General Certificate of Education
(Advanced Level)
BIOLOGY

Unit 9 - Microbiology
Grade 13

Department of Science
Faculty of Science & Technology
National Institute of Education
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G.C.E. (Advanced Level)
Biology
Grade 13
Microbiology
Unit -9

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Message from the Director General

The National Institute of Education takes opportune steps from time to time for the development of quality in education. Preparation of supplementary resource books for respective subjects is one such initiative.

Supplementary resource books have been composed by a team of curriculum developers of the National Institute of Education, subject experts from the national universities and experience teachers from the school system. Because these resource books have been written so that they are in line with the G. C. E. (A/L) new syllabus implemented in 2017, students can broaden their understanding of the subject matter by referring these books while teachers can refer them in order to plan more effective learning teaching activities.

I wish to express my sincere gratitude to the staff members of the National Institute of Education and external subject experts who made their academic contribution to make this material available to you.

Dr. (Mrs.) T. A. R. J. Gunasekara
Director General
National Institute of Education
Maharagama.

Message from the Director

Since 2017, a rationalized curriculum, which is an updated version of the previous curriculum is in effect for the G.C.E (A/L) in the general education system of Sri Lanka. In this new curriculum cycle, revisions were made in the subject content, mode of delivery and curricular materials of the G.C.E. (A/L) Physics, Chemistry and Biology. Several alterations in the learning teaching sequence were also made. A new Teachers' Guide was introduced in place of the previous Teacher's Instruction Manual. In concurrence to that, certain changes in the learning teaching methodology, evaluation and assessment are expected.

When implementing the previous curricula, the use of internationally recognized standard textbooks published in English was imperative for the Advanced Level science subjects. Due to the contradictions of facts related to the subject matter between different textbooks and inclusion of the content beyond the limits of the local curriculum, the usage of those books was not convenient for both teachers and students.

As this book is available in Sinhala, Tamil, and English, the book offers students an opportunity to refer the relevant subject content in their mother tongue as well as in English within the limits of the local curriculum. It also provides both students and teachers a source of reliable information expected by the curriculum instead of various information gathered from the other sources.

This book authored by experienced subject teachers and subject experts from the universities is presented to you followed by the approval of the Academic Affairs Board and the Council of the National Institute of Education. Thus, it can be recommended as a material of high standard.

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Content

09

Microbiology

Nature of microorganisms

Microbiology is the study of organisms that are too small and are not visible clearly to the naked eye or un-aided eye when they exist individually. These organisms are referred to as microorganisms. Microorganisms include bacteria, archaea, cyanobacteria/ Blue green bacteria (BGB), fungi and protists. Mollicutes such as mycoplasmas and phytoplasmas, viruses, viroids and prions are also studied under microbiology.

Microscopic nature of microorganisms

In general, microorganisms are less than 0.1 mm in size and cannot be observed with unaided eye. Therefore, they must be observed with a microscope. Microorganisms and their structural components are measured in micrometers and nanometers.

1 micrometer (μm) = 10^{-6} m

1 nanometer (nm) = 10^{-9} m

Some microorganisms are more readily visible than other because of their larger size.

Ubiquitous nature of microorganisms:

Microorganisms are ubiquitous on earth. They are found in water, soil, air and interior and exterior surfaces of other organisms. Marine and freshwater microorganisms form the basis of food chain in oceans and freshwaters. Some of them do photosynthesis and are primary producers in aquatic environments. Soil microorganisms help recycling of chemical elements between soil, water, air and living organisms. Microorganisms suspended in air as bioaerosols, have the opportunity to travel long distances with the wind current and precipitate. Pathogenic bioaerosols, cause opportunities for disease spreading. Only a minority of microorganisms that associate with other organisms such as plants, animals and human are pathogenic. Majority of them are advantageous or harmless. However, all viruses are harmful to the organisms they attached to. Some microorganisms are capable of inhabiting extreme environmental conditions that are unfavorable or even lethal for other organisms. Such microorganisms are known as extremophiles. Extremophiles have been found inside the Earth's crust, deep sea at high pressures, extreme acidic or extreme basic conditions, hydrothermal vents, frozen sea water and anaerobic conditions. Extremophiles are classified according to the conditions in which they grow.

Table 9.1: Types of extremophiles

Type of Extremophile	Condition
Thermophiles	high temperatures
Psychrophiles	low temperatures
Acidophiles	acid pH
Alkaliphiles	basic pH
Halophiles	require NaCl
Barophies	high pressure

Some of these extreme environments consist of more than one extreme condition. For example,

- Many hot springs are acidic or alkaline in nature at the same time
- Deep seas are cold and remain in high pressure.

Microorganisms live in such environments are adapted to live with more than one extreme condition.

High growth rate of microorganisms:

Rates of growth and reproduction of microorganisms are high. Microorganisms possess a high surface area/volume ratio due to their smaller size. This means that they have large surface area available for exchange of materials from external environment. As a result, flowing rate of materials in to the inside of cells and the exit of waste materials to the outside of the cells increases and results in high metabolic rate. Therefore, average generation time or the time required to double the population of microorganisms is relatively less.

Morphological, nutritional, and physiological diversity of microorganisms:

Microorganisms possess diverse morphological forms. Bacteria possess diversity in their shapes, basically three distinct shapes; rod shape/ bacillus, spherical shape/ coccus and spiral shape/ spirillum. The coccus bacteria may arranged in different forms; coccus/ monococcus, diplococcus, streptococcus, staphylococcus, tetrads and sarcinae. Bacillus bacteria may arranged in to either diplobacillus or streptobacillus. Spiral bacteria may arranged in to either vibrio or spirillum or spirochete.

Cyanobacteria exhibit a great variety of shapes and arrangements, unicellular to long multi cellular filaments. Multi cellular Cyanobacteria may appear as either filamentous or non-filamentous. Filamentous appear as chains and the non-filamentous appear as clusters or colonies forming spherical, cubical, square or irregular shape. Two morphological varieties are found in viruses based on their symmetry of protein coats; Icosahedral and helical. In fungi, some of them are unicellular and others multicellular, consists of a mass of fine tubular branching threads known as hyphae, collectively form mycelium. Hyphae may be septate or aseptate. Prions are smaller proteinaceous particles. Unicellular protists possess wide range of morphological diversity. Mollecutes are pleomorphic (variable shapes).

Microorganisms show a diversity of nutritional types. Based on the sources of carbon and energy, nutritional types of microbes are classified. There are four major nutritional types seen among microorganisms; chemoautotrophs, chemoheterotrophs, photoautotrophs and photoheterotrophs. Based on the utilization of O_2(g), microorganisms can be classified in to four physiological groups; obligate aerobes, obligate anaerobes, facultative anaerobes and microaerophiles. Some microbes capable of fixing atmospheric molecular nitrogen, show physiological diversity; free-living nitrogen fixing microbes and symbiotic nitrogen fixing microbes.

Types of microorganisms

1. Bacteria

Bacteria (singular, bacterium) are single-celled (unicellular) prokaryotic organisms. They show different morphological forms and arrangements. The most obvious structural feature of bacteria is the shape of individual cells. There are three basic shapes.

1. Spherical; coccus (plural- cocci)

2. Rod shape; bacillus (plural-bacilli)

3. Spiral shape; (plural- spirilli)

During cell division, cells can remain attached to each other and form different forms of cell arrangements.

1. Different forms of cell arrangement of coccus bacteria (Figure 9.1)

Coccus	Cells divide in one plane. Divided cells detach from each other after cell division
Diplococcus	Cells divide in one plane. Divided cells remain in pairs
Streptococcus	Cells divide in one plane. Divided cells remain attached in chain like pattern
Tetrad	Cells divide in two planes producing 4 cells remain attached together
Sarcinae	Cells divide in three planes and remain attached in groups of 8 cells
Staphylococcus	Cells divide in multiple planes and form grape like clusters of cells

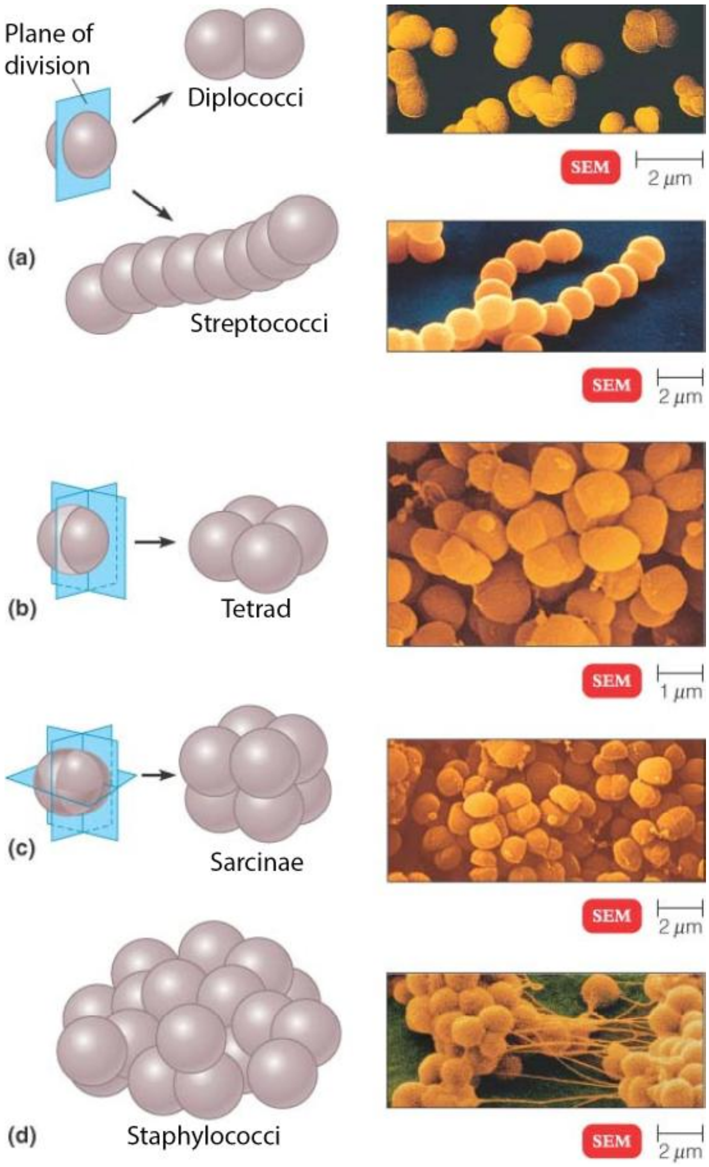

Figure 9.1. Cell arrangement of coccus bacteria. Shown are diagrammatic representations of planes of cell division and various cell arrangements (left) and their scanning electron microscopic (SEM) views (right). Retrieved from Microbiology: An Introduction, 11th edition, by Tortora, Gerard J.; Funke, Berdell R.; Case, Christine L. Pearson Education Ltd (2016).

2. Different forms of cell arrangement of bacillus bacteria (Figure 9.2)

Bacilli divide only across their short axis. Therefore, there are few cell arrangement forms.

Single bacillus	Single rod
Diplobacillus	Remain in pairs after cell division
Streptobacillus	Occur in chains after cell division

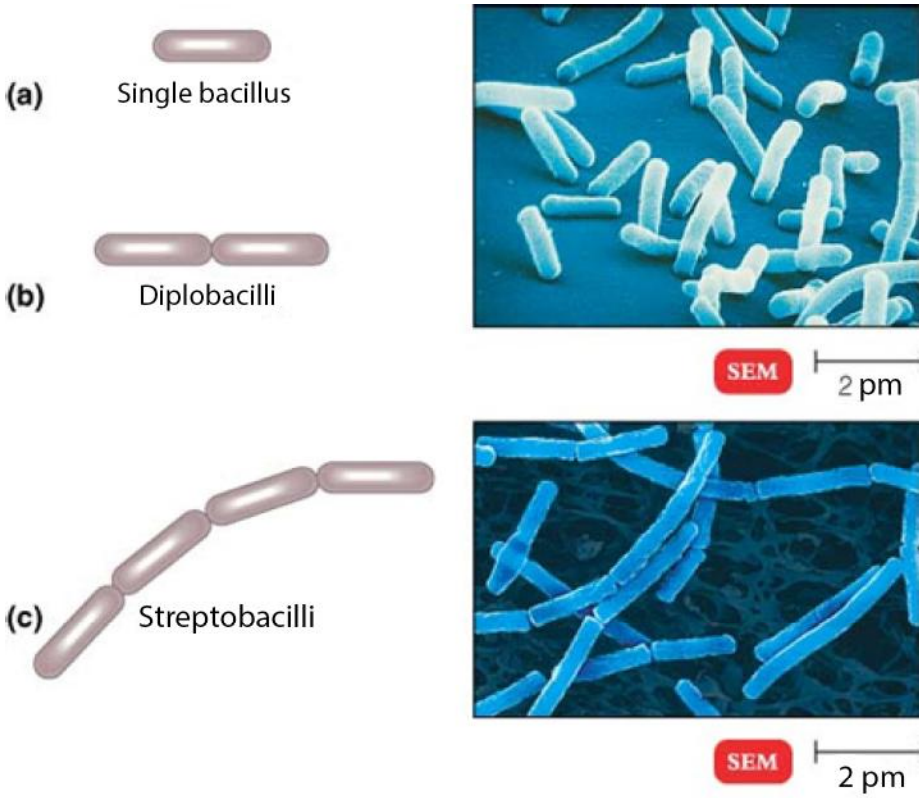

Figure 9.2. Cell arrangement of bacillus bacteria. Shown are diagrammatic representation of (left) and scanning electron microscopic (SEM) views of cells (right). Retrieved from Microbiology: An Introduction, 11th edition, by Tortora, Gerard J.; Funke, Berdell R.; Case, Christine L. Pearson Education Ltd (2016).

3. Different forms of cell arrangement of spiral bacteria (Figure 3)

Spiral bacteria have one or more twists, they are never straight.

Vibrio	Bacteria look like curved rods
Spirillum	Helical shape, like a corkscrew and rigid body
Spirochete	Helical shape, flexible body

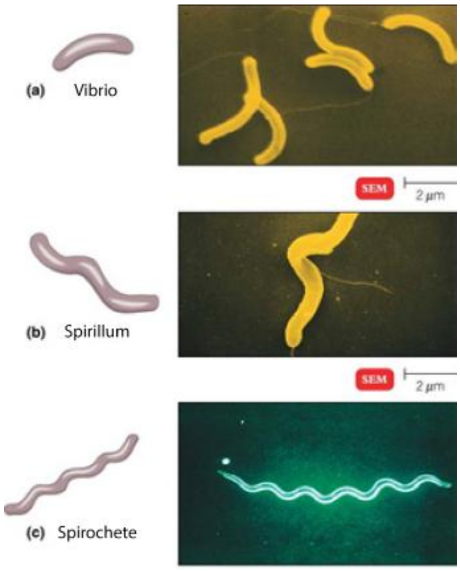

Figure 9.3: Shapes of spiral bacteria. Shown are diagrammatic representation of shapes (left) and scanning electron microscopic (SEM) views of spiral bacteria (right). Retrieved from Microbiology: An Introduction, 11th edition, by Tortora, Gerard J.; Funke, Berdell R.; Case, Christine L. Pearson Education Ltd (2016).

Bacteria show a diversity of nutritional types. Four major nutritional types can be identified among bacteria. They are classified based on the source of energy and carbon.

Nutritional type	Source of energy	Source of carbon	Example
Photoautotrophs	Light	Carbon dioxide (Inorganic Carbon)	Purple sulfur and Green sulfur bacteria
Photoheterotrophs	Light	Organic carbon	Purple non sulfur bacteria
Chemoautotrophs	Inorganic chemicals	Carbon dioxide (Inorganic Carbon)	*Nitrobacter, Nitrosomonas, Thiobacillus thiooxidans*
Chemoheterotrophs	Organic chemicals	Organic carbon	Most bacteria

Microorganisms can be classified into 4 groups on the basis of their tolerance to oxygen.

Physiological Group	Description	Example
Obligate aerobic	These bacteria require oxygen for their survival. They generate energy by oxidative phosphorylation.	*Acetobacter sp*
Obligate anaerobic	They cannot survive in the presence of oxygen. These microorganism generate energy by fermentation	*Clostridium* sp.

Facultative anaerobic	These microorganisms prefer to grow in the presence of oxygen producing energy by oxidative phosphorylation, but they can also grow in anaerobic environments using fermentation	*Escherichia coli*
Microaerophilic	These microorganisms can grow only in oxygen concentrations lower than those in air	*Lactobacillus* sp.

Some bacteria are able to fix atmospheric nitrogen. They show diversity in nitrogen fixation.

- Free-living nitrogen fixing bacteria: *Azotobacter* sp.
- Symbiotic nitrogen fixing bacteria: *Rhizobium* sp. with legume root

Mostly bacteria undergo asexual reproduction by binary fission, and in some occasion, fragmentation or budding. In rare occasions, bacteria of two strains share a portion of genetic material through the process of 'conjugation'.

Cyanobacteria

Cyanobacteria are named for their characteristic blue-green (cyan) pigmentation. Cyanobacteria also exhibit a great variety of shapes and cell arrangements, unicellular to colonial forms (Figure 9.4).

- **Unicellular form-** Cells separate after cell division. However, in nature majority of unicellular forms stay together by copious secretion of mucilage by daughter cells.
- **Colonial form-** Cells remain attached by walls or held in a common gelatinous matrix forming a colony of cells. Colonies may either be non-filamentous or filamentous. Non-filamentous colonial form- depending on the plane of division and direction there are different arrangements such as spherical, cubical, square or irregular shape. Filamentous colonial form is the result of cell division in a single plane and a single direction forming a chain or thread like structure.

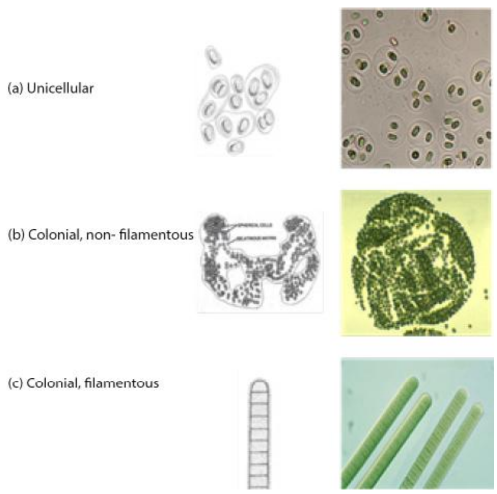

Figure 9.4. Cell arrangement of cyanobacteria. Shown are diagrammatic representations (left) and light microscopic views (left) of cell arrangement.

Cyanobacteria are photoautotrophs that carryout oxygenic photosynthesis similar to plants and algae. Many of cyanobacteria are capable of fixing atmospheric nitrogen. For examples, *Nostoc* sp. is a free living nitrogen fixing cyanobacteria, *Anabaena -Azolla* symbiotically fix nitrogen with its partner, *Azolla* sp. (water fern). In most cases nitrogen fixation takes place in special cells called heterocyst. Nitrogen fixation is catalyzed by the enzyme called nitrogenase in the heterocyst. Nitrogenase is sensitive to oxygen. Heterocyst carry thick cell wall to protect nitrogenase from oxygen that could diffuse from neighboring photosynthetic cells and from air or water.

Cyanobacteria carry another specialized cell type called akinete. They are thick walled resting spores with stored food. Akinete is resistant to drought and high temperature. Therefore, akinete is able to survive during unfavorable environmental conditions although vegetative cells dries out.

Cyanobacteria reproduce only by asexual methods. Single unicellular and colonial non-filamentous types undergo simple cell division while colonial filamentous and colonial unicellular forms reproduce by fragmentation.

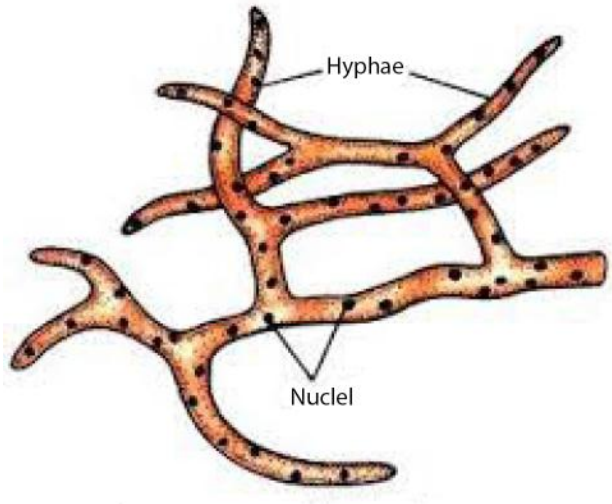

figure 9.5 Fungi fillamentous thallus, branched mycellium

Fungi (singular, fungus) are eukaryotes. They may be unicellular (yeast) or multicellular (molds). Some multicellular fungi form mushrooms. Molds form visible masses called mycelia, which are composed of long filament like structures called hyphae. Many molds contain cross-walls called septa (singular, septum). Septa divide hyphae into distinct single nucleate cell-like units. Some molds do not contain septa in their hyphae resulting in long continuous cells with many nuclei. These are called coenocytic hyphae. The cottony growths sometimes found on bread and fruit are mycelia of molds.

Fungi are chemoheterotrophs and acquire food by absorption. They possess saprophytic mode of nutrition. They play important role in food chain by decomposing dead plant materials by secreting enzymes and thereby recycle vital elements. Parasitic (plant and animal pathogens) and mutualistic (lichens and mycorrhizae) modes of nutrition also found among fungi.

Unicellular fungi reproduce asexually by fission or budding, on the other hand, filamentous fungi (molds) reproduce asexually and/or sexually by producing spores.

a.

Figure 9.6 (a). Reproduction method of *Penicillium (*spore formation)

b.

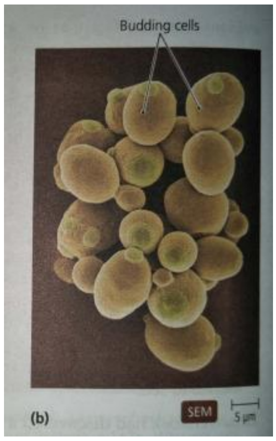

Figure 9.6 b. Reproduction method of Yeast (budding)

Unicellular protists

a.

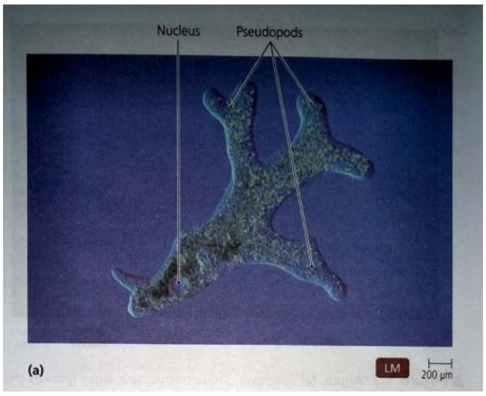

Figure 9. 7 a. *Amoeba*

b.

Figure 9. 7 b. *Paramecium*

c.

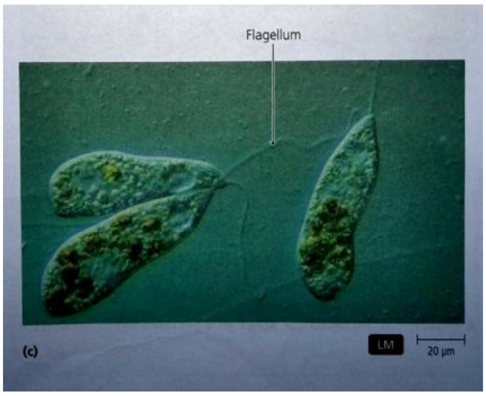

Figure 9. 7 c. *Euglena*

Unicellular Protists are pleomorphic, vary in their shapes and possess locomotive structures such as pseudopods, cilia or flagella. They exist either individually or form colonies. Some join together and form filaments.

Photoautotrophic, heterotrophic and mixotrophic modes of nutrition are found among protists.

There are aerobic, anaerobic and facultative anaerobic respiratory modes found among protists.

Some algae contribute to the symbiotic interactions in lichens.

They reproduce sexually by gametes and asexually by fission.

Mollicutes

Mollicutes are prokaryotes included in the domain bacteria. Mycoplasma and phytoplasma, are considered unique due to absence of cell walls.

Mycoplasma and Phytoplasma

Mycoplasma are pleomorphic, vary in shape from spherical to filamentous. They are the smallest prokaryotic group of organisms invisible under light microscope. Mycoplasma do not contain flagella. Almost all mycoplasma are parasites of humans and animals. Mycoplasma require high amount of organic growth factors. They reproduce by budding and binary fission and do not produce spores. Mycoplasma are aerobic or facultative anaerobic.

Phytoplasma resembles to mycoplasma in many ways. They are similar in size to mycoplasma. Both can only be seen under electron microscope. Shape varies from spherical to filamentous. Phytoplasma only infect plants and are generally present in the phloem sap. They cannot grow in artificial media. They are transmitted mostly by leafhoppers. Therefore, they reproduce in both leafhoppers as well as plant body. They reproduce by budding and binary fission. They possess aerobic or facultative anaerobic mode of respiration.

Virus

(a) Characteristic features

Viruses are neither prokaryotes nor eukaryotes and do not show any cellular organization. They do not possess any metabolic activity or reproduction when they are out of living host cells. Thus, they are not considered as living organisms. However, once they get in to the host cells, they multiply and cause infection through various metabolic pathways, shows characteristics of living organisms. Since viruses can only multiply within a living host cell, they are obligate parasites. Viruses are very small can only be seen through an electron microscope. Viruses possess simple structures, usually are composed of a central core of a nucleic acid and surrounded by a protein coat called the capsid which is made up of a fixed number of protein subunits called capsomeres. Viruses may have either DNA or RNA as their genetic material. They do not have protein synthesis machinery such as additional RNAs or enzymes for protein synthesis. Therefore, they depend on host cell's protein synthesis machinery. RNA viruses consist of reverse transcriptase enzymes for reverse transcribing RNA in to DNA.

(b) Morphology and types of viruses

On the basis of capsid architecture two basic morphological symmetries can be identified (Figure 9.8).

1. Helical

2. icosahedron

Based on the above symmetries viruses show four types of morphological forms; helical, polyhedron, complex and enveloped.

- Helical viruses- long rigid or flexible rods. e.g; Rabies virus
- Icosahedron/ polyhedral- icosahedron symmetry. e.g; Adeno virus
- Complex viruses-Exhibits more than one form of symmetry with additional structures. e.g; bacteriophage
- Enveloped viruses. e.g; roughly spherical. Capsid covered by envelopes. e.g; Herpes simplex virus

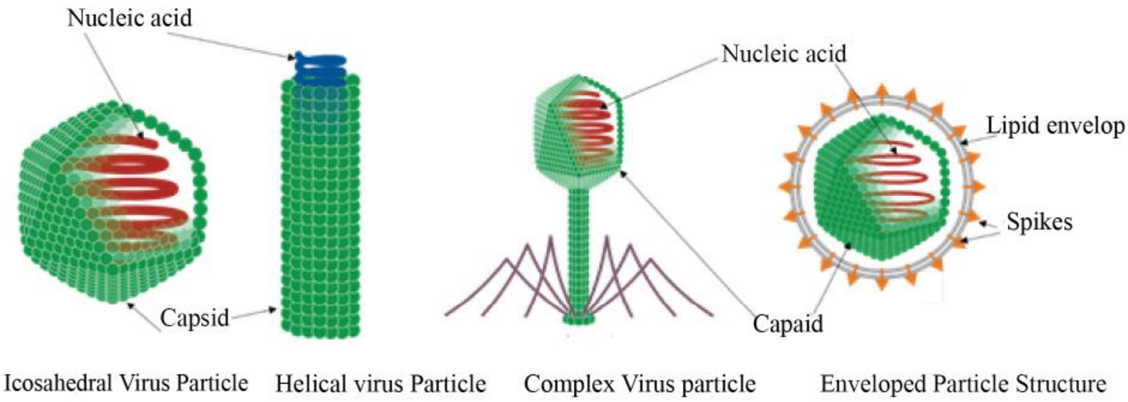

Figure 9.8 Diagrammatic representation of four morphological forms of virus.

Multiplication of viruses

A single virus can give rise to thousands of similar viruses in a single host cell. Therefore, viruses cause serious damages to their host leading to severe diseases in plants, animals and bacteria. Bacteriophages are typical group of viruses that are capable of infecting bacteria. They multiply by two distinct mechanisms; lytic cycle or lysogenic cycle.

Lytic cycle involves with the lysis of the host cell, whereas the lysogenic cycle allows viral DNA incorporating into host DNA and multiply without causing lysis of the host cell.

Lytic cycle of a bacteriophage

There are five distinct steps in the lytic cycle ; attachment, penetration, biosynthesis, maturation and release (Figure 9.9).

- **Attachment:** The first step is the attachment of virus to a matching receptor site on the bacterial cell.
- **Penetration**: After attachment, bacteriophage injects its DNA into the bacterial cell. This is facilitated by an enzyme which breaks down bacterial cell wall.
- **Biosynthesis:** The next step is the biosynthesis of viral DNA and proteins in the host cytoplasm using host resources. This stage induces degradation of host cell DNA.
- **Maturation and assembly:** Once bacteriophage DNA and proteins are synthesized, DNA and capsid are assembled to form complete virus particles. This is called maturation.
- **Release:** Finally, bacteriophage induce bacterial cell to break open (lyse). Newly produced bacteriophages are released from the host cell. These released bacteriophages can start another lytic cycle in cells in the vicinity.

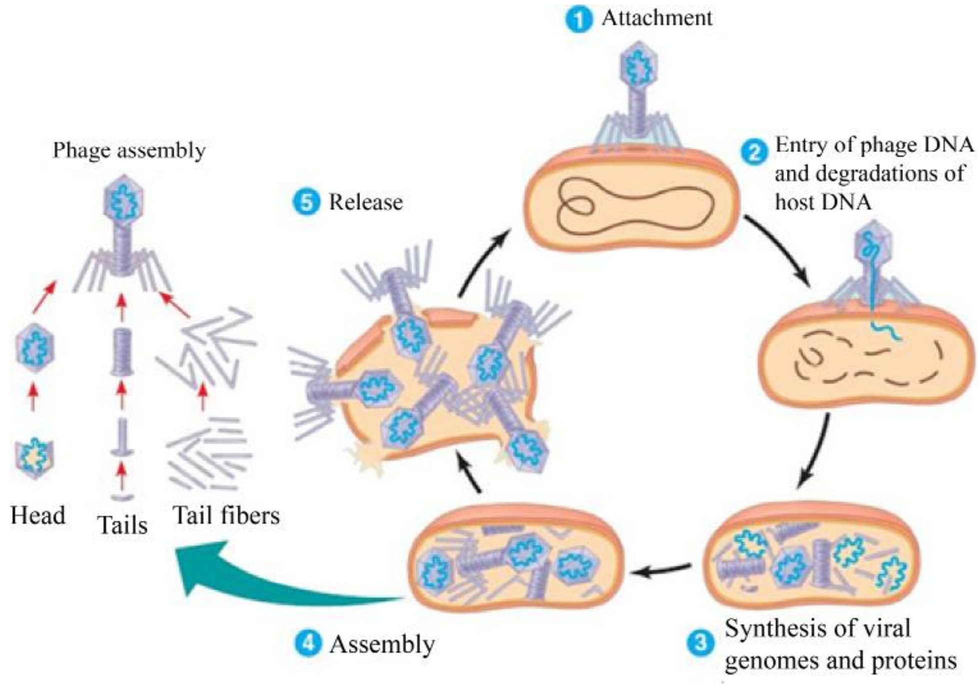

Figure 9.9 Stages of lytic cycle of a bacteriophage

Viroids

Viroids consist only of short piece of naked RNA with no protective layer such as a protein coat. Viroids can only multiply within a living host cell using host cell resources. However, viroids do not contain any gene and only carry signals for their multiplication. Viroids infect plants, but no other life forms till to date.

Prions

Prions are proteinaceous infectious particles. Their size is smaller than virus. Although prions lack nucleic acid they can replicate with the help of a host's gene that encodes the prion protein. They are found as disease causing agents in some birds and mammals. All these diseases are neurological diseases. Eg;

- Transmissible Spongiform Encephalopathies (TSEs), because large vacuoles develop in the brain giving sponge-like appearance.
- Mad cow disease was one of the serious disease emerged in cattle in 1987.
- Creutzfeldt-Jakob disease (CJD) is one of the human diseases caused by prions.

Human to human disease transmission has been associated with transfusion of infected blood and tissue and organ transplantation. Some TSE infections may be transmitted from cow to human.

Basic laboratory Techniques

For the study of morphology and biochemical properties of microorganisms, it is essential to culture them on artificial media. There are some basic laboratory techniques such as preparation of artificial culture media and sterilization techniques, to be followed in culturing of the microorganism of interest without any contamination. This section describes such basic laboratory techniques.

Methods of sterilization

Sterilization is the process of removal or destruction of all forms of microbial life including endospores.

There are two types of sterilization, physical and chemical.

I. Physical methods of sterilization

Sterilization by moist heat, dry heat, filtering using membrane filters, exposure to UV radiation are some of the physical methods used in sterilization.

- **Moist heat sterilization**

  Here, moist heat is used to destroy the microorganisms present in the desired materials such as culture media, temperature labile reagents/ fluids and various laboratory utensils. This is done by denaturing of proteins by high temperature and pressure.

E.g. autoclaving- In an autoclave, steam with 121 ºC temperature at the pressure of 1 atm/ 15 psi is used for sterilization. Extending the above condition for 15 minutes is sufficient to kill all microorganisms (except prions) and their endospores.

Autoclaving is used to sterilize culture media, solutions, syringes and needles, healthcare instruments and various other items that can withstand high temperatures and pressure. Glassware can also be sterilized with an autoclave if care is taken to ensure that the steam contacts all surfaces.

Pressure cooker also can be used for moist heat sterilization.

- **Dry heat sterilization**

  Here, dry heat is used to destroy the microorganisms present in the desired materials such as glasswares, petridishes, pipettes, inoculation loops, inoculation needles, scalpels, etc.

1. **Direct flaming**

   It is a simple method of dry heat sterilization. This is used in laboratories to sterilize inoculating loops, inoculating needles and scalpel blades by heating them on the flames of Bunsen burners / hot spirit lamp until they reach red hot.

2. **Incineration**

   It is mostly done in an incineration oven. Incineration is used to sterilize hospital waste. Microorganisms are burned to ash during direct flaming and incineration.

3. **Hot-air sterilization**

   Microorganism are killed by oxidation. Items to be sterilized are heated to about 170 ºC and maintain for 2 hours in a dry air oven. This type of sterilization is often used to sterilize glassware such as Petri plates, flasks, beakers, bottles and glass pipettes.

- **Pasteurization**

  Louis Pasteur found spoilage of beer and wine can be prevented by applying mild heat that kills organisms causing spoilage without seriously damaging the taste, texture and nutritional content of the product. Later, the same principle was applied to milk products, now known as pasteurized milk. The objectives of milk pasteurization are to eliminate pathogenic microorganisms and reduce microbial number which prolongs milk quality under refrigeration.

  High temperature short-time (HTST) pasteurization, which uses temperature of at least 72 ºC for 15 seconds and **low temperature long time (LTLT)** 63 ºC for 30 minutes are the two main methods of pasteurization. Milk can also be sterilized by **ultra-high-temperature (UHT)** pasteurization. Here, milk is heated to about 140 ºC in less than 5 seconds by flashing steam. This milk can be stored for several months without refrigeration.

- **Boiling**

  Boiling the materials such as surgical instruments to 100 ºC. Most of the pathogenic microorganisms are killed at boiling temperature.

- **Filtration- e.g. Membrane filters**

  Filtration is used to sterilize heat sensitive liquids such as solutions containing enzymes, vitamins, antibiotics, vaccines and some culture media. Material to be sterilized is passed through a filter by using vacuum. Filter retains microorganism and the liquid is passed through the filter.

  Membrane filters are widely used to sterilize heat sensitive solutions. Pores of membrane filters are from 0.01 µm to 0.45 µm size, retain almost all the microorganisms including viruses and some large protein molecules.

- **UV Radiation**

  UV Radiation kills microorganisms that falls in to direct exposure, either through destruction or damaging DNA. However, a major disadvantage of UV is that radiation does not penetrate through solid surfaces and coverings such as paper, glass and textile. Therefore, anything to be sterilized should have direct contact with radiation. UV radiation is commonly used to sterilize air in hospital rooms such as operating theaters and nurseries.

II. Chemical methods of sterilization

Few chemicals such as ethylene oxide and chlorine dioxide (both are gases) are currently used as chemical sterilizing agents. Majority of chemical agents reduce microbial populations to a safe levels or remove vegetative forms of pathogens.

Ethylene oxide kills microorganisms and endospores. It is also highly penetrating. Therefore ethylene oxide is used to sterilize mattresses in hospitals.

Chlorine dioxide has been used to fumigate enclosed building areas contaminated with endospores of Bacillus anthracis. It is most commonly used in water treatment prior to chlorination.

Preparation of culture media

Microorganisms cannot be studied in their natural habitat such as in soil, water or air. Therefore, we need to bring them to the laboratory and provide similar conditions for their growth and reproduction. A nutrient material prepared for providing nutrition and anchorage essential to the growth of microorganisms at laboratory condition is called a culture medium.

Not all microorganism can be grown on a laboratory culture media. They are called non-culturable microorganisms. Some microorganisms grow well on any culture medium whereas other microorganisms require special medium.

Suppose we want to grow a culture of microorganisms present in a certain soil sample. The culture medium should contain necessary nutrients, sufficient moisture and suitable pH. This medium must initially be sterile which means it should not contain any living microorganisms. Therefore, when preparing a culture medium all glassware and liquid nutrient solutions should be sterilized.

Nutrient agar (NA) and potato dextrose agar (PDA) are two general media, commonly used to grow bacteria and fungi respectively. Nutrient agar is made up of peptone, meat extract, sodium chloride, agar and distilled water. Whereas, PDA is made up of potato, glucose, agar and distilled water.

Here, agar is used as a solidifying agent. Agar solidifies at temperatures below 40 ºC which means a culture medium containing agar is a solid medium. For growing microorganisms, solid culture medium is usually contained in Petri dishes or test tubes.

Because most microorganisms appear almost colorless when viewed through a standard light microscope, we have to prepare them for observations. One of the ways this can be done is by staining, means coloring the microorganisms with a dye.

However, before the microorganisms stain, they must be fixed (attached) to the microscopic slide. A simple stain is an aqueous or alcohol solution of a single basic dye. The primary purpose of a simple stain is to highlight the entire microorganisms so that cellular shapes, cell arrangements and basic structures are visible. Some of the simple stains commonly used in the laboratory are methylene blue., crystal violet and safranin.

Microorganisms and diseases

Generally, humans are free of microorganisms at birth. However, during birth, the newborn first get in contact with microorganisms present on the vagina of mother. Usually, these are Lactobacilli. Lactobacilli colonize the intestine of the newborn. After birth, many other microbial populations begin to establish inside or on the surface of the body. These are called normal microbiota of human body. However, internal tissues of healthy human body are free of microorganisms.

A part of these microorganisms colonize on the skin, and majority enter the body and colonize on the inner surfaces such as mucous membranes of nose, throat, upper respiratory tract, intestinal tract and genitourinary tract.

A normal healthy body contains a large number of microorganisms. It has been estimated that the human body consists of 1×10^{14} microbial cells for 1×10^{13} total body cells, which means a 10 times more microbial cells than human cells.

Majority of these organisms are generally harmless or even beneficial. For example, minimal colonization of *Escherichia coli* at large intestine prevent colonization of pathogenic bacteria such as Salmonella typhi. *E. coli* in large intestine synthesizes vitamin K and some of the B vitamins that are absorbed into the bloodstream and used by body cells.

Recent interest in the importance of bacteria to human health has led to the study of probiotics. Probiotics are live microbial cultures. e.g. Yoghurt exert a beneficial effect. Serveral studies have shown that ingestion of certain lactic acid bacteria can alleviate diarrhea and prevent colonization by Salmonella enterica during antibiotic theraphy.

Although majority of human microbiota are harmless, some of them may change their interactions with human body under certain conditions and cause infections. Those microorganisms are called opportunistic pathogens. For example, E. coli is generally harmless as long as it remains in the large intestine. However, they may cause diseases, if enter other body parts (urinary bladder- urinary tract infection, lungs- pulmonary infection).

Terms related to infectious diseases

- **Pathogen:** An organisms or entity (non-living entities such as virus and prions) that is capable of causing disease
- **Host:** Organism within which infected pathogens live on or in and multiply.
- **Pathogenicity:** The ability of a pathogen to cause disease in the host by overcoming the defence of a host.
- **Parasite:** An organism or entity living on or in another living organism (host) and obtain nutrients and other resources from the host.

Characteristics of pathogenic microorganisms

- Having optimal growth conditions (e.g. temperature) that corresponds to the body conditions of the host.
- Having structures to adhere to the host cells and protect against host's defense mechanisms. e.g. capsule, pilli
- Produce toxins; endotoxins or exotoxins.
- Having enzymes for invasiveness such as phospholipase, lesithinase, and hyaluronidase.
- Having enzymes such as DNase to alter the host's metabolic processes.

Virulence and virulent factors

Microbes express their pathogenicity by means of their virulence. Virulence is the degree of pathogenicity of the pathogen. Some pathogens are highly virulent (chicken pox virus) whereas others are less virulent/ non virulent.

Few genes of pathogenic microorganisms express factors which provide them the ability to infect their host and cause disease. Such factors are called virulent factors.

The relationship between a host and a pathogen is dynamic, each modifies the activities and functions of the other. As a result, outcome of such a relationship depends on the virulence of the pathogen and the effectiveness of the host defense mechanisms.

Virulence factors enhance the pathogenicity and allows pathogen to invade and colonize host tissues and disrupt normal body function. Pathogens use two major mechanisms for pathogenicity.

1. Invasiveness

It is the ability of pathogens to invade tissues by overcoming host's defense mechanisms and multiply for colonizing.

Several extracellular enzymes produced by pathogens contribute to invasiveness.

e.g.

- Phospholipase- destroy animal cell membranes.
- Lecithinase- Hydrolyzes the lecithin component of the lipid in the cell membrane.
- Hyaluronidase- destroys the body tissue by breaking down the hyaluronic acid which is cementing substance between cells.

Pathogenic microorganisms do enter passively through various portals or natural openings such as wounds on skin, respiratory, gastrointestinal and genito-urinary tracts.

2. Toxigenecity

Ability of microorganisms to produce biochemical substances known as toxins that disrupt the normal functions of cells. These are proteins or lipopolysaccharides that produce specific harmful effects on the host, thus are called biological poisons. They may be,

- Endotoxins- Endotoxins are lipopolysaccharides. These are thermos-stable toxins which are part of the microbial cell. Toxins are released when the bacteria die and the cell wall breaks apart. All endotoxins cause the same signs of symptoms regardless of the species of pathogen. These symptoms include chills, fever, weakness, generalized aches and sometimes shock and death. Endotoxins are produced only by gram-negative bacteria

  e.g. Lipopolysaccharides of the cell walls of *Salmonella typhi*

- Exotoxins- Exotoxins are produced inside bacterial cells as part of their growth and metabolism and are secreted or released to the surrounding environment after cell lysis. Exotoxins are proteins. Majority of them are enzymes. Due to their catalytic nature even a small amount of toxin is quite harmful. These are thermo-labile protein toxins, being inactivated by boiling. Exotoxins are most commonly produced by gram-positive bacteria and a few gram negative bacteria

- Exotoxins are classified into three types
  - Neurotoxins- interfere with normal transmission of nerve impulses. E.g., toxins produced by *Clostridium tetani*
  - Enterotoxins- stimulates cells of the gastrointestinal tract in an abnormal way. E.g. Toxins produced by *Vibrio cholera*
  - Cytotoxins- kills host cells by enzymatic attack. E.g. Toxins produced by *Corynebacterium diptheriae*

Important diseases of human caused by microorganisms

Organ	Disease	Causal agent
Skin	Chickenpox Rubella Measles	Herpesvirus varicella-zoster Rubella virus Measles virus
Eye	Conjunctivitis (bacteria/virus)	Haemophilus *influenzae/Adenoviruses*
Nervous system	Bacterial meningitis	*Streptococcus pneumonia* *Haemophilus influenzae* *Neisseria meningitidis*
Nervous system	Tetanus Rabies	*Clostridium tetani* Rabies virus
Cardiovascular system	Rheumatic fever	*Streptococcus pyogenes*
Respiratory system	Tuberculosis Influenza Pneumonia	*Mycobacterium tuberculosis* Influenza virus *Streptococcus pneumoniae*
Digestive system	Hepatitis Food poisoning Cholera Typhoid	*Hepatitis A virus* Staphylococcus aureus Vibrio cholerae Salmonella typhi
Urinary system	Leptospirosis	*Leptospira interrogans*
Reproductive system	Gonorrhea Genital herpes	*Neisseria gonorrhoeae* Herpes simplex virus
Immune system	AIDS	Human immune deficiency virus (HIV)

Microbial disease control starts from avoidance and prevention of opportunities of getting infection to treatment or curative methods after infection.

Avoidance and prevention of microbial diseases

Good hygienic practices in day to day life is the best method to avoid infectious diseases. Antiseptics, disinfectants and immunization play import role in prevention of infection.

Methods of controlling microbial diseases of human

- **Use of antiseptics and disinfectants**

Antiseptics and disinfectants are chemical substances that are used to kill or reduce microbial population in order to prevent infection. However, such chemicals are not effective against some microorganisms. For example, polio virus, tuberculosis bacterium, spores of bacteria and fungi are not destroyed by most antiseptics and disinfectants.

The major difference between antiseptics and disinfectants is that antiseptics can be safely and directly applied to the human body, whereas disinfectants cannot. Therefore, antiseptics are used in disinfection of living surfaces such as skin. Disinfectants are used in disinfection of non-living surfaces such as operation theatres, bathing areas, sinks, kitchen tops, cutlery, drains etc.

Antiseptics and disinfectants are generally formulated as liquids. Their effectiveness varies with concentration, duration of exposure, temperature and presence of organic matter.

Some examples of antiseptics and disinfectants are given below.

Antiseptics: ethanol, isopropanol, chloroxylenol

Disinfectants: phenol, hypochlorites (calcium hypochlorite and sodium hypochlorite),

- **Use of Antibiotics in controlling microbial diseases**

When the body's defense fails to protect body from the infection or overcome the disease, it has to be treated by chemotherapy with antimicrobial drugs. Antimicrobial drugs kill or interfere with the growth of microorganism without damaging the host. Antibiotics are effective antimicrobial drugs against bacteria.

Some antibiotics affect against a broad range of bacteria and they are termed as broad-spectrum antibiotics, while others affect only against a specific group of bacteria and are called narrow-spectrum antibiotics.

Antibiotics show various modes of action. Some examples are given below.

- Inhibition of cell wall synthesis- Penicillin
- Inhibition of protein synthesis- Erythromycin, Tetracycline
- Disrupting plasma membrane- Daptomycin
- Inhibition of DNA/RNA synthesis- Rifampin

- **Immunization: Vaccines**

A vaccine is a suspension of weakened pathogens or fractions of organisms that is used to induce immunity. Vaccines are frequently used to control diseases caused by viruses because there is no other control methods once infected. There are several types of vaccines.

1. **Live attenuated vaccines**

   Vaccine contains live pathogens which were deliberately weakened for its pathogenicity. These vaccines, mimic an actual infection. Since the pathogen is active inside the host, such vaccines provide lifelong immunity. More often a booster (secondary) immunization is not required. Examples for live attenuated vaccines are, vaccines for,

   - Measles, Mumps and Rubella (MMR)
   - Chickenpox

2. **Inactivated vaccines**

   Pathogenic microorganism is inactivated or killed in the vaccine. Compared to live attenuated vaccines, inactivated killed vaccines often require repeated booster doses. Examples for inactivated vaccines are vaccines for,

   - Virus diseases such as Rabies, Influenza, Polio
   - Bacterial diseases such as Cholera

3. **Subunit vaccines**

   Subunit vaccines contain only the antigenic fragments of a pathogen that can induce immunity in the recipient. Toxoid vaccines are the best examples for subunit vaccines, those have been used for a long time. Toxoids contain inactivated toxins derived from a pathogen. Examples for Toxoid vaccines are vaccines for Tetanus, Diphtheria, etc. Presently, subunit vaccines are produced using genetic engineering. E.g. Hepatitis-B Vaccines. Subunit vaccines usually require repeated booster dose to obtain full immunity.

Use of microorganisms in industry, agriculture and environment

Microorganisms have been exploited for various purposes long before their discovery. Babylonian and Sumerian civilizations used yeast to make alcohol in as early as 6000 BC. After the discovery of microorganisms, in the latter part of the nineteenth century, pure cultures of microorganisms are being used in the food production. This increased the understanding of microorganisms, their processes and products. At present various industries based on selected microorganisms and their qualities are in operation.

1. **Advantages of using microbial processes over chemical processes**

- Simple nutritional requirements are sufficient for their growth.
- They are able to convert (metabolize) a wide range of raw materials.
- They are able to convert cheap raw materials into industrially important products.
- Due to higher growth rate, they can convert the raw materials into products within a short period of time.
- Their growth conditions can be controlled to obtain desired end products.

- Reactions can be carried out at low temperatures, energy and pressures compared to the conventional industrial methods.
- They give higher yield with higher specificity when compared to the conventional industrial methods.
- Microbes are amendable to genetic manipulation to obtain desired yield and quality with high efficiency.

Basic principles of metabolic processes of microorganisms for product formations

1. Microbial cells are used as the end products. e.g. single cell proteins
2. Microbial metabolic products are used as end products- they may be either primary end products or secondary metabolites. e.g. primary end products- alcoholic beverages, secondary metabolites- antibiotics.
3. Microbial metabolic processes are used as end products. e.g. bioremediation (heavy metal remediation) , metal extraction (Cu, Fe), retting (production of fibers).
4. Genetically modified microorganisms are used to produce end products. e.g. productions of commercial enzymes (amylase from *Aspergillus niger*), vaccines (hepatitis B), hormones (insulin).

Applications of microorganisms in industries

Industrial microbiology is the large scale production of economically important products using microorganisms and their metabolic processes. Recent technological and biotechnological advances expanded the scope of industrial microbiology. Bacteria, fungi, algae and viruses are used in industry.

In industrial microbiology, microorganisms can be considered as miniature chemical factories, where various energy releasing (catabolic) and energy acquiring (anabolic) chemical reactions take place. Within this factory, raw materials (substrates) are converted into end products, one or more byproducts and wastes. End products can be separated from byproducts and wastes, by purification to obtain a purified industrial products.

Commercial products made by microorganisms and their processes

1. Single cell proteins

- Microbial cells are grown in large scale as food supplement and rich in proteins are called single cell proteins. e.g. Yeast, *Spirulina* sp. and *Chlorella* sp

$$C_6H_{12}O_6 \xrightarrow{\text{Yeast}} 2C_2H_5OH + CO_2$$

2. Alcohol and alcoholic beverages

Microorganisms are involved in the production of almost all alcoholic beverages such as beer, wine, sake, toddy and ethanol. Yeast, *Saccharomyces cerevisiae* ferment sugars into ethanol and carbon dioxide.

Globally, more than 70% of ethanol is produced by fermentation. Sucrose derived from sugarcane is the most widely used fermentation substrate. In addition, simples sugars derived from plants and dairy waste are also used

e.g. 1. Beer- by the fermentation of cereal grain malt.

2. Wine - produced by the fermentation of grapes or other suitable fruits.

3. Toddy - produced by the fermentation of sap of palms such as Palmyra and coconut.

4. Arrack- produced by fermentation of palm tree sap such as coconut and sugarcane.

3. Production of vinegar

There are two steps in vinegar production.

1. Alcoholic fermentation: sugars in malted grain, sap of palms, sugarcane and fruit juices is fermented by *S. cerevisiae*. Ethanol is subjected to acetic acid fermentation.

2. Acetic acid fermentation: Ethanol derived from alcoholic fermentation undergoes incomplete oxidation and is converted to acetic acid. This process is highly aerobic and involves *Acetobacter sp*. and *Gluconobacter sp.*

$$C_2H_5OH \xrightarrow{\text{Acetic acid bacteria}} CH_3COOH + H_2O$$

4. Dairy products

Dairy products are made by the fermentation of milk. Sugar lactose in milk is fermented by lactic acid producing bacteria into lactic acid. These bacteria are killed during pasteurization, therefore, added externally when making dairy products.

e.g.

- Curd and yoghurt is produced by fermentation of lactose sugar in milk by a mix population of *Lactobacillus bulgaricus, Lactococcus* lactis and *S. thermophilus. L. bulgaricus* add flavor and Streptococcus spp. add creamy texture and flavor.
- Production of cheese- *Streptococcus sp, Penicillium molds*
- Lactic acid is commercially produced by using waste products from cheese and butter industry. *L. bulgaricus* ferment lactose into lactic acid.

5. Organic acids

Majority of commercially produced organic acids are obtained through microbial fermentation. Fermentation substrates such as beat or cane molasses and organisms such as Aspergillus niger are used.

E.g. Citric acids- sucrose fermented by *Aspergillus niger*

6. Metal extraction

Some metals from ore are extracted with the help of microorganisms. This process is called leaching. One of the best example is extraction of copper from lower grades of copper ore for which other extraction methods are unprofitable. Thiobacillus ferrooxidans recover copper from the ore that contains iron and sulfur. About 70% of copper in the ore can be recovered by this microbial process. Uranium, gold and cobalt ore are also leached in similar manner using microbial processes.

7. Production of vitamins

Microbes can provide a source of an inexpensive source of vitamins for individual food supplements. E.g. vitamin *B12 – Pseudomonas sp*. and *Propionibacterium* sp

Riboflavin- fermentation by fungi

Vitamin C- *Acetobacter* sp

8. Vaccines

Commercial production of vaccine is done by variety of microbial antigens used in active immunization against various diseases. Some of them are genetically engineered vaccines. E.g. Hepatitis B vaccines.

Commercial production of various antibody preparation used for passive immunization. E.g. anti toxins against tetanus, Botulism toxin and immunoglobulin against rabies.

9. Enzymes

A range of enzymes are commercially produced by microorganisms. For example,

- Amylase: *Aspergillus niger, A. oryzae, Bacillus subtilis*
- Protease: *A. oryzae*
- Lipase: *Rhizopus spp.*
- Invertase: *Saccharomyces cerevisiae*
- Cellulase: *A. niger*

10. Antibiotics

Antibiotics is the most important secondary metabolites of microorganisms. Many antibiotics are still produced by microbial fermentation.

- Tetracycline: *S. aureofaciens*
- Penicillin: *Penicillium chrysogenum*
- Streptomycin: *Streptomyces griseus*

11. Hormones

a. Human insulin

Conventionally, insulin is extracted from animal pancreas. However, this is expensive and often cannot meet the demand. At present, insulin is produced cheaply by using genetically modified *E. coli* and *S. cerevisiae*. This insulin is identical to human insulin.

b. Human growth hormone

Earlier animal-derived hormones were used as alternatives to human growth hormone, but with very less efficiency. At present this hormone is successfully produced by genetically engineered E. coli in large scale.

12. Retting

Retting is the process of loosening fibers from woody stem or other plant material such as coir. For retting, plant materials are immersed in water for varying periods of time depending on the plant material. Heterogeneous bacterial populations participate in the process under aerobic or anaerobic conditions. Bacteria secrete enzymes, mainly pectinases to facilitate loosening.

13. Biogas production

Anaerobic digestion of organic waste produces various gases, called biogas. The type of biogas produced depends on the substrate biodegraded. Activity of acetogenic bacteria on the organic waste produce carbon dioxide and hydrogen, whereas, activity of methanogenic bacteria produce methane.

14. Biofuel production

Petroleum-based fuel supply is expensive and sometimes uncertain. As a result, much attention has been given to renewable replacement fuels such as ethanol, butanol, biodiesel and biogas etc. In Brazil a large amount of ethanol is produced by microbial fermentation of sugarcane to be used as a source of fuel. Also efforts have been made to produce ethanol and butanol from cellulosic materials such as wood, wastepaper and cornstalks by using genetically modified bacteria. Many researches are going on to produce biodiesel from microalgae.

15. Bakery products

Sugars in bread dough is fermented by S. cerevisiae, bakers's yeast. The primary function of fermentation in bread is to generate carbon dioxide. Bread dough is made with grain flour such as wheat, rye and rice. Dough traps carbon dioxide and rises due to the pressure during baking and form open crumb texture. .

Applications of microorganisms in environment management

Industries and agriculture release various chemicals substances that are not readily degradable in nature. For example, plastic is a synthetic substance that is not biodegraded. Residues of pesticides such as heavy metals, insecticide DDT, herbicide 2,4-D are some other examples of chemicals that are not degraded or very slowly degraded by microorganisms and retain in the soil for long periods and also contaminates groundwater.

1. Bioremediation

Bioremediation is a technology that applies of living organisms to remove, degrade or detoxify pollutants. Bioremediation naturally occurs in the soil. In most occasions microorganisms are used in bioremediation processes. Growth of microorganisms in polluted soils and water can be stimulated to promote biodegradation/ bio-removal of pollutants. Microorganisms with selected properties or genetically modified microorganisms with selected properties can be used to degrade/ remove a specific pollutant from the polluted sites. Bioremediation is currently used to,

- remediate soil and water contaminated with oil spills, toxic metal waste, hazardous organic waste etc.
- decompose wastewater from food processing and chemical plants.

2. Solid waste treatment

Accumulation of solid waste from house hold (garbage) possesses various environmental and health issues. In waste treatment, waste is degraded by microorganisms either aerobically or anaerobically. Composting, degrades waste aerobically. At the end, waste is converted to a stable material like humus.

Mostly garbage are placed as large compacted landfills or piles, where conditions are mostly anaerobic. In such situations, waste is degraded anaerobically using methanogenic bacteria. Methane gas produced as a byproduct of the degradation process. It may be used to generate electricity or as natural gas.

Applications of microorganisms in agriculture

Microorganisms have various applications in agriculture for improving yield, agronomic characteristics such as improved nitrogen and phosphorus absorption, resistance to pest and diseases and tolerance to drought etc.

1. Bio-fertilizers

Nitrogen and phosphorus are the most limiting nutrients in soil for plant growth and development. Therefore, chemical fertilizers are applied to the soil, to improve bioavailability of these nutrients. However, intensive use of synthetic fertilizers may result environmental problems such as deprivation of soil and water quality. Hence, much attention has been given for microorganisms that can be applied into the cropping systems to improve bioavailability of N and P. These microbial inoculants are called bio -fertilizers.

a. Phosphate solubilizing bacteria and mycorrhizae:

Phosphorus is the most limiting nutrient among all major plant nutrients. Bioavailability of phosphorus in any type of soil is negligible. (Very low amount of phosphorus applied to the soil remain available for plants). Phosphate solubilizing bacteria and Mycorrhizae contributes in improving the solubility of phosphorus in the soil solution. These bacteria and fungi secrete organic acids that dissolve minerals containing phosphorus and chelate cationic partners of the phosphate ions, thereby release phosphorus into the soil solution. At present, commercial formulations of microbial bio-fertilizers are available in the market.

b. Nitrogen fixing microorganism:

Biological nitrogen fixation is a process, where microorganisms convert atmospheric molecular nitrogen into its soluble form. These soluble forms of nitrogen can be assimilated by some plants directly or converted into desired soluble forms of nitrogen. e.g.

- Symbiotic nitrogen fixing
  - o *Rhizobium* sp. form intimate relationships with leguminous plants. The fixed nitrogen is released to the soil when plants die, making nitrogen available for other plants. Variousrhizobium inoculations are commercially available.
  - o Nitrogen-fixing cyanobacteria, *Anabaena* sp. form symbiotic association with water fern *Azolla*. This system has been successfully used in rice cultivation in many countries.

- Free living nitrogen fixing
    - o Free living nitrogen fixing bacteria such as Azotobacter present in high concentrations at rhizosphere.

c. Plant growth promoting bacteria

Many microorganisms in the plant rhizosphere produce plant growth-promoting substances like auxins (indole-3-acitic acid), cytokinins. These bacteria include,

Pseudomonas putida, P. fluorescens: auxin

Azotobacter sp., Rhizobium sp., B. subtilis, P. fluorescens: cytokinins

Acetobacter sp., Azospirillum sp: Gibberellin

(Names of the above microorganisms are not expected to be memorized by the students)

2. Bio-pesticides/ Bio Control Agents (BCA)

Extensive use of chemical pesticides leads to hazardous side effects to people. These substances or their residues persist in food and the environment. The residual toxicity may affect the non-target organisms. Further, over use of pesticides develop resistance against pesticides among the pests.

Therefore, environmentally friendly and less toxic alternative strategies are required to replace synthetic chemicals as pesticides. Naturally occurring microorganisms are being explored to control pests and diseases. Some microbial formulations are currently commercially available and widely used in many cropping systems. Those include, Entomopathogenic fungi, bacteria and viruses

- .Entomopathogenic fungi: These fungi infect a broad range of insects leading to insect death. They have been formulated into myco-insecticides.
- Entomopathogenic bacteria: *Bacillus thuringiensis*: insecticidal and toxic for many insect larvae. Protein crystals produced by this bacterium are toxic to larvae when ingested. This toxin is called Bt toxin. After ingestion, toxin is dissolved and lyse tissues of the guts of larvae. Majority of biopesticide formulations currently in use are Bt-based.

3. Composting

Composting is a process used to convert plant remains into the equivalent of natural humus by microbial degradation. Degradation of organic matter by a mix population of microbes under warm, moist and aerobic conditions.

Initial activity of thermophilic bacteria on the plant remains increase temperature of the pile to 55-60 °C. As a result, thermophilic bacteria dominate in the degradation process for few days. When temperature decreases over time, thermophilic microbial population is replaced by mesophilic microbial populations. The process can be enhanced by addition of moisture and supply of oxygen through turning the pile. In addition to bacteria, microbes such as fungus, Actinomycetes and protozoa also contribute to the breakdown of organic matter into compost.

Nature, distribution and role of soil microorganisms

Soil provides an adequate physical and chemical environment for growth of microorganisms in terms of space and nutrients which include minerals, decomposing organic materials, water, gases such as carbon dioxide, oxygen and nitrogen. Within a few centimeter depth of soil, there are different amounts of oxygen, moisture, light and nutrition, increasing the diversity of soil microorganisms.

The top few centimeters of the soil contains the largest community of bacteria. Microbial number declines rapidly with increasing depth. Majority of soil microflora is represented by bacteria. In addition, there are fungi, algae, protozoa and Actinomycetes. Actinomycetes, despite of being a member of domain bacteria, usually it is mentioned separately due to their significances. These microorganisms play a major role in decomposition of complex organic substances and participate in cycling of elements in biogeochemical cycles. Elements are oxidized and reduced by microorganisms for their metabolic requirements.

1. Mineralization

Mineralization is the decomposition of plant and animal residue by using extracellular enzymes of bacteria and fungi. These enzymes break down complex organic materials into simple inorganic materials such as CO_2 and H_2O. This is the major process by which plant nutrients are made available and recycling. Mineralization helps in following ways;

- helps to remove plant and animal debris from the earth surface allowing other organisms to live.
- recycle minerals which are found in limited quantities on the earth.

2. Role of microorganisms in the carbon cycle

- All organisms contain a large amount of carbon in organic compounds such as cellulose, starch, proteins and fat.
- Photosynthesis is the important first step in carbon cycle, in which, the atmospheric inorganic carbon dioxide is reduced/fixed to form organic compounds by photosynthetic organisms. Photoautotrophs such as plants, cyanobacteria, algae and photosynthetic bacteria fix carbon dioxide using energy from sunlight.
- Chemoheterotrophs such as animals and protozoa, depend on organic compounds produced by autotrophs to utilize them as their carbon source.
- Through food chain, carbon fixed from carbon dioxide by autotrophs, transferred from organism at lower trophic levels to the organisms at higher trophic levels.
- Both autotrophs and chemoheterotrophs, release a part of their fixed carbon as carbon dioxide to the atmosphere through respiration. This carbon dioxide is again made available for autotrophs.
- In chemoheterotrophs, undigested food is released to the environment as faeces which is later decomposed by soil microorganisms.

- Rest of the carbon fixed in organisms, remain within them until they die. Once the organisms are dead, these organic compounds are decomposed and carbon dioxide is returned back to the atmosphere.
- Microorganisms, mainly bacteria and fungi play a major role in organic matter decomposition.
- Microorganisms play another major role in carbon cycle in relation to methane gas- Ocean sediments contain a large amount of methane. However, about 80% of methane generated within ocean is consumed by microorganisms called methanotrophs before it reaches to the atmosphere.
- Despite of the above, methanogenic bacteria in the ocean's depths are constantly producing more methane.

3. Role of microorganisms in the nitrogen cycle

All organisms require nitrogen to synthesize protein, nucleic acid and other nitrogen-containing compounds. About 80% of molecular nitrogen available in the atmosphere. This is not biologically available for organisms. Therefore, it is essential to convert that atmospheric molecular nitrogen into bioavailable forms of nitrogen. Certain groups of microorganisms are able to fix gaseous molecular nitrogen into bioavailable forms of nitrogen such as ammonia, nitrate and nitrite. Therefore, nitrogen available on the earth, organisms and the atmosphere must follow cyclic paths.

There are four key steps in the nitrogen cycle, they are, ammonification, nitrification, denitrification and nitrogen fixation.

- **Ammonification**

More than 90% of organic nitrogen in the soil exist as proteins. Proteins from dead plants and animals are decomposed by extracellular proteolytic enzymes secreted by microorganisms into amino acids. Resulting amino acids are taken into microbial cells and are then subjected to ammonification in which, amino groups of amino acids are converted into ammonia (NH_3). In moist soil, ammonia is solubilized in water to form ammonium ions (NH_4^+). This ammonium ions are utilized by plants and soil microorganisms. Ammonia in dry soil rapidly disappear into the atmosphere.

- **Nitrification**

Nitrification is the process of oxidation of nitrogen in the ammonium ion to produce nitrate. This process is done by nitrifying bacteria living in soil in two stages.

In the first stage, microorganisms such as *Nitrosomonas*, oxidizes ammonium ions into nitrites.

$$NH_4^+ \xrightarrow{\textit{Nitrosomonas}} NO_2^-$$

In the second stage, microorganisms such as Nitrobacter oxidizes nitrites into nitrates.

$$NO_2^- \xrightarrow{\textit{Nitrobacter}} NO_3^-$$

Plants utilize nitrate as their source of nitrogen. Therefore, microorganisms play essential roles in providing nitrogen in bioavailable forms for plants and animals.

- **Denitrification**

  In the absence of atmospheric oxygen, some microorganism use nitrate and reduces it into N2. This process is called denitrification. Denitrification causes loss of nitrogen into the atmosphere. Therefore, it reduces available nitrogen in the soil. Denitrification frequently occurs in waterlogged soils, where limited oxygen is present. Pseudomonas sp converts nitrate ions into molecular nitrogen through following steps; nitrate to nitrite, nitrite to nitrous oxide and nitrous oxide to nitrogen gas

$$NO_3^- \longrightarrow NO_2^- \longrightarrow N_2O \longrightarrow N_2$$

- **Nitrogen fixation**

  The process of conversion of nitrogen gas to ammonia is termed as nitrogen fixation. Bacteria that are able to fix nitrogen require nitrogenase enzyme. Nitrogenase enzyme is inactivated by oxygen. There are two types of nitrogen fixing microorganisms, free-living and symbiotic.

  o **Free-living nitrogen fixing bacteria-** They are abundantly found in the rhizosphere. Rhizosphere is the soil immediately surrounding plant root. e.g. *Azotobacter sp*. Many cyanobacteria are also able to fix nitrogen. e.g. *Nostoc*. These organisms, exhibit mechanisms to prevent the exposure of nitrogenase enzyme to the atmospheric oxygen; *Azotobacter sp* - high rate of aerobic respiration, Cyanobacteria- heterocyst. Some anaerobic bacteria such as *Clostridium sp* also fixes nitrogen.

  o **Symbiotic nitrogen-fixing microorganism-** They play an important role in agricultural crops such as leguminous crops; soybean, beans, peas and peanuts. These symbiotic nitrogen-fixing bacteria are commonly known as rhizobia. Leguminous plants are specially adapted to facilitate symbiotic nitrogen fixation. Plants form root nodules where, anaerobic conditions and nutrients for bacteria are given. Bacteria fixes nitrogen and make it bio available to be used by the plant. Some non-leguminous plants can also fix nitrogen symbiotically with different microbial combinations. Lichen, a combination of fungus and an algae or cyanobacteria also fixes nitrogen. In rice paddy waters a small free-floating fern, *Azolla* fixes nitrogen symbiotically with *Anabaena sp*.

Interactions of soil microorganisms relevant to plant growth

Microorganisms in the soil directly interact with plants. Interactions in the soil such as rhizosphere, mycorrhizae and endophytes with symbiotic relationship of bacteria, fungi and plant cells. These microorganisms are beneficial to the plants in numerous ways such as nitrogen fixation, increased uptake of water and nutrients such as phosphorous, secretion of plant hormones such as indole acetic acid, increases iron uptake at iron limiting conditions and defense against pathogens. In return plants provide organic compounds essential for the microorganisms.

Rhizosphere

This is a kind of symbiotic interaction between plant roots, and soil surrounding the root surface for about few millimeters. This micro-ecological zone is called rhizosphere. Rhizosphere is considered as the most biodiverse and dynamic habitat on earth.

Microorganisms in the rhizosphere feed on the compounds exuded by roots such as sugars, amino acids and various aromatic compounds. Microorganisms compete –antagonize using antimicrobial compounds with each other for resources such as nutrients, space, and water in the rhizosphere. Bacteria are the most numerous organisms in the rhizosphere. Three most common genera of bacteria inhabiting rhizosphere are Pseudomonas, Bacillus and Agrobacterium. Root exudates act as chemical signals for bacteria to move towards the root surface. Both pathogenic and symbiotic fungi associate with the rhizosphere.

Mycorrhizae

Mycorrhizae (myco = fungus, rhiza = root) are symbiotic association between plant roots with fungi. All most all land plants have symbiotic association with one or more mycorrhizal fungi. Mycorrhizal fungi extend the surface area over which nutrients and water can be taken up by the plant. They can reach small pores in soil where plant roots cannot reach and uptake nutrients. Most significantly, mycorrhizae increase uptake of immobile nutrients such as phosphorous, zinc and copper. In return, mycorrhiza receive organic carbon from the plant.

Role of soil microorganisms in improving soil quality

Free living soil microorganisms and those associated with root surfaces, play a major role in improving soil quality. Microorganisms are involved in the formation of stable soil-aggregates, which are characteristics of good soil structure in fertile soils. Fungal filaments, Actinomycetes filaments and polysaccharide gums/slimes produced by bacteria are involved in soil aggregation formation.

Microbiology of domestic water and waste water

Contamination routes of drinking water

Drinking water resources can be contaminated with infectious disease causing organisms or chemical pollutants. Since water undergoes a filtering of microorganisms when pass through the soil into deeper layers, generally, water from springs and deep wells are of good quality in microbial point of view. Drinking water can be contaminated by dangerous pathogens when feces enter the water supply. Many diseases are disseminated by the fecal - oral route of transmission, in which, pathogens are shed in human or animal feces, contaminate water, and ingested via drinking. Examples for diseases spread by water (water-borne diseases) are typhoid fever, cholera, diarrhea etc.

Contamination of drinking water by chemical pollutants is a global issue. Industry, domestic and agriculture sectors release a large amount of chemicals that leach from the soil surface to groundwater. Most of these chemicals are resistant to biodegradation. Most freshwaters, such as tanks, often contain excessive amounts of nitrates and phosphates that come from agricultural fertilizer and household chemicals such as detergents. Accumulation of excessive amounts of such nutrients cause eutrophication, and flourish the growth of cyanobacteria and algae. They are toxic to humans. This kind of dense growth of cyanobacteria and algae, is called algal blooms. Various industries also release chemicals that are non-biodegradable. They too can contaminate drinking water supplies.

Microorganisms as indicators of water quality

Water supplies can be contaminated with pathogenic microorganisms such as Salmonella spp., *Shigella* sp. and *Vibrio* sp. that causes infectious diseases such as typhoid and Cholera. Therefore, it is essential to detect the presence of such microorganisms before the consumption to prevent disease outbreak.

However, it is not practical to test water samples to look for pathogens because, pathogens may present only in small numbers and might not be included in the test sample. On the other hand, since the testing for pathogens take time, discovery of pathogens in the laboratory takes time and, it will be too late to prevent an outbreak of the disease. Therefore, it is important to test water samples routinely for "indicator organisms, which will indicate potential contamination of water supply by pathogens.

One of the major criteria for an indicator organism is that it should be consistently present in human feces in large numbers. This ensures the, presence of indicator organism providing evidence for contamination of water supply with human feces.

Sri Lanka, and many other countries use coliform bacteria as an indicator organism to test drinking water quality. Coliforms are aerobic or facultative anaerobic, gram-negative, non-endospore forming, rod shaped bacteria that ferment lactose to form gas within 48 hours in a lactose broth medium at 35 °C. Coliforms constitute a large portion of human intestinal microflora. They are nonpathogenic line in the intestine. Therefore, presence of coliforms in water indicates fecal contamination. However, there are some coliforms found in plant and soil samples. There are specialized test to distinguish coliforms that has fecal origin from coliforms present in plant and soil samples. Coliform test is routinely carried out by laboratories of the national water supply and drainage board to determine water quality of drinking water.

Water-borne diseases

Pathogenic organisms that are frequently transmitted through water cause infections in the intestinal tract such as typhoid, paratyphoid, cholera, gastroenteritis and dysentery.

Process of drinking water treatment

Drinking water that comes from various water supplies can be polluted at any time. Therefore, water needs to be purified before consumption for our health and safety. The idea of water purification is not to sterilize water, but to make water free of disease-causing organisms. There are three main steps in an urban water treatment plant. They are,

- Sedimentation and coagulation,
- Filtration
- Disinfection.

I. Sedimentation & Coagulation

This is the first step. If water is turbid (cloudy), water is allowed to stand in a holding tank for a period of time to allow settling down of particles suspended in water. This occurs in large reservoirs, where water remains for a holding period during which large particulate matter settles to the bottom. Sedimentation is enhanced by adding alum (Aluminum potassium sulphate) which produces a sticky precipitate. Many microorganisms as well as finely suspended matter are removed in this manner.

II. Filtration

After sedimentation and coagulation, water is filtered by passing through beds of fine sand. Filtration removes protozoan cysts and other microorganism. Microorganisms are trapped by surface absorption onto the sand particles. This removes about 99% of bacteria. Some urban treatment plants additionally use activated carbon for the removal of toxic chemicals.

III. Disinfection

The final step in water treatment is disinfection. Water can be disinfected by several methods. Chlorination is one of the most commonly used method. It kills pathogenic bacteria. Disinfection by ozone (O_3) is another method. Ozone is highly reactive. It kills microorganisms by oxidation. Ozone has become a preferred method of disinfection since it leaves no taste or odor and has little residual effect.

Wastewater (sewage) management

Wastewater includes water from household use such as water used for toilets and washing, water from urban drainage systems and from industry. Many developing countries and some developed counties still do not have proper wastewater treatment mechanisms.

- **Principles and main steps in purification of industrial waste water**

  I. Primary treatment- Following steps are involved in the primary treatment.

  - Large floating materials are screened out
  - Removal of sand
  - Removal of oil and grease
  - Solid matter settles out in sedimentation tanks
  - Sludge collected and removed
  - No biological activity is used here
  - Primary treatment removes 25-35% organic matter

  II. Secondary treatment- Following steps are involved in the Secondary treatment.

  - The liquid flowing out of primary treatment, enters the secondary treatment stage.
  - During this treatment, waste water is aerated to facilitate the growth of aerobic bacteria and rapid microbial oxidation. One of two systems used are, activated sludge and trickling filter.
  - In activated sludge, vigorous aeration is done mechanically. In trickling filters, waste water is slowly sprinkled or sprayed over a bed of rocky material and allowed to trickle. In this process, microorganisms grow on the filter bed and oxidize organic matter.
  - In secondary treatment 75-95 % of organic material is oxidized.
  - The liquid flowing through these systems are then disinfected and allowed to flow

into natural waters.

- Sludge remaining from both treatments is sent to an anaerobic sludge digester where anaerobic decomposition convert the organic material in the sludge, finally to methane and CO_2 (biogas).
- Digested sludge can be used as fertilizer.

Adverse effects of discharging large amount of waste water into natural water bodies.

- Dissemination of pathogenic microorganisms
- Water pollution due to accumulation of biodegradable material and their decomposition products
- Decomposition which may consume large amounts of oxygen in water affecting aquatic organisms (high BOD – biological oxygen demand)
- Anaerobic decomposition leading to bad smells

Solid waste treatments

Nature of solid wastes

Solid waste include materials such as plant and animal residues, food waste, paper, plastic, polythene and glass. Among these, organic wastes such as plant and animal residues and food wastes are rapidly degradable. However, synthetic materials such as plastic and polythene are not easily degraded and tend to accumulate continuously. Proper management of solid waste, ensure community health and environmental safety. Accumulation of unmanaged waste in big piles in the open environment can lead to soil, air and water pollution which is harmful for the environment and organisms in contact with the polluted environment.

Environmental and hygenic importance of recycling solid wastes

- Open dumping of waste provide breeding grounds for mosquitoes, flies, other insects andrats. These organisms serve as vectors for dangerous diseases such as dengue, chikungunya, various food borne diseases and leptospirosis.
- Contaminated water resources have the risk of spreading water borne diseases such as typhoid, paratyphoid, cholera, dysentery and gastroenteritis.
- Piles of waste at public and residential area often create social issues due to unpleasant smell generated during anaerobic digestion of waste.
- Heavy piles of waste sometimes can be dangerous due to accumulation of methane, generated during anaerobic digestion of waste. Accumulation of methane can cause explosion and fire.
- Groundwater can be polluted by the leachate of waste piles. Leachate means liquid pass through the waste, extracting the dissolved and the suspended matter from the waste.

Therefore, solid waste must be properly managed using appropriate technology which is socially accepted and environment friendly.

Methods of minimizing problems created by solid wastes (solid waste management)

1. **Sorting and recycling**

   In many countries municipal solid waste is sorted into kitchen waste, plant material, paper, plastic, glass etc. and collected into separate containers. Sorting allows separation of waste that can be recycled such as paper, plastic and glass. Although paper is presumably biodegradable, it is not easily degraded since microorganisms cannot effectively attack papers when the waste is placed into large compacted dumping sites.

2. **Degradation/ decomposition of organic matter**

   Readily degradable organic matter such as kitchen and garden waste is subjected to natural microbial degradation by the process of composting. Resulting compost is used in agriculture and gardening. Compost generate additional income to the waste management authorities such as municipal council. Anaerobic condition in the pile of compost, promote the activity of methanogenic bacteria. The methane gas they produce can be collected to generate electricity.

3. **Sanitary land filling**

   Sanitary landfills are one of the most popular forms of waste disposal, primarily because, they are the least expensive way to dispose the waste. More than four-fifths of municipal solid waste is disposed in landfills. A sanitary landfill is an engineered means of disposal of waste. In a sanitary landfill, waste is spread in layers on a piece of property, usually on marginal or sub marginal land. The objective is to spread the layers and then compact them tightly, greatly reducing the volume of the waste. The waste is then covered by soil. A landfill should not be located in areas with high ground water levels. Much of the waste in a sanitary landfill will decompose through biological and chemical processes that produce solid, liquid and gaseous products.

Microorganisms and food

Why food is spoiled by microorganisms

All food materials used for human consumption are of plant or animal origin. Microorganisms inhabit nearly every niche on earth, and food supply which are of plant or animal origin is no exception. Food have a natural micro biota and also get contaminated with microorganisms in nature during handling and processing. Food materials contain nutrients and water, act as culture media for growth of microorganisms. The nutrients present in the food materials, the same that is required for the growth of many microorganisms. Therefore, food serves as a culture medium such as nutrient broth. Numerous bacteria, yeast and , molds will grow in this broth.

Physical, chemical and biological changes occur in food spoilage

Growth of microorganisms in food changes physical, chemical and biological structures of food and making them unfit for consumption which is termed as food spoilage. Microorganisms growing in food are heterotrophic bacteria and fungi. During this process, they breakdown carbohydrates, proteins and fats, to obtain energy and other requirements for their own growth. Various extra cellular enzymes such as amylase pectinase, cellulase, protease and lipase are secreted by contaminant microorganisms, in order to complete the above process. As a result of this, major constituents of food, undergo chemical, physical and biological changes.

Chemical changes take place in food

1. **Putrefaction**

   Protein present in the food source is broken down by the proteolytic enzymes secreted by proteolytic microorganisms into Amino acids, Amines, Ammonia and hydrogen sulphide (H S).

2. **Fermentation**

   complex carbohydrates present in the food source are broken down into simple carbohydrates by amylase secreted from microorganisms. Simple carbohydrates (sugars) then converted into carbohydrate food acid, alcohol and gases by enzymes secreted from saccharolytic microorganisms.

3. **Rancidity**

   Lipids present in food source is converted into fatty acids and glycerol by enzymes secreted by lipolytic microorganisms.

Physical changes that may take place in food

1. Softening of food
2. Pigmentation
3. Ropiness
4. Slime or gum formation (polysaccharide)
5. Toxin accumulation

External factors influence on food spoilage

External factors are environmental factors that affect both food and microorganisms.

1. Temperature of storage- Growth of microorganisms is affected by a wide range of temperatures, at low temperature growth is slow, therefore, spoilage is also slow, at ambient temperature growth is high, and therefore, spoilage is also high. However, even at low temperature (e.g. food stored at 4 ^{0}C in the refrigerator getting spoiled) microbial spoilage occurs by psychrophilic bacteria.

2. Relative humidity (RH) of environment- Relative humidity of the storage environment is important, as it relates to the availability of moisture, which is an important factor for food spoilage due to microbial growth. Low moisture containing food should not be stored in high RH environments, as the food will pick moisture from the atmosphere and the microbial growth will be facilitated.

3. Presence and concentrations of gases in the environment- presence or absence of O_2 determine the type of contaminants causing food spoilage. There are both aerobic as well as anaerobic microbes cause food spoilage.

Internal factors influence on food spoilage

Internal factors are those factors that are present in food itself.

1. pH- Most microorganisms grow best at pH values around 7.0 (6.6 – 7.5) and very few grow below 4.0. In general, molds and yeasts can grow over a wide range of pH from very low to very high (pH 2 - 10). Bacteria in general, grow between pH 5 – 7. Fruits such as limes, orange and banana are likely to be spoiled by molds and yeast. Most animal foods such as beef, chicken, fish, milk are susceptible to bacterial, mold and yeast spoilage.

2. moisture content- Drying is the oldest method of preserving food which is based on reducing moisture content. High moisture containing food such as meat and fish are spoiled by bacteria. Low moisture containing food such as biscuits and bread are spoiled by molds. Dried milk powder or flour which contains very low moisture are not easily spoiled by bacteria or molds. Salt and sugar containing foods (available water is low) are generally spoiled by halophillic bacteria (salted foods), osmophillic and xerophillic molds/ yeast (sugary foods).

3. Nutrient content- Water, source of energy, nitrogen, vitamins and minerals are essential

   nutrients for growth of microorganisms. Nutrient rich food are easily spoiled by microorganisms. e.g.; Milk, meat

4. Biological structure- The natural covering of some foods protect the food from the entry and the damage cause by the spoilage microorganisms. e.g., outer covering of fruits, shell of egg

Food borne pathogens of humans

- Typhoid - *Salmonella typhi*
- Dysentery - *Shigella*
- Cholera - *Vibrio cholera*
- Food poisoning - *Staphylococcus aureus*
- Botulism - *Clostridium botulinum*
- Aflotoxin - *Aspergillus flavus*

Effects of food spoilage on human health

Some microorganisms produce various toxic substances in food, which cause food poisoning or food intoxication when consumed. Illness may result from consuming food spoiled by microorganisms. Microorganisms grow and multiply in food, increasing the numbers of microbial cells, and also produce toxic chemical substances. Ingestion of large numbers of microbial cells and their toxic chemicals by any individual through highly contaminated food, may develop disease. These diseases/ illnesses are of two kinds;

(a) food borne infections- In food borne infections, the contaminating pathogenic microorganisms enter the body of the individual consumed spoiled food, and inside the host, they grow, multiply and also produce toxins which cause symptoms of the characteristic disease.

e.g. *Salmonella typhi* - typhoid fever

Shigella - dysentery

Vibrio cholerae - cholera

(b) Food intoxication- In food intoxication, the spoiled food contains toxins, produced due to microbial growth. Any individual who consumes such contaminated food containing toxins will develop symptoms of the disease, within a short period of time. e.g;

- Food poisoning - *Staphylococcus aureus*
- Botulism - *Clostridium botulinum*
- Aflatoxins - *Produced by fungi Aspergillus flavus*

In general all the above illnesses are referred to as due to food poisoning.

(c) Viruses also (entero viruses) cause some food borne illnesses.

References

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Notes:

This is to acknowledge that some of the diagrams used in this book have been taken from various electronic sources using internet . This book is not published to make profit and sold only to cover cost.

The resource book is prepared according to the subject content and learning outcomes of the G.C.E. (A.L) Biology new syllabus which is implemented from 2017.

The content of this Resource book declares the limitation of the G.C.E. (A.L) Biology new syllabus which is implemented from 2017.