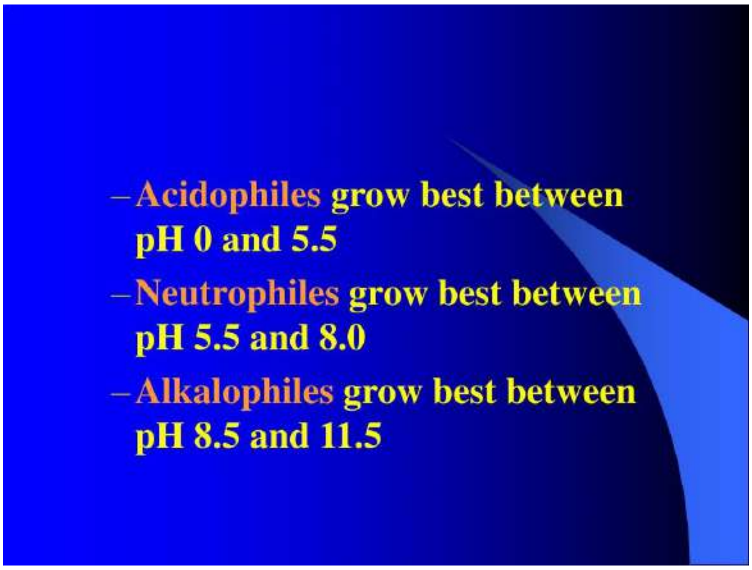
Thermophile : *Bacillus stearothermophilus*

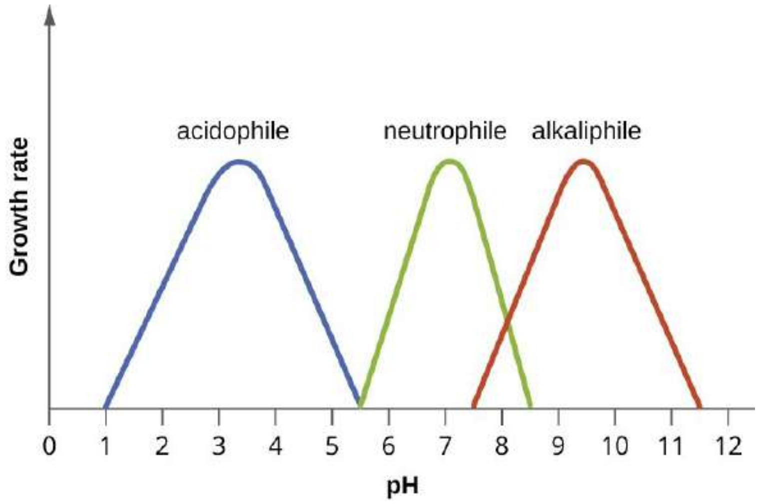
Hyperthermophile : *Thermococcus celer*

pH

- The activity of microbial enzymes depends on the charge present on the surface of amino acids
- Any change in environmental pH alters enzyme activity ---- affect the growth







Examples

Acidophile : *Lactobacillus acidophilus*

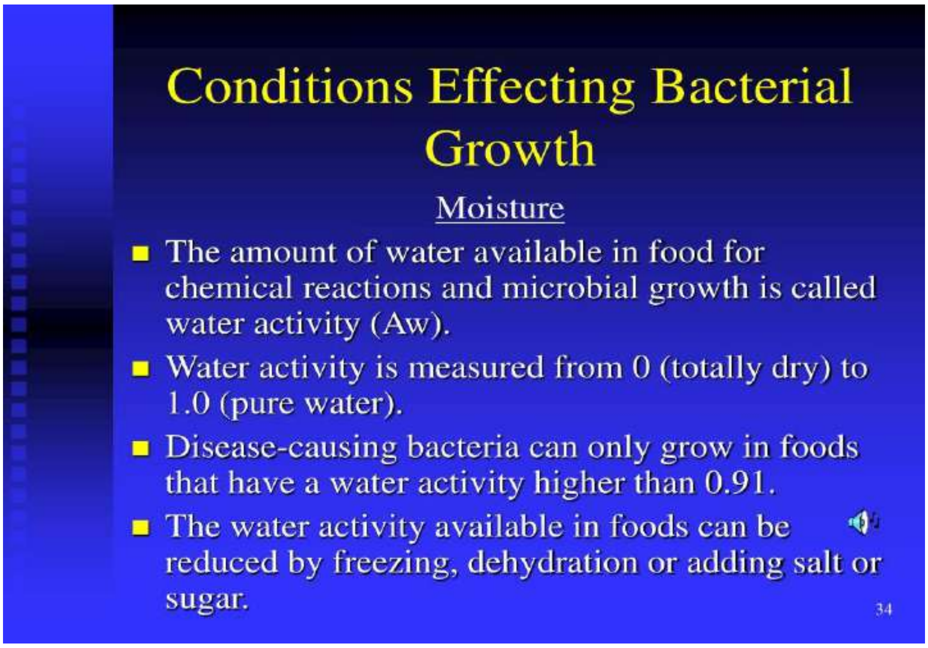
Neutrophile : *E. coli*

Alkaliphile : *Vibrio cholera*

- Growing microorganisms produce acidic and basic metabolic waste products.
- These wastes become inhibitory agents ------ alter the pH of environment
- Thus, citrate or phosphate buffers are added to maintain constant pH of the medium

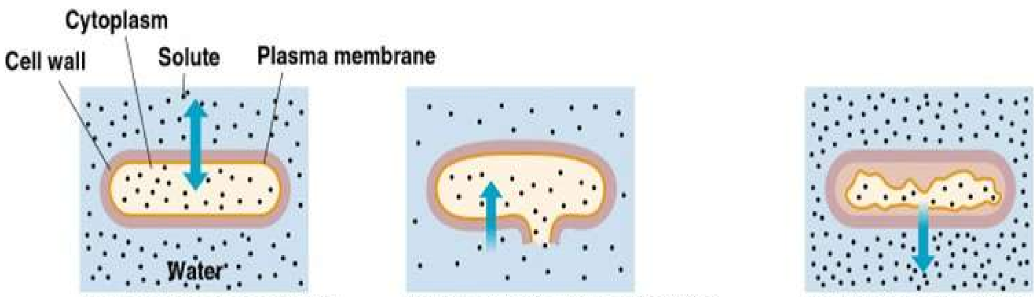
Osmotic pressure

- Pressure exerted on bacteria by their environment
- One of the major agents exerting such pressure is solute concentration
- Microorganism obtain almost all their nutrients in solution from the surrounding water
- They require water for growth and are made up of 80 – 90% water
- Availability of water depends on two factors: # water content at the surrounding environment # Conc. of solutes (salt, sugars etc.) dissolved in the water
- Water activity inversely proportional to osmotic pressure
- Most microorganisms grow well only near pure water activity (around 1)



Osmotic pressure

- Microbes obtain their nutrients in solution from the surrounding water
- Higher osmotic pressures remove water from a cell
- Hypertonic, cellular water passes out through causing shrinkage
- **Plasmolysis**
- in food preservation, addition of salts helps to prevent microbial growth
- extreme halophiles have good adaptation at high salt concentrations



(c) Isotonic (isosmotic) solution— no net movement of water

(d) Hypotonic (hypoosmotic) solution—water moves into the cell and may cause the cell to burst if the wall is weak or damaged (osmotic lysis)

(e) Hypertonic (hyperosmotic) solution— water moves out of the cell, causing its cytoplasm to shrink (plasmolysis)

Osmotic Pressure

Isotonic solutions :

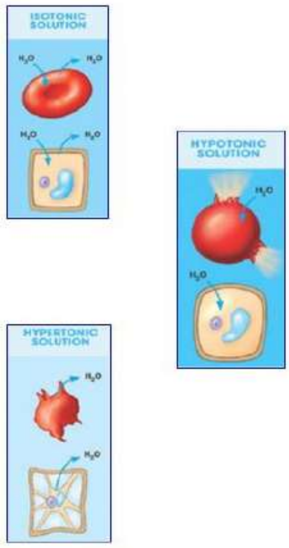
solutions where the concentration of the solute is equal to that of normal cells found in it; thus no osmotic pressure is exerted. Most organisms prefer isotonic solutions.

Hypotonic solutions:

Solutions where solute concentration outside the cell is less than that inside the cell. This cause microbial cells to swell then burst (die).

Hypertonic solutions :

Solutions where solute concentration outside the cell is more than that inside the cell. This cause microbial cells to shrink (inhibiting growth).



Salt Concentration

- A specific concentration of NaCl is required for microbial growth in vitro
- It is equal to normal saline salts concentration (0.9% NaCl)
- Halophilic bacteria resist high salt concentration

Bacterial growth
Physical requirements – Salt

- **Halophiles:** specific requirement for the sodium ion (microorganisms found in the sea)
  - Mild halophile → 1-6% NaCl
  - Moderate halophile → 6-15% NaCl
  - Extreme halophile → 15-30% NaCl **(archaebacteria)**
- **Halotolerant:** grow best in the absence of NaCl, but can tolerate some NaCl

Halophiles

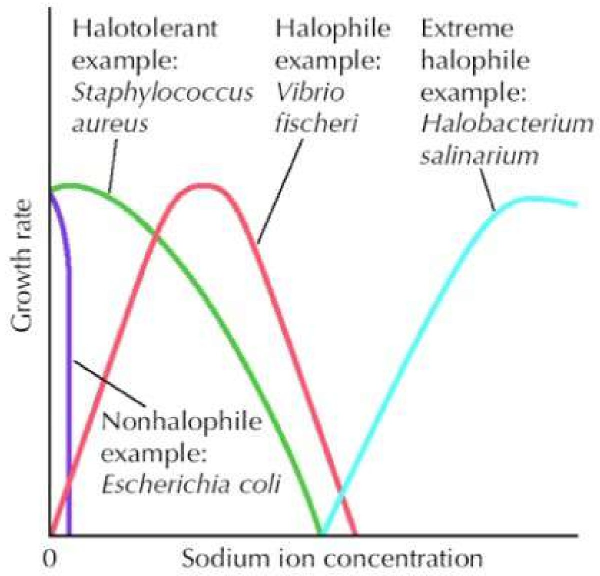
- **Salt-loving organisms which require moderate to large quantities of salt (sodium chloride)**
- **Membrane transport systems actively transport sodium ions out of cells and concentrate potassium ions inside**
- **Why do halophiles require sodium?**

1. **Cells need sodium to maintain a high intracellular potassium concentration for enzymatic function**
2. **Cells need sodium to maintain the integrity of their cell walls**

Salt Tolerance

- The effect to which salt concentration causes changes in bacterial growth depends on the osmotic balance required for such growth.
- Some bacteria require a high level of salt to grow, whereas other bacteria would be killed in high levels of salt.

Optimal salt concentration for different species



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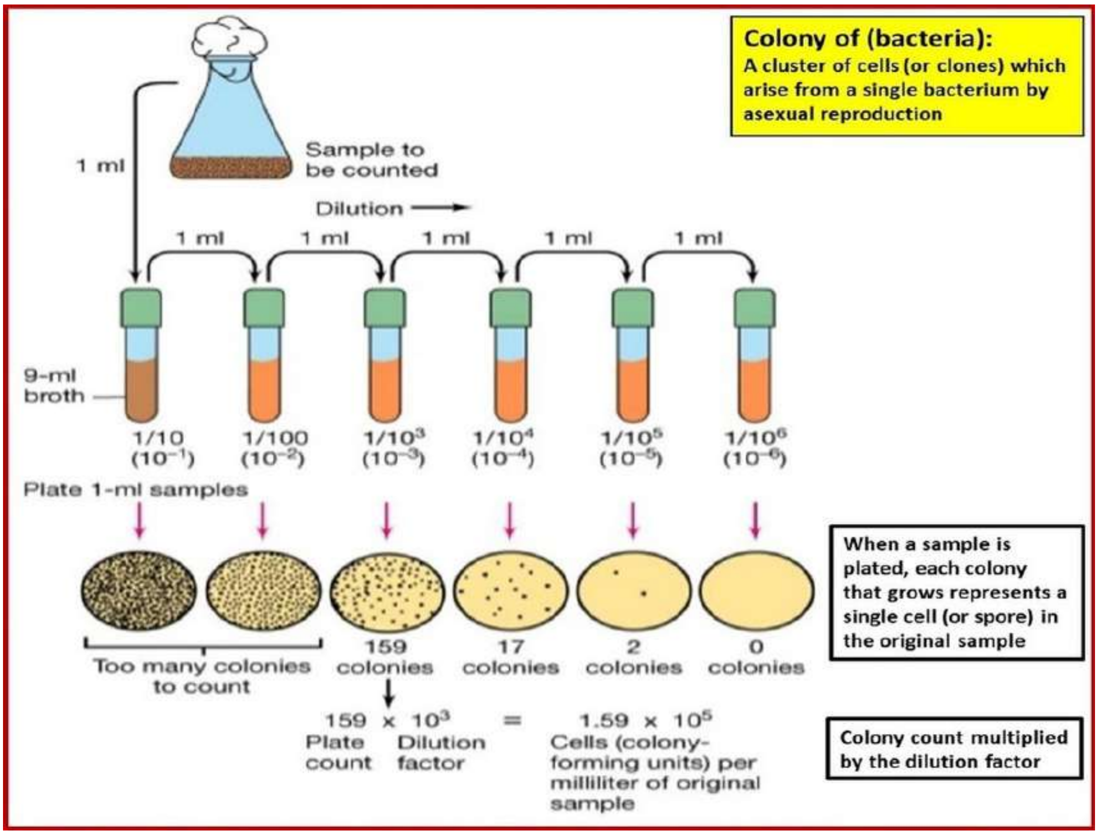
- Halophilic organisms (grow optimally at > 0.2 M NaCl) can be divided into:
- Obligate : requiring a high salt conc.
- Extreme : requiring very high levels of salt (5-6 M NaCl)
- Facultative or tolerant : can grow either with or without elevated salt levels

CFU



Calculation of Bacterial Count

- The aim is to calculate how many colony forming units (C.F.U.) of organism which present in the original sample.
- Colony forming units (CFU) = **number of bacteria** that were able to replicate many times and give one single, visible colony.

(Colony: is a group of bacteria that grew from one original bacterium).

- **B = N/ V x D**
  B = number of bacteria
  N = number of colonies counted on a plate
  V= volume
  D = dilution factor

Sterilization

Definition

- A physical or chemical process that completely destroys or removes all living microbial cells, viable spores, viruses etc. from an object or habitat.

Examples

- When sterilization is achieved by a chemical agent ----called sterilant ---such as Hydrogen peroxide, Nitrogen dioxide, Formaldehyde solutions.

Disinfection

Definition

- A process of destruction or removal of all living pathogenic microorganisms or organism capable of giving rise to infection.
- It does not destroy bacterial spores.

Application

- In **hospital environment**, for cleaning of bedpans, surfaces, also for skin disinfection before surgical operation.
- In **microbiology laboratory**, for skin disinfection, instrument disinfection, laboratory surfaces cleaning and others.
- water purification and domestic disinfection in food preservation area

Examples

An agent (chemical or physical) used to carry out disinfection called **disinfectant (**which is used only on inanimate objects) such as Alcohol, Formaldehyde solution, Phenol, Sodium hypochlorite (household bleach).

Antiseptic

Definition

An agent that causes destruction or inhibition of growth of microorganisms (bacteria, viruses, fungi) on living surfaces such as skin and mucous membranes.

Application

Applied to **living tissue** for the purpose of preventing infection.

Example

Hydrogen peroxide, Tincture of Iodine, Chlorhexidine gluconate, Alcohol.

Sanitizer

Definition

An agent that reduces the number of microorganisms to a safe level

Application

- Food contact sanitizers are commonly used in the food industry to treat surfaces such as those of mixing and cooking equipment, dishes and utensils.
- Non-food contact sanitizers are used to treat surfaces such as those of counters, floors, walls, carpets, air and laundry.
- Hand sanitizer used to decrease infectious agents on the hands

Example

Peroxyacetic acid, Hydrogen peroxide, Chlorine dioxide, Isopropyl alcohol

Germicide

Definition

An agent that destroys germs, harmful microorganisms

Application

- Germicides can be used to inactivate microorganisms in or on living tissue (i.e. antiseptics) or on environmental surfaces (i.e. disinfectants).
- Used in dentistry
- Used in hand wash

Example

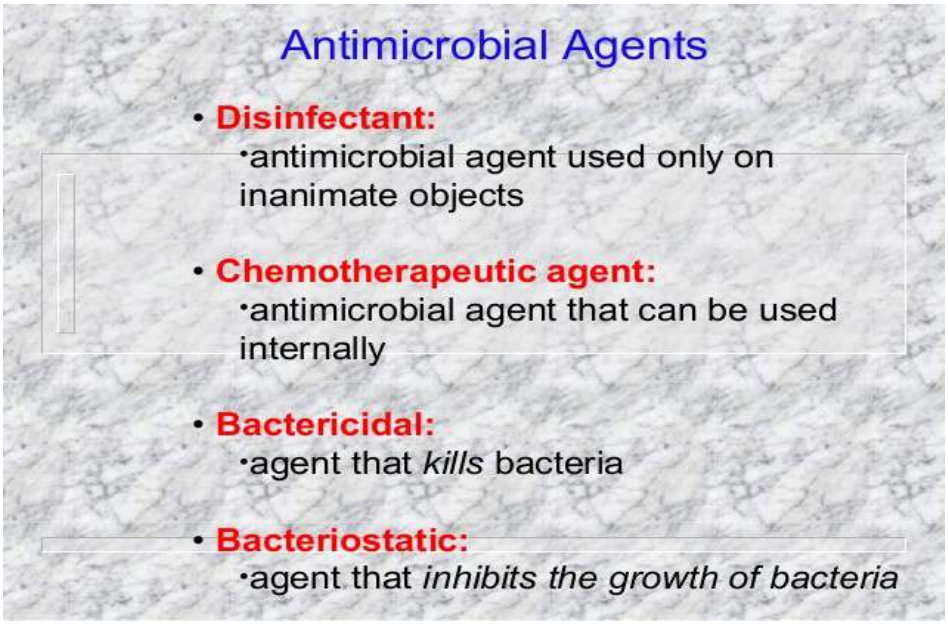
Gluteraldehyde, Chlorhexidine gluconate (0.12- 4%), Alcohol, Triclosan.

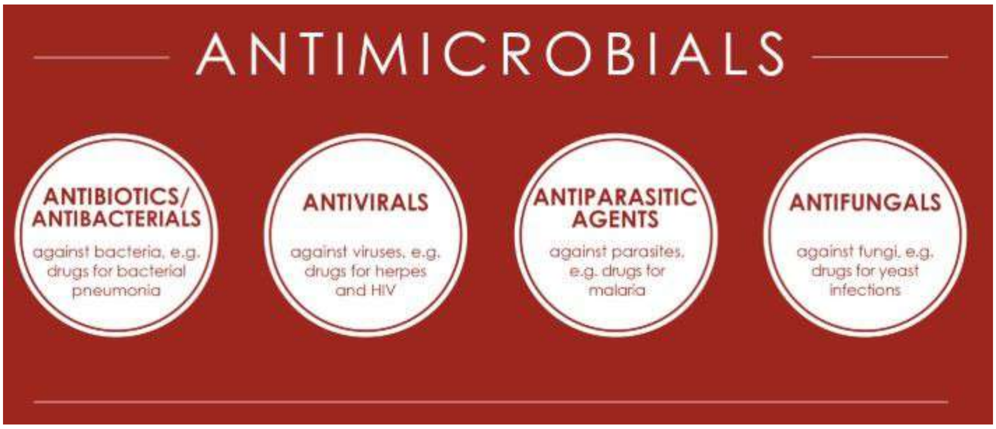
Antimicrobial agent

Definition

A natural or synthetic substance that kills microorganisms (bacteria, viruses, fungi, protozoa) or inhibits their growth.

Application

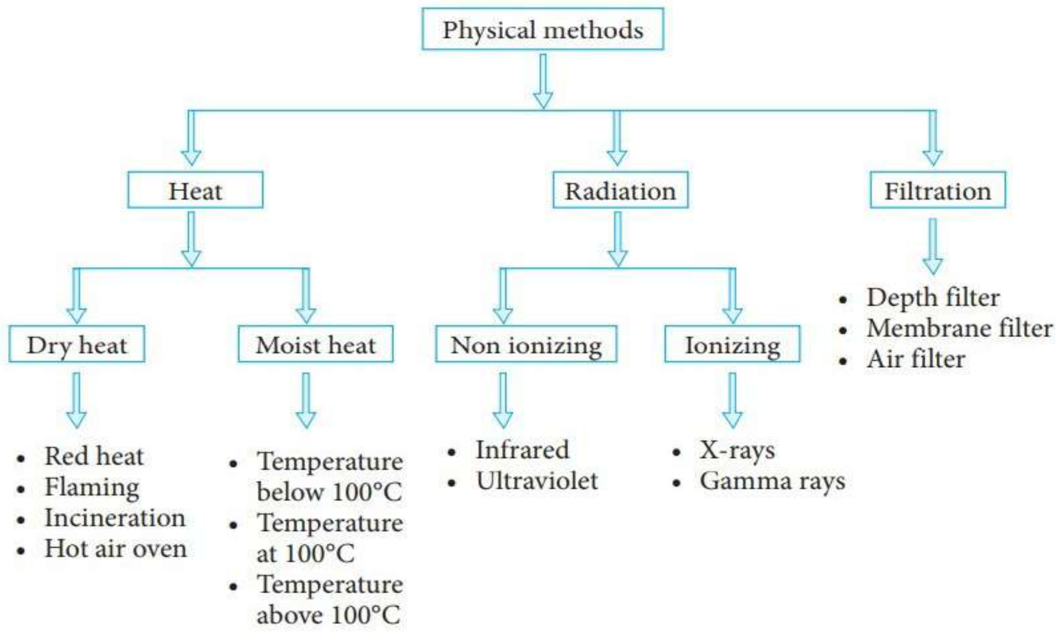




Example

Antibiotics (like Penicillin, Tetracycline), Sulfonamides, Quinolones, Benzoic acid, Phenol, Phenyl mercuric acetate, Formaldehyde solution.

Physical method of Disinfection and Sterilization



Flowchart 4.1: Physical Methods of Sterilization

Heat sterilization is the most widely used and reliable method of sterilization, involving destruction of enzymes and other essential cell constituents.

Dry heat

Principle

- Sterilizing by dry heat is accomplished by conduction. The heat is absorbed by the outside surface of the item, then passes towards the Centre of the item, layer by layer. The entire item will eventually reach the temperature required for sterilization to take place.

- Dry heat does most of the damage by oxidizing molecules. The essential cell constituents are destroyed and the organism dies. The temperature is maintained for almost an hour to kill the most difficult of the resistant spores.

- It employs higher temperatures in the range of 160-180°C and requires exposures time up to 2 hours, depending upon the temperature employed.
- The vital constituents of cells such as proteins (enzymes) and nucleic acids are denatured by oxidation.
- The benefit of dry heat includes good penetrability and non-corrosive nature.

Dry heat sterilization:

Dry heat is believed to kill microorganisms by causing a destructive oxidation of essential cellular components.

Types of dry heat sterilization:

1- Hot air Oven sterilization:

Oven is a double walled steel chamber (with a door) that is electrically heated and thermostatically controlled.

The **optimum conditions** for sterilization by hot air oven will be: For 2 hours at **160°C**, one hour at **170°C** and 30 minutes at **180°C**.

2- Red heat sterilization and flaming: In the laboratory. Inoculating wires, loops and points of forceps are sterilized by holding them in the flame of **Bunsen burner** until they are red hot.

Dry heat sterilization

Applications

✓Dry heat is used to sterilize *Glass ware* (e.g. test tubes, Pasteur-pippettes, petridishes, flasks, glass syringes etc)

✓The glass wares should be prewashed with apyrogenic water.

✓Porcelain and metal equipment such as forceps, scalpels, scissors etc.

✓Fats, oils and greasy materials (like petroleum jelly) those are **impermeable** to **moisture**.

Moist heat

- Moist heat is more effective than dry heat because water is good conductor of heat than air
- It kills microorganisms by coagulating and denaturing their enzymes and structural proteins.

Mode of action

- Denaturation of proteins
- Destruction of membranes
- Destruction of DNA

Types

- Boiling
- Steam under pressure (autoclaving)

Temperatures at 100⁰C (boiling)

- Vegetative bacteria are killed almost immediately at 90-100⁰C, but sporing bacteria require prolonged periods of boiling.
- Boiling water is not considered as a sterilizing agent because destruction of bacterial spores and inactivation of viruses cannot always be assured. It is considered as a method for disinfection
- A minimum exposure period of 30min. is recommended to kill vegetative bacteria.
- Sodium bicarbonate 2% conc. is added to increase the efficiency of process.

Pasteurization

- Controlled heating at **temperatures** well below boiling (**<100ºC**) ----reduces total microbial population and thereby increases shelf life of treated material

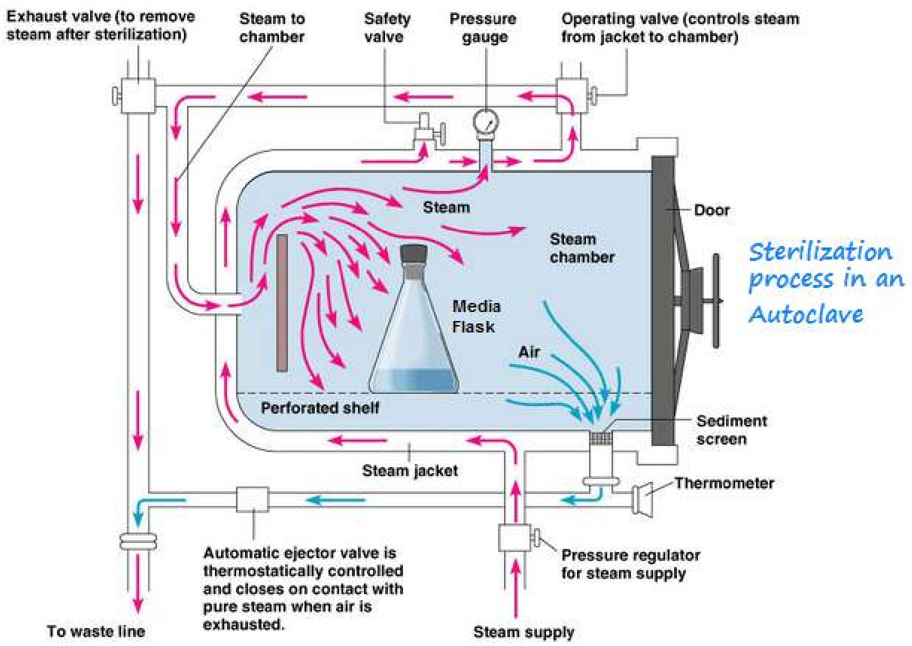
Pasteurization

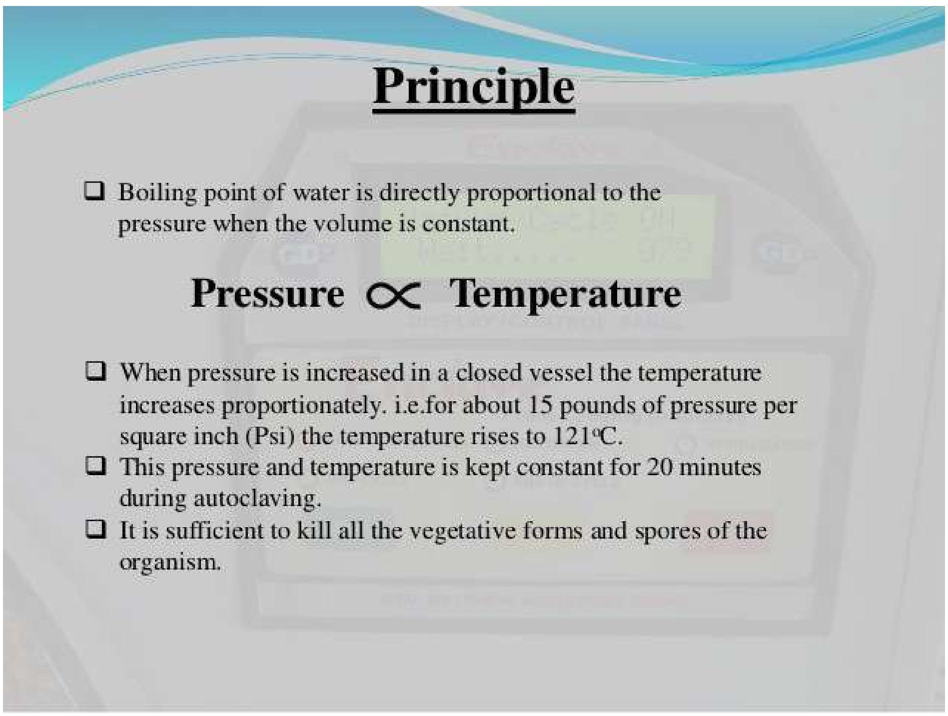
- Purpose: Inactivation of bacterial pathogens (target organisms *Coxiella burnettii*)
  - assurance of longer shelf life (inactivation of most spoilage organisms and of many enzymes)
- Pasteurization
  - Heat treatment of 72ºC (161°F) for 15 sec (HTST) or 63ºC (145°F) for 30 min (or equivalent)
  - does not kill all vegetative bacterial cells or spores (*Bacillus* spp. and *Clostridium* spp.)
  - Pasteurization temperature is continuously recorded

2. Pasteurization

- **Pasteurization** is a relatively mild heat treatment, in which food is heated to below 100ºC.
- **In low acid foods** (pH>4.5, for example milk) it is used to minimise possible health hazards from pathogenic micro-organisms and to extend the shelf life of foods for several days.
- **In acidic foods** (pH<4.5, for example bottled fruit) it is used to extend the shelf life for several months by destruction of spoilage micro-organisms (yeasts or moulds) and/or enzyme inactivation.
- **In both types of food,** minimal changes are caused to the sensory characteristics or nutritive value.

Autoclave (Above 100ºC temperature)





Applications of autoclave

- ✓ It is used to sterilize anything, which is not injured by steam and high temperature of sterilization. These includes, Aqueous parenteral solutions e.g. distilled water, saline solutions.
- ✓ Aqueous liquid media e.g. liquid media with or without carbohydrate and gelatin.
- ✓ Surgical dressings and fabrics.
- ✓ Plastic and rubber closures.
- ✓ Metal instruments.
- ✓ Glass apparatus and containers.

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Filtration Sterilization

- Filtration process does not destroy but removes the microorganisms. It is used for both the clarification and sterilization of liquids and gases as it is capable of preventing the passage of both viable and non viable particles.
- The major mechanisms of filtration are sieving, adsorption and trapping within the matrix of the filter material.
- Ex:HEPA FILTERS

Membrane Filters

- *The* ***membrane filter*** *is the most common type of filtration system used in modern microbiology laboratories. These are made from high tensile strength polymers of cellulose acetate, cellulose nitrate, polycarbonate, polyester, polypropylene or polysulfone.*

MEMBRANE FILTERS

- ✓ Membrane filters are made of cellulose-derivative (acetate or nitrate). They are very fine. They are fixed in some suitable holders.
- ✓ **Nominal pore size is 0. 22 ± 0. 02 mm** or less is required.
- ✓ The membranes are brittle when dry. In this condition they can be stored for years together. They become very tough when dipped in water.
- ✓ They are sterilized by autoclaving or by ethylene oxide gas. They cannot be sterilized by dry heat as they decompose above 120^{0} C.
- ✓ They are suitable for sterilizing aqueous and oily solutions but not for organic solvents such as alcohol, chloroform etc.

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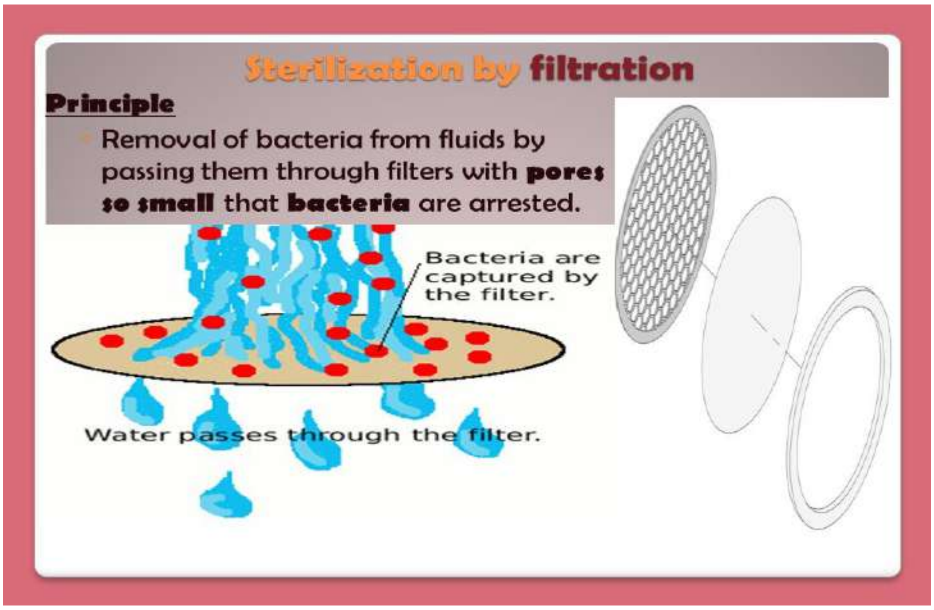
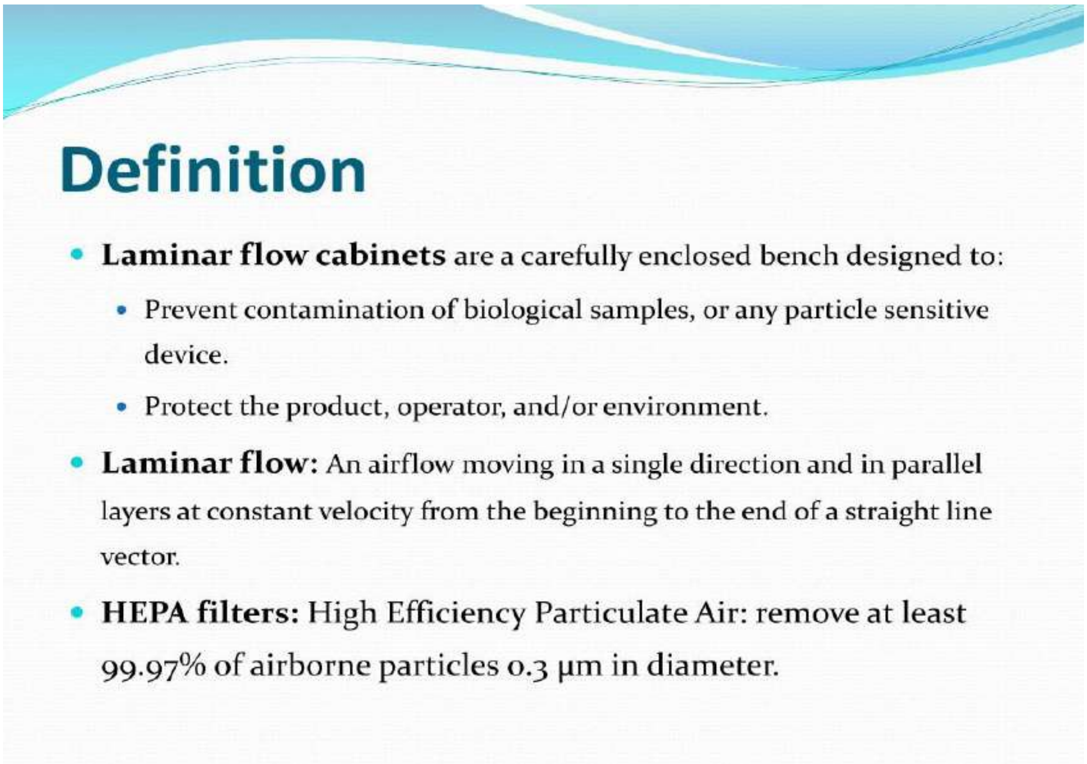
Filtration

- Bacteria can be removed from liquids by passing them through filters
- Useful for sterilizing heat labile fluids like vitamins, antibiotics, sera, urea, protein solution etc
- Types of filters
  - Earthernware candle filters - eg. Berkefeld filter, Chamberland filter
  - Asbestos pad filters - eg. Seitz filters
  - Sintered glass filter
  - Membrane filters
    - made from cellulose acetate or cellulose nitrate
    - membranes of 0.45 μ m or 0.22 μ m are used



Filtration of air

To make air free from unwanted microbes, a device called **Laminar air flow** is used at appropriate times in laboratories.



HEPA in Laminar air flow systems

- Laminar air flow systems are used for cleaning air in aseptic rooms.
- In laminar air flow systems,clean air is obtained through HEPA filters
- This air flows at a speed of 100 ft/min & sweeps dust particles making entire room free of particulate matter

Applications of filtration

1. Production of sterile products:- (HEPA) filters (or) laminar air bench provide sterile environment during manufacture of sterile products.
2. Bacteria proof filters are used for filtration of thermo labile substances where heat sterilization is not applicable.
3. Production of bulk drugs:- Filtration is an essential step in the removal of impurities from the products.
4. Production of liquid orals:- Filtration is very important in the production of liquid orals and other for obtaining clear solutions. Eg. In aromatic water preparations, syrups, elixirs, and eye drops, where removal of foreign impurities is very important.
5. Effluent and waste water treatment

STERILIZATION BY RADIATION

Radiation can be divided into two groups:

1. Electromagnetic waves:
   - (i) infra-red radiation (IR)
   - (ii) ultraviolet radiation (UV)
   - (iii)X-rays
   - (iv)gamma rays

2. Streams of particulate matter
   - (i) alpha radiation
   - (ii) beta radiation

For sterilization infrared, ultraviolet, gamma radiation and high velocity electrons (a type of beta radiation) are used for sterilization.

Radiation	Wave length	Energy
UV-radiation	190 to 370 nm	5 eV
Gamma radiation	1 to 10^{-4} nm	1.3 MeV*
High velocity electrons		4MeV

- Radiation refers to transmission of energy in a variety of forms through space or a medium, the most effective type of radiation to sterilize or reduce microbial growth is through **electromagnetic radiation**.

- Streams of minute particles of matter are **α and β radiation**
- Radiation sterilization causes damage to DNA and results in cell death

Types of Radiation

- 1. Ionizing Radiation
  - X-rays and gamma rays
  - Disrupts DNA and RNA
  - Used for Sterilization Procedures

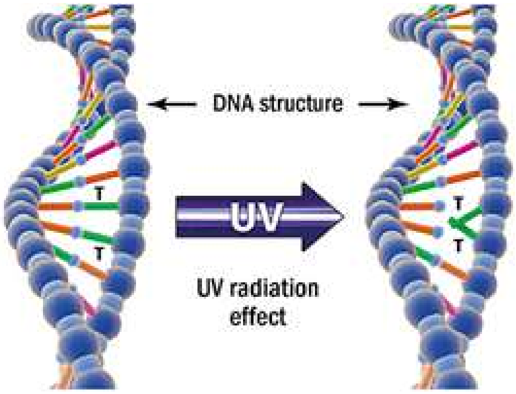
- 2. Non-Ionizing Radiation
  - UV light
  - Does not penetrate plastic, glass and proteinaceous matter (very well)
  - Not used for sterilization procedures, but is used to **reduce microbial populations**
  - lethal mutations – Thymine Dimers

Non-Ionising radiation:

1. Infra red rays
2. Ultraviolet (UV) rays

– Infra red is used for rapid mass sterilisation of syringes and catheters.

– Ultraviolet radiation is used for disinfecting enclosed areas such as bacterial laboratory, inoculation hood, laminar flow and operation theatres.



When exposing microorganisms to UVC light, the light penetrates through their cell wall and disrupts the structure of their DNA molecules, prohibiting reproduction.

Radiation Sterilisation

- **Sterilisation by U.V light:**
- U.V rays for sterilisation is produced by passing a low current at high voltage through mercury vapour in an evacuated glass tube.
- The antimicrobial activity of U.V. light depends on its wave length.

Applications:

- It is used for sterilisation of air to prevent cross infection in hospitals.
- It is used in maintain the aseptic area in the pharmaceutical industry & also used for sterilisation.
- It is used for sterilisation of thermolabile substances before packing.

IONIZING RADIATION

- It is effective for heat labile items .
- It is commonly used by industry to sterilize disposable materials such as needles, syringes, swabs, catheters, suture material, cannulas.
- High energy gamma rays from cobalt 60 are used to sterilize such article.

USE OF IONIZING RADIATION

Mode of action:

- Affects on bacteria, yeasts and moulds.
- Main sites of damages: nucleic acid and the lipids of the cell membrane
  - *Membrane lipid degradation*
  - *Change the permeability of the cell membrane*
  - *Leach out of cell components and*
  - *Inhibition of the DNA replication*
- Indirect effect: inhibitory effects of free radicals produced by the radiolysis of water.

Application

- Radiation sterilization is generally applied to articles in the dry state; including surgical instruments, sutures, prostheses, unit dose ointments, plastic syringes and dry pharmaceutical products.
- UV light, with its much lower energy, and poor penetrability finds uses in the sterilization of air, for surface sterilization of aseptic work areas, for treatment of manufacturing grade water, but is **not suitable** for sterilization of pharmaceutical dosage forms.

Reference

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