Microbiology

What is a Unicellular or single cell Organism?

unicellular organisms are living organisms that exist as single cells. Examples include such bacteria as *Salmonella* and protozoa like *Entamoeba coli*. Being single celled organisms, various types possess different structures and characteristics that allow them to survive.

- *Unicellular are organisms consisting of one cell
- *Bacteria, Protists, Amoeba, and Algae are all unicellular
- *Unicellular organisms are also known as Single cell
- *Can usually only be seen through a microscope
- *Can preform all life functions (Eating, breathing, moving, growing)

What is microorganisms?

Microorganisms or microbes are microscopic organisms that exist as unicellular, multicellular, or cell clusters. Microorganims are widespread in nature and are beneficial to life, but some can cause serious harm. They can be divided into six major types: bacteria, archaea, fungi, protozoa, algae, and viruses. Microorganisms are beneficial in producing oxygen, decomposing organic material, providing nutrients for plants, and maintaining human health, but some can be pathogenic and cause diseases in plants and humans.

Identification and classification:

There are various types of microorganisms like bacteria, fungi, viruses, algae, archaea, and protozoa.

Bacteria

Bacteria are unicellular organisms. The cells are described as prokaryotic because they lack a nucleus. They exist in four major shapes: bacillus (rod shape), coccus (spherical shape), spirilla (spiral shape), and vibrio (curved shape). Most bacteria have a peptidoglycan cell wall; they divide by binary fission; and they may possess flagella for motility. The difference in their cell wall structure is a major feature used in classifying these organisms.

According to the way their cell wall structure stains, bacteria can be classified as either Gram-positive or Gram-negative when using the Gram staining. Bacteria can be further divided based on their response to gaseous oxygen into the following groups: aerobic (living in the presence of oxygen), anaerobic (living without oxygen), and facultative anaerobes (can live in both environments).

According to the way they obtain energy, bacteria are classified as heterotrophs or autotrophs. Autotrophs make their own food by using the energy of sunlight or chemical reactions, in which case they are called chemoautotrophs. Heterotrophs obtain their energy by consuming other organisms. Bacteria that use decaying life forms as a source of energy are called saprophytes.

Archaea

Archaea or Archaebacteria differ from true bacteria in their cell wall structure and lack peptidoglycans. They are prokaryotic cells with avidity to extreme environmental conditions. Based on their habitat, all Archaeans can be divided into the following groups: methanogens (methane-producing organisms), halophiles (archaeans that live in salty environments),

thermophiles (archaeans that live at extremely hot temperatures), and psychrophiles (cold-temperature Archaeans). Archaeans use different energy sources like hydrogen gas, carbon dioxide, and sulphur. Some of them use sunlight to make energy, but not the same way plants do. They absorb sunlight using their membrane pigment, bacteriorhodopsin. This reacts with light, leading to the formation of the energy molecule adenosine triphosphate (ATP).

Fungi

Fungi (mushroom, molds, and yeasts) are eukaryotic cells (with a true nucleus). Most fungi are multicellular and their cell wall is composed of chitin. They obtain nutrients by absorbing organic material from their environment (decomposers), through symbiotic relationships with plants (symbionts), or harmful relationships with a host (parasites). They form characteristic filamentous tubes called hyphae that help absorb material. The collection of hyphae is called mycelium. Fungi reproduce by releasing spores.

Protozoa

Protozoa are unicellular aerobic eukaryotes. They have a nucleus, complex organelles, and obtain nourishment by absorption or ingestion through specialized structures. They make up the largest group of organisms in the world in terms of numbers, biomass, and diversity. Their cell walls are made up of cellulose. Protozoa have been traditionally divided based on their mode of locomotion: flagellates produce their own food and use their whip-like structure to propel forward, ciliates have tiny hair that beat to produce movement, amoeboids have false feet or pseudopodia used for feeding and locomotion, and sporozoans are non-motile. They also have different means of nutrition, which groups them as autotrophs or heterotrophs.

Algae

Algae, also called cyanobacteria or blue-green algae, are unicellular or multicellular eukaryotes that obtain nourishment by photosynthesis. They live in water, damp soil, and rocks and produce oxygen and carbohydrates used by other organisms. It is believed that cyanobacteria are the origins of green land plants.

Viruses

Viruses are noncellular entities that consist of a nucleic acid core (DNA or RNA) surrounded by a protein coat. Although viruses are classified as microorganisms, they are not considered living organisms. Viruses cannot reproduce outside a host cell and cannot metabolize on their own. Viruses often infest prokaryotic and eukaryotic cells causing diseases.

Microorganisms are small organisms invisible to the naked eye because of their extremely small size. They are grouped into various groups like Bacteria, fungi, algae, protozoa and viruses. Many of these organisms are useful to human and nature while some are harmful and even cause diseases.

Type	**Useful microorganisms (one example)**	**Harmful microorganisms**(One example)
Bacteria	Lactobacillus (curd bacteria)	Salmonella typhi (causes typhoid)
Fungi	Penicillium notatum (to make antibiotics)	Trichophyton rubrum (Athlete's foot)
Algae	Anabaena (biological nitrogen fixation)	Blue-green algae and red tides (produce toxins)
Protozoa	Paramecium (kills harmful bacteria)	Plasmodium (malaria fever)
Virus	polio, chicken pox (to make vaccines)	HIV (causes AIDS)

Useful effects of microorganisms

1. **Bacteria:** In industries, they are needed to make curd, cheese, vinegar, lactic acid, alcohol and even in ripening of tea leaves, etc.
2. **Fungi:** Some useful fungi are yeast, used to make dough in bakery and alcohol etc. Some fungi are useful for preparing antibiotics. Some mushrooms which are useful in making vitamins and organic acids.
3. **Algae:** Some useful algae are chlorella (produce protein and vitamin), red algae (agar) and brown algae (Iodine and potassium).
4. **Protozoa:** Some help in the final degradation of waste and sewage while some are symbiotic in nature.
5. **Virus:** They are used for scientific research such as in biotechnology, genetic engineering, gene therapy and for making antibiotics etc.

Harmful effects of microorganisms:

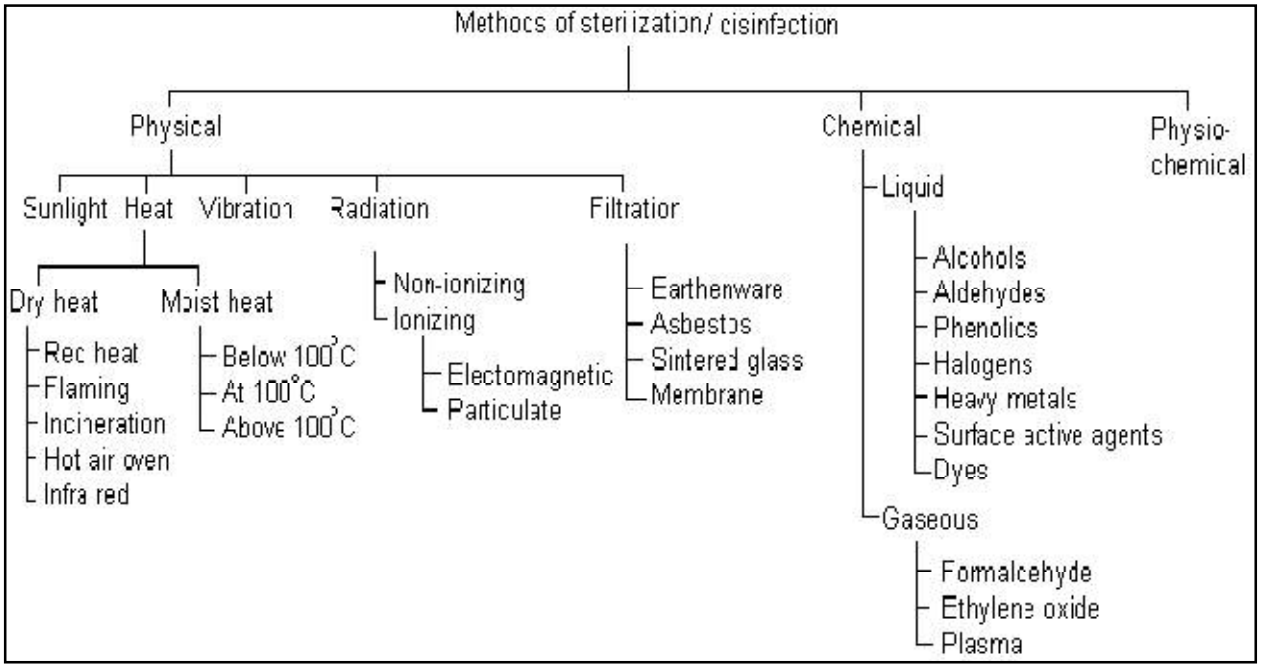
1. **Bacteria:** Causes various diseases such as typhoid, diarrhea, and cholera.
2. **Fungi:** Causes a large number of diseases in plants and in animals such as rust diseases in plants, fruit rot in apple, red rot in sugar cane and ring worm disease in human beings.
3. **Algae:** Algal boom in water (rapid growth of algae) causes poisonous effect after they die, which in turn results in the death of aquatic organisms.
4. **Protozoa:** Causes Amoebic dysentery, pyorrhoea and sleeping sickness etc.
5. **Virus:** Cause small fox, common cold, influenza, herpes, hepatitis, polio and rabies.

Sterilization

Sterilization is defined as the process where all the living microorganisms, including bacterial spores are killed. Sterilization can be achieved by physical, chemical and physiochemical means. Chemicals used as sterilizing agents are called chemisterilants.

Disinfection is the process of elimination of most pathogenic microorganisms (excluding bacterial spores) on inanimate objects. Disinfection can be achieved by physical or chemical methods. Chemicals used in disinfection are called disinfectants. Different disinfectants have different target ranges, not all disinfectants can kill all microorganisms. Some methods of disinfection such as filtration do not kill bacteria, they separate them out. Sterilization is an absolute condition while disinfection is not. The two are not synonymous.

Methods



PHYSICAL METHODS OF STERILIZATION:

Sunlight: The microbicidal activity of sunlight is mainly due to the presence of ultra violet rays in it. It is responsible for spontaneous sterilization in natural conditions. In tropical countries, the sunlight is more effective in killing germs due to combination of ultraviolet rays and heat. By killing bacteria suspended in water, sunlight provides natural method of disinfection of water bodies such as tanks and lakes. Sunlight is not sporicidal, hence it does not sterilize.

Heat: Heat is considered to be most reliable method of sterilization of articles that can withstand heat. Heat acts by oxidative effects as well as denaturation and coagulation of proteins. Those articles that cannot withstand high temperatures can still be sterilized at lower temperature by prolonging the duration of exposure.

Action of heat:

Dry heat acts by protein denaturation, oxidative damage and toxic effects of elevated levels of electrolytes. The moist heat acts by coagulation and denaturation of proteins. Moist heat is superior to dry heat in action. Temperature required to kill microbe by dry heat is more than the moist heat. **Thermal death time** is the minimum time required to kill a suspension of organisms at a predetermined temperature in a specified environment.

DRY HEAT:

Advantages: It is an effective method of sterilization of heat stable articles. The articles remain dry after sterilization. This is the only method of sterilizing oils and powders. Disadvantages:

- Since air is poor conductor of heat, hot air has poor penetration.
- Cotton wool and paper may get slightly charred.
- Glasses may become smoky.
- Takes longer time compared to autoclave.

MOIST HEAT:

Moist heat acts by coagulation and denaturation of proteins.

Advantages of steam: It has more penetrative power than dry air, it moistens the spores (moisture is essential for coagulation of proteins), condensation of steam on cooler surface releases latent heat, condensation of steam draws in fresh steam.

RADIATION:

Two types of radiation are used, ionizing and non-ionizing. Non-ionizing rays are low energy rays with poor penetrative power while ionizing rays are high-energy rays with good penetrative power. Since radiation does not generate heat, it is termed "cold sterilization".

FILTRATION:

Filtration does not kill microbes, it separates them out. Membrane filters with pore sizes between 0.2-0.45 μm are commonly used to remove particles from solutions that can't be autoclaved. It is used to remove microbes from heat labile liquids such as serum, antibiotic solutions, sugar solutions, urea solution. Various applications of filtration include removing bacteria from ingredients of culture media, preparing suspensions of viruses and phages free of bacteria, measuring sizes of viruses, separating toxins from culture filtrates, counting bacteria, clarifying fluids and purifying hydatid fluid. Filtration is aided by using either positive or negative pressure using vacuum pumps. The older filters made of earthenware or asbestos are called depth filters.

CHEMICAL METHODS OF DISINFECTION:

Disinfectants are those chemicals that destroy pathogenic bacteria from inanimate surfaces. Some chemical have very narrow spectrum of activity and some have very wide. Those chemicals that can sterilize are called chemisterilants. Those chemicals that can be safely applied over skin and mucus membranes are called antiseptics.

Classification of disinfectants:

1. Based on consistency
    a. Liquid (E.g., Alcohols, Phenols)
    b. Gaseous (Formaldehyde vapor, Ethylene oxide)
2. Based on spectrum of activity

a. High level
b. Intermediate level
c. Low level

3. Based on mechanism of action
   a. Action on membrane (E.g., Alcohol, detergent)
   b. Denaturation of cellular proteins (E.g., Alcohol, Phenol)
   c. Oxidation of essential sulphydryl groups of enzymes (E.g., H2O2, Halogens)
   d. Alkylation of amino-, carboxyl- and hydroxyl group (E.g., Ethylene Oxide, Formaldehyde)
   e. Damage to nucleic acids (Ethylene Oxide, Formaldehyd

ALCOHOLS:

Mode of action: Alcohols dehydrate cells, disrupt membranes and cause coagulation of protein.

Examples: Ethyl alcohol, isopropyl alcohol and methyl alcohol

Application: A 70% aqueous solution is more effective at killing microbes than absolute alcohols. 70% ethyl alcohol (spirit) is used as antiseptic on skin. Isopropyl alcohol is preferred to ethanol. It can also be used to disinfect surfaces. It is used to disinfect clinical thermometers. Methyl alcohol kills fungal spores, hence is useful in disinfecting inoculation hoods.

Disadvantages: Skin irritant, volatile (evaporates rapidly), inflammable

ALDEHYDES:

Mode of action: Acts through alkylation of amino-, carboxyl- or hydroxyl group, and probably damages nucleic acids. It kills all microorganisms, including spores.

Examples: Formaldehyde, Gluteraldehyde

Application: 40% Formaldehyde (formalin) is used for surface disinfection and fumigation of rooms, chambers, operation theatres, biological safety cabinets, wards, sick rooms etc. Fumigation is achieved by boiling formalin, heating paraformaldehyde or treating formalin with potassium permanganate. It also sterilizes bedding, furniture and books. 10% formalin with 0.5% tetraborate sterilizes clean metal instruments. 2% gluteraldehyde is used to sterilize thermometers, cystoscopes, bronchoscopes, centrifuges, anasethetic equipments etc. An exposure of at least 3 hours at alkaline pH is required for action by gluteraldehyde. 2% formaldehyde at 40oC for 20 minutes is used to disinfect wool and 0.25% at 60oC for six hours to disinfect animal hair and bristles.

Disadvantages: Vapors are irritating (must be neutralized by ammonia), has poor penetration, leaves non-volatile residue, activity is reduced in the presence of protein. Gluteraldehyde requires alkaline pH and only those articles that are wettable can be sterilized.

PHENOL:

Mode of action: Act by disruption of membranes, precipitation of proteins and inactivation of enzymes.

Examples: 5% phenol, 1-5% Cresol, 5% Lysol (a saponified cresol), hexachlorophene, chlorhexidine, chloroxylenol (Dettol)

Applications: Joseph Lister used it to prevent infection of surgical wounds. Phenols are coal-tar derivatives. They act as disinfectants at high concentration and as antiseptics at low concentrations. They are bactericidal, fungicidal, mycobactericidal but are inactive against spores and most viruses. They are not readily inactivated by organic
matter. The corrosive phenolics are used for disinfection of ward floors, in discarding jars in laboratories and disinfection of bedpans. Chlorhexidine can be used in an isopropanol solution for skin disinfection, or as an aqueous solution for wound irrigation. It is often used as an antiseptic hand wash. 20% Chlorhexidine gluconate solution is used for pre-operative hand and skin preparation and for general skin disinfection. Chlorhexidine gluconate is also mixed with quaternary ammonium compounds such as cetrimide to get stronger and broader antimicrobial effects (eg. Savlon). Chloroxylenols are less irritant and can be used for topical purposes and are more effective against gram positive bacteria than gram negative bacteria. Hexachlorophene is chlorinated diphenyl and is much less irritant. It has marked effect over gram positive bacteria but poor effect over gram negative bacteria, mycobacteria, fungi and viruses. Triclosan is an organic phenyl ether with good activity against gram positive bacteria and effective to some extent against many gram negative bacteria including Pseudomonas. It also has fair activity on fungi and viruses.

Disadvantages: It is toxic, corrosive and skin irritant. Chlorhexidine is inactivated by anionic soaps. Chloroxylenol is inactivated by hard water.

HALOGENS:

Mode of action: They are oxidizing agents and cause damage by oxidation of essential sulfydryl groups of enzymes. Chlorine reacts with water to form hypochlorous acid, which is microbicidal.

Examples: Chlorine compounds (chlorine, bleach, hypochlorite) and iodine compounds (tincture iodine, iodophores).

Applications: Tincture of iodine (2% iodine in 70% alcohol) is an antiseptic. Iodine can be combined with neutral carrier polymers such as polyvinylpyrrolidone to prepare iodophores such as povidone-iodine. Iodophores permit slow release and reduce the irritation of the antiseptic. For hand washing iodophores are diluted in 50% alcohol. 10% Povidone Iodine is used undiluted in pre and postoperative skin disinfection. Chlorine gas is used to bleach water. Household bleach can be used to disinfect floors. Household bleach used in a stock dilution of 1:10. In higher concentrations chlorine is used to disinfect swimming pools. 0.5% sodium hypochlorite is used in serology and virology. Used at a dilution of 1:10 in decontamination of spillage of infectious material. Mercuric chloride is used as a disinfectant.

Disadvantages: They are rapidly inactivated in the presence of organic matter. Iodine is corrosive and staining. Bleach solution is corrosive and will corrode stainless steel surfaces.

HEAVY METALS:

Mode of action: Act by precipitation of proteins and oxidation of sulfydryl groups. They are bacteriostatic.

Examples: Mercuric chloride, silver nitrate, copper sulfate, organic mercury salts (e.g., mercurochrome,

merthiolate)

Applications: 1% silver nitrate solution can be applied on eyes as treatment for opthalmia neonatorum (Crede's method). This procedure is no longer followed. Silver sulphadiazine is used topically to help to prevent colonization and infection of burn tissues. Mercurials are active against viruses at dilution of 1:500 to 1:1000. Merthiolate at a concentration of 1:10000 is used in preservation of serum. Copper salts are used as a fungicide.

Disadvantages: Mercuric chloride is highly toxic, are readily inactivated by organic matter.

SURFACE ACTIVE AGENTS:

Mode of actions: They have the property of concentrating at interfaces between lipid containing membrane of bacterial cell and surrounding aqueous medium. These compounds have long chain hydrocarbons that are fat soluble and charged ions that are water-soluble. Since they contain both of these, they concentrate on the surface of membranes. They disrupt membrane resulting in leakage of cell constituents.

Examples: These are soaps or detergents. Detergents can be anionic or cationic. Detergents containing negatively charged long chain hydrocarbon are called anionic detergents. These include soaps and bile salts. If the fat-soluble part is made to have a positive charge by combining with a quaternary nitrogen atom, it is called cationic detergents. Cationic detergents are known as quaternary ammonium compounds (or quat). Cetrimide and benzalkonium chloride act as cationic detergents.

Application: They are active against vegetative cells, Mycobacteria and enveloped viruses. They are widely used as disinfectants at dilution of 1-2% for domestic use and in hospitals.

Disadvantages: Their activity is reduced by hard water, anionic detergents and organic matter. Pseudomonas can metabolise cetrimide, using them as a carbon, nitrogen and energy source.