# UNIT –I

**Isolation of Microorganisms:**

There are over a million species of micro-organisms widely distributed in nature. Less than 1% of the world's microorganisms have been studied. In fact, only a few hundred species are important for industrial use. A selected list of organisms along with their products is given in Table 19.3.

**Table 19.3 A selected list of important microorganisms and their products**

| *Microorganism* | *Product* |
|---|---|
| **Algae** | |
| *Chlorella sorokiniana* | Single-cell protein |
| *Spirulina maxima* | Single-cell protein |
| **Bacteria** | |
| *Acetobacter aceti* | Acetic acid |
| *Acetobacter woodii* | Acetic acid |
| *Bacillus subtilis* | Bacitracin |
| *B. brevis* | Gramicidin |
| *B. thuringiensis* | Endotoxin |
| *Clostridium aceticum* | Acetic acid |
| *Methylophilus methylotrophus* | Glutamic acid |
| *Pseudomonas denitrificans* | Vitamin $B_{12}$ |
| **Actinomycetes** | |
| *Streptomyces aureofaciens* | Tetracycline |
| *S. griseus* | Streptomycin |
| *S. tradiae* | Neomycin |
| *Nocardia mediterranei* | Rifamycin |
| *Micromonospora purpurea* | Gentamycin |
| **Fungi** | |
| *Aspergillus niger* | Citric acid |
| *A. oryzae* | Amylase, cellulase, single-cell protein |
| *Candida lipolytica* | Lipase |
| *C. utilis* | Single–cell protein |
| *Penicillium chrysogenum* | Penicillin |
| *Saccharomyces cerevisiae* | Ethanol, wine, single-cell protein |
| *S. lipolytica* | Citric acid, single-cell protein |
| *Rhizopus nigricans* | Steroids |
| *Gibberella fujikuroi* | Gibberellin |
| *Trichoderma viride* | Cellulase |

The good sources for the isolation of microorganisms are soils, lakes and river muds. It is estimated that a gram of soil contains $10^6$—$10^8$ bacteria, $10^4$—$10^6$ actinomycete spores and $10^2$—$10^4$ fungal spores.

The common techniques employed for the isolation of microorganisms are given below:

1. Direct sponge of the soil
2. Soil dilution
3. Gradient plate method
4. Aerosol dilution
5. Flotation
6. Centrifugation

The actual technique for the isolation of microorganisms depends on the source and the physiological properties of microorganisms. The general scheme adopted for isolating microorganisms from soil or water source is given below:

I. The sample (soil or water) is diluted with sterile water to which an emulsifying agent (Tween) is added.
II. Sample is thoroughly mixed and allowed to stand at room temperature.
III. Supernatant is diluted, $10^{-1}$ to $10^{-10}$
IV. Various culture media are inoculated with diluted samples and incubated.
V. Colonies from the plates are isolated and identified.
VI. The required pure strains are maintained and preserved.

Screening for New Metabolites, and Isolation of Microorganisms:

Industrial microbiologists continue their search for newer metabolites produced by microorganisms. Research work is particularly directed for identifying chemotherapeutically

important products for the treatment of tumors, bacterial diseases (newer antibiotics against resistant strains) and viral diseases, besides several other substances (e.g. hormones, enzyme inhibitors). In addition, isolation of microorganisms for improvement of food industry, and for efficient degradation of the environmental pollutants and hazardous chemicals also assumes significance.

Preservation of Microorganisms:

There are distinct methods for preservation of microorganisms. The most important being storage by refrigeration, freezing and lyophilization.

**Microbial strain improvement**

Microorganisms can convert various carbon sources from side and waste streams into valuable chemicals, biopolymers and biofuels. Some research institutes optimises conversion into the desired end product by developing specific microorganisms and cultivation conditions.

The Science and Technology of designing, breeding, manipulating, and continuously improving the performance of microbial strains for biotechnological applications is referred to as "strain improvement.". The science behind developing improved cultures has been enhanced recently by a greater understanding of microbial biochemistry and physiology, coupled with advances in fermentation reactor technology and genetic engineering. In addition, the availability and application of user-friendly analytical equipment such as high pressure liquid chromatography (HPLC) and mass spectroscopy, which raised the detection limits of metabolites, have also played a critical role in screening improved strains.

**Introduction**

The use of microbes for industrial processes is not new. Improving the commercial and technical capability of microbial strains has been practiced for centuries through selective breeding of microbes. In making specialty foods and fermented beverages (such as alcohol, sake, beer, wine, vinegar, bread, tofu, yogurt, and cheese), specific strains of bacteria and fungi isolated by chance have been employed to obtain desirable and palatable characteristics.

Microbes are now routinely used in large-scale processes for the production of lactic acid, ethanol fuel, acetone–butanol, and riboflavin as well as for the commercial production of enzymes such as amylases, proteases, and invertase. Efforts were also made by chemical engineers to improve fermenter designs on the basis of understanding the importance of culture media components, sterile operations, aeration, and agitation.

Today, production of hormones, steroids, vaccines, monoclonal antibodies, amino acids, and antibiotics are testimonies to the important role of strain improvement in the pharmaceutical industry. The intent of this article is to briefly describe strategies employed in strain improvement, the practical aspects of screening procedures, and the overall impact that strain improvement has on the economics of fermentation processes.

**Attributes Of Improved Strains**

Microbial strain improvement cannot be defined simply in terms of modifying the strain for overproduction of bioactive compounds. Strain improvement should also be viewed as making the fermentation process more cost-effective. Some of the traits unique to fermentation process that make a strain "improved" are the ability to

a) Assimilate inexpensive and complex raw materials efficiently
b) Alter product ratios and eliminate impurities or by-products problematic in downstream processing
c) Reduce demands on utilities during fermentation (air, cooling water, or power draws)
d) Excrete the product to facilitate product recovery
e) Provide cellular morphology in a form suitable for product separation
f) Create tolerance to high product concentrations
g) Shorten fermentation times and
h) Overproduce natural products or bioactive molecules not synthesized naturally, for example, insulin.

**Need for strain improvement**

Microbes (fungi, bacteria, actinomyces) that live freely in soil or water and produce novel compounds of commercial interest, when isolated from their natural surroundings, are not ideal for industrial use. In general, wild strains cannot make the product of commercial interest at high enough yields to be economically viable. In nature, metabolism is carefully controlled to avoid wasteful expenditure of energy and the accumulation of intermediates and enzymes needed for their biosynthesis. This tight metabolic and genetic regulation, and synthesis of biologically active compounds, is ultimately controlled by the sequence of genes in the DNA that program the biological activity. To improve microbial strains, the sequence of these genes must be altered and manipulated. In essence, microbial strain improvement requires alteration and reprogramming of the DNA (or the genes) in a desired fashion to shift or bypass the regulatory controls and checkpoints. Such DNA alterations enable the microbe to devote its metabolic machinery to producing the key biosynthetic enzymes and increasing product yields.

In some cases, simple alteration in DNA can also lead to structural changes in a specific enzyme that increases its ability to bind to the substrate, enhance its catabolic activity, or make itself less sensitive to the inhibitory effects of a metabolite.

**Strategies For Strain Development**

Several procedures are employed to improve microbial strains. All bring about changes in DNA sequence. These changes are achieved by mutation, genetic recombination, or the modern DNA

splicing techniques of "genetic engineering." In some cases, a combination of one or more techniques is employed to attain maximum strain improvement.

**A. Mutation**

Microbes, generation after generation, generally inherit identical characteristics as their parents. However, when changes occur in the DNA, they too are passed on to daughter cells and inherited in future generations. This permanent alteration of one or more nucleotides at a specific site along the DNA strand is called a genetic mutation. The strain that harbors the mutation is called a mutant strain. Although a gene consists of hundreds or even thousands of base pairs, a change in just one of these bases can have a significant change in the function, operation, and expression of the gene or in the function of its protein product.

**B. Genetic recombination**

In addition to the manipulation of microorganisms by mutation, the techniques of genetic recombination can be employed to get new strains containing novel combinations of mutations and superior microbial strains. Generally, genetic recombination methods include those techniques that combine two DNA molecules having similar sequences (homologs). Through the special event of crossing-over, they are reunited to give a new series of nucleotide sequences along the DNA that are stable, expressible genetic traits. This mechanism of gene alteration and strain modification is called genetic recombination.

**C. Cloning and genetic engineering**

By employing restriction endonucleases and ligases, investigators can cut and splice DNA at specific sites. Some endonucleases have the ability to cut precisely and generate what are known as "sticky ends." When different DNA molecules are cut by the same restriction enzyme, they possess similar sticky ends. Through a form of biological "cut and paste" processes, the lower parts of the one DNA is made to stick well onto the upper part of another DNA. These DNA molecules are later ligated to make hybrid molecules. A useful aspect of this cut and paste process involves the use of plasmid, phage, and other small fragments of DNA (vectors) that are capable of carrying genetic material and inserting it into a host microbe such that the foreign DNA is replicated and expressed in the host.

Media Formulation

Thorough analysis is essential to establish a suitable medium for an individual fermentation process. All most all microbes need water, energy sources, sources of carbon and nitrogen, certain mineral elements and perhaps vitamins plus oxygen if microbes are aerobic. It is easy to devise a medium containing pure compounds on a small scale but this medium may be unsuitable for use in a large scale fermentation processes. Following are the criteria imperative to consider while designing a medium for large scale production

- The medium should support the maximum production of yield of product per gram of substrate used
- It should promote maximum accumulation of the product

- The maximum rate of product formation should be achieved
- There should be minimum production of unwanted products
- Constituents of the medium should be available throughout the year at cheaper rate and nearby area
- There should not be any undesirable changes in the consistency of the medium during preparation of media and after sterilization
- There should not be any difficulty in the operations like aeration, agitation during the production process and downstream operations like detection, isolation, extraction, purification and waste treatment

Carbon Sources

- The rate at which the formation of the product take place is influenced by the rate at which the carbon source is metabolized
- Many time, sugars which metabolize rapidly results in fast growth of the organism and less productivity of secondary metabolites

Preparation of the Inoculums

Microbial inoculums have to be prepared from the preservation culture so that it can be used for the fermentation process. The aim of inoculum preparation is to select microorganisms with high productivity and to minimize low productive, mutant strains. The process involves several steps:

I. First generation culture is prepared from the preservation culture on agar slants which is then sub-cultured to prepare "working culture". At this stage the microorganisms start growing. In small-scale fermentation processes working culture is used as inoculum, but for large scale fermentation inoculum preparation involves additional steps.

II. Second, sterile saline water or liquid nutrient medium containing glass beads is added to the agar slants and shaken so that microbial suspension is prepared. This suspension is transferred to a flatbed bottle, which contains sterile agar medium. The microorganisms are allowed to grow by incubating the bottle.

III. Third, the microbial cells from the flat bed bottles are transferred to a shaker flask containing sterile liquid nutrient medium and is placed on a rotary shaker bed in an incubator. Microorganisms grow at a rapid rate due to aeration.

IV. Fourth, microbial cells from the shaker flask can be used as seed culture which are then added to small fermenters and allowed to grow for 1-2 days. This simulates conditions that exist in the larger fermenters to be used for production of metabolites. Finally, the microorganisms are transferred to the main fermentation vessel containing essential media and nutrients.

Culture Medium

Media requirements depend on the type of microorganism being used in the fermentation process, but the basic requirements remain the same source of energy, water, carbon source, nitrogen source, vitamins, and minerals. Designing the media for small-scale laboratory purpose is relatively easy, but media for industrial purpose are difficult to prepare.

The culture medium should:

- Allow high yield of the desired product and at fast rate,
- Allow low yield of undesired products,
- Be sterilized easily,
- Yield consistent products i.e., minimum batch variation,
- Be cheap and readily available,
- Be compatible with the fermentation process,

- Not pose environmental problems before, during, or after the fermentation process.

The culture medium will affect the design of the fermenter. For example, hydrocarbons in the media require high oxygen content so an air-lift fermenter should be used. Natural media ingredients are cheap but they have high batch variation. On the other hand pure ingredients (also called defined media or formulated media) have very little batch variation but are expensive. The media should support the metabolic process of the microorganisms and allow bio-synthesis of the desired products.

Carbon & Energy source + Nitrogen source + Nutrients Product(s) + Carbon Dioxide + Water + Heat + Biomass

Media are designed based on the above equation using minimum components required to produce maximum product yield. Important components of the medium are carbon sources, nitrogen sources, minerals, growth factors, chelating agents, buffers, antifoaming agents, air, steam, and fermentations vessels.

Carbon Sources:

Product formation is directly dependent on the rate at which the carbon source is metabolized; also the main product of fermentation determines the type of carbon source to be used. Carbon sources include carbohydrates, oils and fats, and hydrocarbons.

Carbohydrates:

These are the most commonly used carbon sources in the fermentation process. Starch is easily available carbohydrate obtained from maize, cereals, and potatoes. It is widely used in alcohol fermentation. Grains like maize are used directly in the form of ground powder as carbohydrate. Molasses is one of the cheapest sources of carbohydrate. It contains high sugar concentration and other components like nitrogenous substances and vitamins and is used in alcohol, SCP (Single-cell Protein), amino acid, and organic acid fermentations.

Oils and Fats:

Vegetable oils are used as a carbon source. Oils provide more energy per weight compared to sugars. They also have anti-foaming properties but are generally used as additives rather than as the sole carbon source. Examples are olive oil, cotton seed oil, soya bean oil, linseed oil, and lard (animal fat).

Hydrocarbons:

$C_{12}$-$C_{18}$ alkanes can be used as carbon sources. They are cheap, and have more carbon and energy content per weight than sugars. They can be used in organic acids, amino acids, antibiotics, enzymes, and proteins fermentation.

Nitrogen Sources:

Ammonia, ammonium salts, and urea are the most commonly used nitrogen sources in the fermentation process. Ammonia also serves the purpose of pH control. Other substances used as nitrogen sources are corn-steep liquor, soya meal, peanut meal, cotton seed meal, amino acids, and proteins.

Minerals:

Calcium, chlorine, magnesium, phosphorous, potassium and sulfur are the essential minerals for all media. Other minerals like copper, cobalt, iron, manganese, molybdenum, and zinc are needed in trace amounts and are generally present as impurities in other components. The specific concentration on these elements depends on the micro- organism being used.

Growth Factors:

Vitamins, amino acids, and fatty acids are used as growth factors in the fermentation process to complement the cell components of the microorganisms.

Chelating Agents: Chelating agents prevent formation of insoluble metal precipitates. They form complexes with the metal ions present in the medium and can be utilized by the microorganisms. Chelating agents are not required in large scale fermentation processes since some of the other ingredients like yeast extract will perform the function of forming complexes with the metal ions.

Buffers:

Buffers are used to maintain the pH of the medium as microbial growth is affected by the pH changes. Optimum pH for most microorganisms is 7.0. Commonly used buffers are calcium carbonate, ammonia, and sodium hydroxide.

Antifoaming Agents:

Microbial process produces a large amount of foam in the fermentation vessel. This is due to microbial proteins or other components of the media. Foaming causes removal of cells from the media and their autolysis, thus, releasing more microbial foam-producing proteins, and aggravating the problem. Foam will reduce the working volume in the fermentation vessel, decrease rate of heat transfer, and deposit cells on the top of the fermenter. The air filter exits become wet allowing growth of contaminating microorganisms. Antifoaming agents are also called surfactants, i.e. they reduce the surface tension in the foam and destabilize the foam producing proteins.

Commonly used antifoaming agents are stearyl alcohol, cotton seed oil, linseed oil, olive oil, castor oil, soy bean oil, cod liver oil, silicones, and sulphonates.

Air:

Air is required for aeration and is supplied to the fermenter by means of pumps or compressors. It is sterilized by passing through filters before being introduced. The amount of air required and the extent of purity depends on the fermentation process being carried out.

Steam:

Steam is used to sterilize fermenters and other equipment and to control temperature. Continuous dry steam supply is required for the fermentation process and care should be taken to prevent condensation.

MEDIA STERILIZATION Sterilization is defined as the complete destruction or elimination of all viable organisms (in or on an object being sterilized). There are no degrees of sterilization: an object is either sterile or not. Sterilization procedures involve the use of heat, radiation, chemicals or physical removal of cells. Media for industrial fermentations are usually sterilized. In some cases the economics of the fermentation makes it unrealistic to sterilize. The fermentations can proceed, however, these fermentations employ low pH and other contamination inhibitors (lactic acid) to hold in check the numbers of contaminating microorganisms. In other cases, sterilization is not required as the media components are poorly utilized by contaminating microorganisms. Fermentation media are sterilized by the use of: filtration, radiation, ultrasonic treatment, chemical treatment or heat (boiling or passing live steam through the medium, or by subjecting the medium to steam under

pressure - autoclaving). Steam is used almost universally for the sterilization of fermentation media. The major exception is the use of filtration for the sterilization of animal cell culture.

Heat: Heat is the most important and widely used method. For sterilization, the type of heat, time of application and temperature required to ensure destruction of all microorganisms must always be considered. Endospores of bacteria are the most thermo-resistant of all cells so their destruction usually guarantees sterility.

Incineration: In this process, organisms are burned and physically destroyed. It is widely used for needles, inoculating wires, glassware, tubes etc. and objects that cannot be destroyed in the incineration process.

Boiling: Boiling is done at >100˚C for 20-30 min. It kills everything except for some endospores. To kill endospores and therefore perfectly sterilize the solution, very long or intermittent boiling is required.

Autoclaving: Autoclaving is the process of using steam under pressure in an autoclave or pressure cooker. It involves heating at 121˚C for 15-20 min under 15 psi pressure and can be used to sterilize almost anything. However heat labile substances will be denatured or destroyed. Sterilization of nutrient media is usually done using this process.

Dry Heat (Hot Air Oven): The process involves heating at 160˚C for 2 hours or at 170˚C for 1 hour. It is used for glassware, metal and objects that will not melt.

Sterilization in industry-scale fermenters (or bioreactors) is more complex. Steam is used to sterilize fermentation media. The medium can be sterilized in situ within the bioreactor. However, if the medium is sterilized in a separate vessel, the bioreactor needs to be sterilized before the sterile medium is added to it. Bioreactors are sterilized by passing steam through spargers. Spargers are devices that distribute gas bubbles (usually sterile air or steam) in a liquid

phase. They have particular design criteria, e.g., providing small sized bubbles (the sparger breaks the incoming air into small bubbles).

Various designs can be used such as porous materials made of glass or metal. However, the most commonly used type of sparger used in modern bioreactors is the sparge ring. A sparge ring consists of a hollow tube in which small holes have been drilled and is easier to clean than porous materials and is also less likely to block during fermentation. During sparging, steam pressure is held at 15 psi in the vessel for 20 min.

Definition of Upstream

The upstream stage of the production process involves searching for and extracting raw materials. The upstream part of the production process does not do anything with the material itself, such as processing the material. This part of the process simply finds and extracts the raw material. Thus, any industry that relies on the extraction of raw materials commonly has an upstream stage in

its production process. In a more general sense, "upstream" can also refer to any part of the production process relating to the extraction stages.

## UPSTREAM PROCESSING AND DOWNSTREAM PROCESSING

### 26.1 Introduction

Industrial fermentation involves upstream and downstream processes (Figure 26.1).

**Upstream processes,** include selection of a microbial strain characterized by the ability to synthesize a specific product having the desired commercial value. This strain then is subjected to improvement protocols to maximize the ability of the strain to synthesize economical amounts of the product. Included in the upstream phase is the fermentation process itself which usually is carried out in large tanks known as fermenters or bioreactors. In addition to mechanical parts which provide proper conditions inside the tank such as aeration, cooling, agitation, etc., the tank is usually also equipped with complex sets of monitors and control devices in order to run the microbial growth and product synthesis under optimized conditions. The processing of the fermentation reactions inside the fermenter can be done using many modifications of engineering technologies. One of the most commonly used fermenter types is the stirred-tank fermenter which utilizes mechanical agitation principles, mainly using radial-flow impellers, during the fermentation process.

**Downstream processing,** the various stages that follow the fermentation process, involves suitable techniques and methods for recovery, purification, and characterization of the desired fermentation product. A vast array of methods for downstream processing, such as centrifugation, filtration, and chromatography, may be applied. These methods vary according to the chemical and physical nature, as well as the desired grade, of the final product.

**Fig. 26.1 Stages in fermentation process (Waites et al., 2001)**

### 26.2 Overview of Upstream Processing

Upstream processing normally deals with three important points.

The first relates to fermentation media, especially the selection of suitable cost effective carbon and energy sources, along with other essential nutrients. The media optimization is a vital aspect of process development to ensure maximization of yield and profit.

The second aspect involves aspects associated with the producer microorganism. They include the strategy for initially obtaining a suitable microorganism, industrial stain improvement to enhance productivity and yield, maintenance of strain purity, preparation of a suitable inoculums and continuing development of selected strains to increase the economic efficiency of the process.

The third component relates to the **fermentation** which is usually performed under rigorously controlled conditions developed to optimize the growth of the organism or the production of a target microbial product.

### 26.2.1 Fermentation medium

The medium used for fermentation may be classified as defined, complex or technical medium. Defined medium consists only of precisely chemically defined substrates. Complex medium is composed of substrates with undefined composition, such as extracts or hydrolysates from waste products, which are cheap substrates commonly used in industrial production. Relatively expensive substrates, such as yeast extract, brain heart infusion, peptone, and tryptone are often used for complex medium. Technical media are used on an industrial scale and are cheaper. The substrate sources can also be derived from industrial waste, and are often highly impure mixtures, requiring pretreatment before they could be used for a fermentation process. Examples are soy meal, whey, fishmeal, malt extract, and sulfite waste liquor. Wastewater from monosodium glutamate production, which contains high levels of chemical oxygen demand (COD), sulphate, and ammoniacal nitrogen at a low pH, has been used as the nitrogen and water source, with sugar beet pulp as the carbon source, for the production of pectinase.

Media sterilization is necessary to ensure that only the desired microorganism is present to carry out the fermentation, that products are made of predicted quality, that the environment is protected from undesirable contamination, and that deterioration (microbial spoilage) of products is prevented. Sterilization by high temperature achieved by direct or indirect steam or electric heating, membrane filtration, microwave irradiation, high voltage pulses and photosemiconductor powders which involve the rupture of the cell membrane by increasing the transmembrane electric field strength beyond a certain threshold.

Inoculation is the transfer of seed material or inoculum into the fermentor. Inoculation of a laboratory fermentor is generally done using presterilized tubing and a peristaltic pump. However, on a larger scale, inoculum transfer is done by applying a positive pressure on the inoculum fermentor and connecting it aseptically to the production fermentor. The connecting lines are sterilized before being used for transfer of inoculum. Heat susceptible substances such as amino acids and some vitamins must be dissolved in small volumes of water, sterilized by filtration and added separately to the final medium aseptically.

### 26.2.2 Fermentation systems

A fermentation system is usually operated in one of the following modes: batch, fed batch, or continuous fermentation. The choice of the fermentation mode is dependent on the relation of consumption of substrate to biomass and products. The systems are batch, continuous and fed batch systems that were described earlier in lesson 21.

Today the most common type of upstream processing of proteins utilizes two tools: bioreactors a nd suspension (or attached) cells transformed with expression vectors genetically engineered to contain one (or more) human genes that produce copious amounts of their protein(s).

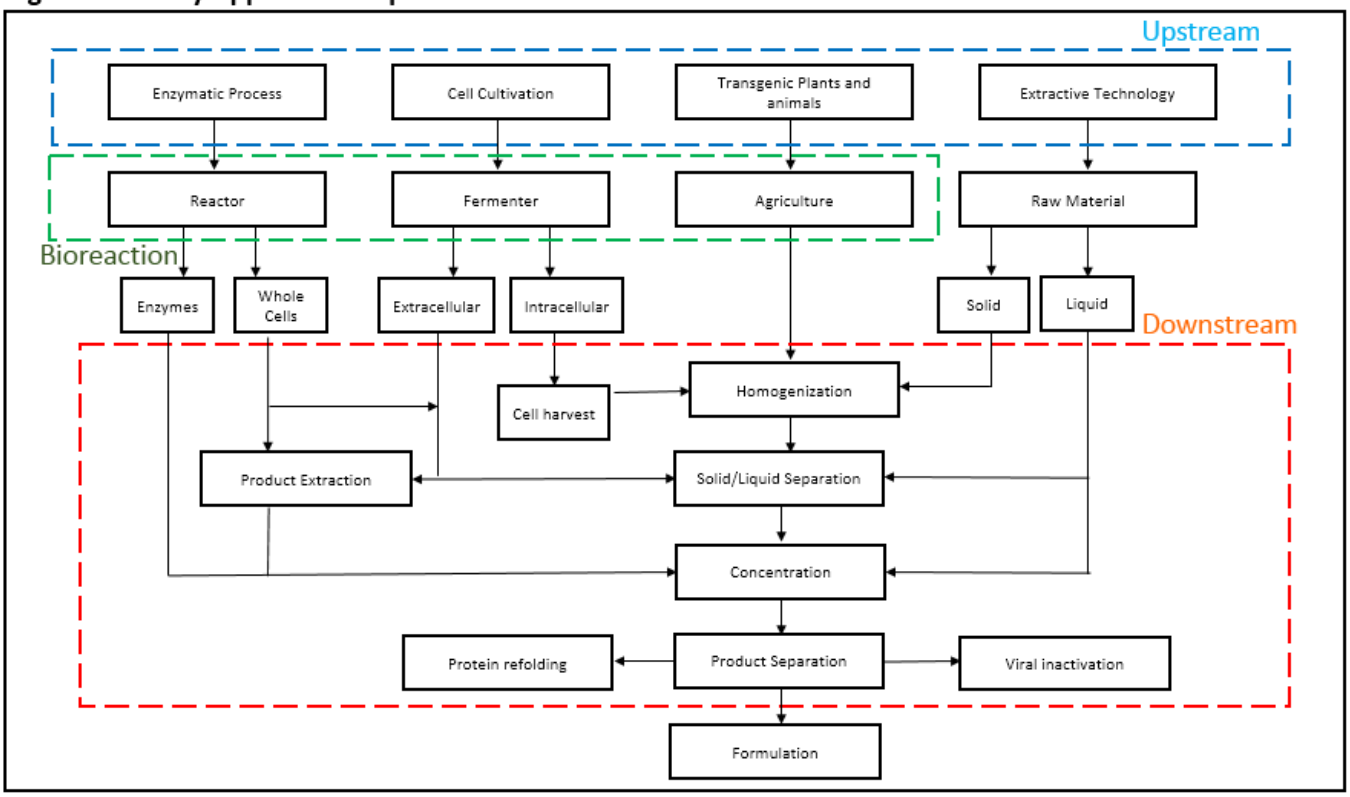
### 26.2.3 Innoculum

Upstream processing of proteins using bioreactors and cells usually begins with the preparation of the inoculum which proceeds in scale-up steps until enough inoculum is made to aseptically inoculate the final, sterile, media-filled bioreactor.

During the culture period samples are removed, aseptically, and various parameters are measured by fermentation technicians or operators including optical density (OD) and live cell count. Samples are also brought to quality control where other parameters may be measured such as the levels of glucose, lactate and ammonia, as well as the identity and concentration of the human protein that the cells are producing. Also part of upstream processing are the initial purification steps which could include centrifugation and/or filtration in order to separate cells from media. The cells or the media would be discarded to the kill tank, depending on where the protein was located. In this course we are using glass bioreactors and representative of three types of cells used in upstream processing of human protein pharmaceuticals: bacterial, animal, and fungal cells. In bacteria, such as biotechnology's workhorse, *Escherichia coli* , *Pichia pastoris* , proteins are secreted into the media so the media is saved for later isolation and purification of the protein of interest in downstream

processing. proteins remain inside the cell so the cells are separated from the media and the media is discarded to the kill tank. In animal cells, such as Chinese Hamster Ovary (CHO) cells, and in fungal cells, such as the yeast

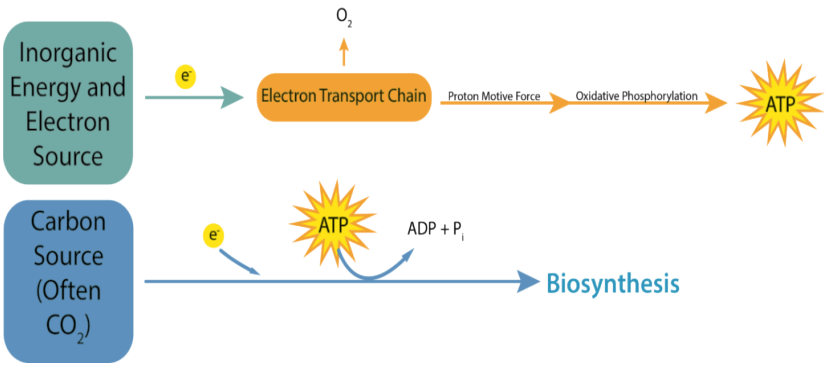
**Fig. 1: Generally Applicable Bioprocess Tree**



# UNIT –II

## Chemolithotrophy

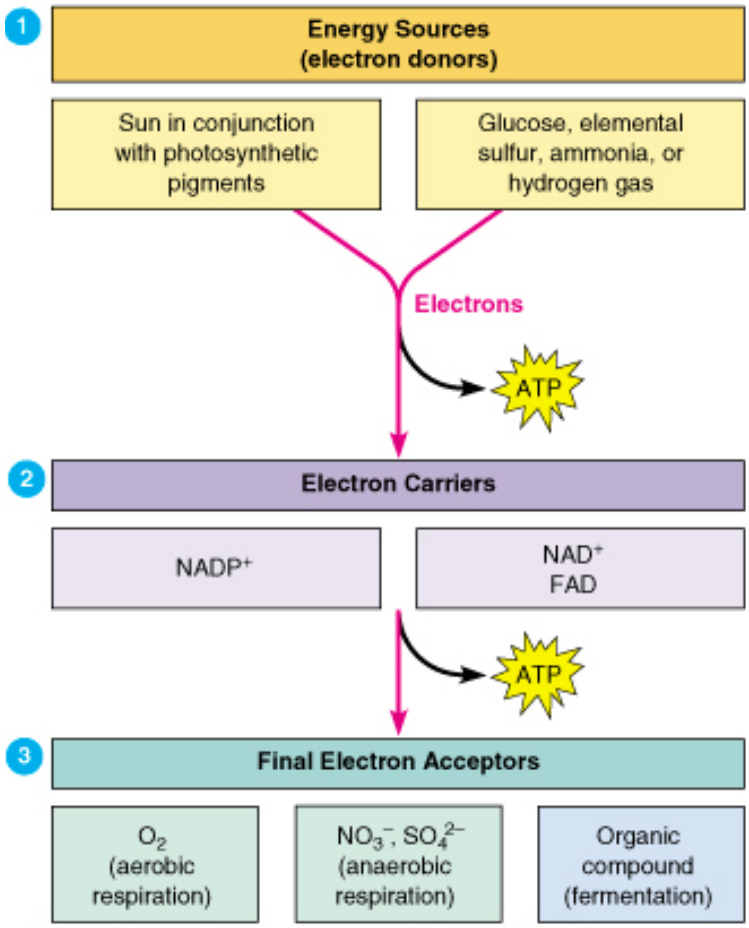
Chemolithotrophy is the oxidation of inorganic chemicals for the generation of energy. The process can use oxidative phosphorylation, just like aerobic and anaerobic respiration, but now the substance being oxidized (the electron donor) is an inorganic compound. The electrons are passed off to carriers within the electron transport chain, generating a proton motive force that is used to generate ATP with the help of ATP synthase.




## Chemolithotrophy Pathways

- Electrons donors

Chemolithotrophs use a variety of inorganic compounds as electron donors, with the most common substances being hydrogen gas, sulfur compounds (such as sulfide and sulfur), nitrogen compounds (such as ammonium and nitrite), and ferrous iron.




- Hydrogen oxidizers – these organisms oxidize hydrogen gas (H2) with the use of a hydrogenase enzyme. Both aerobic and anaerobic hydrogen oxidizers exist, with the aerobic organisms eventually reducing oxygen to water.
- Sulfur oxidizers – as a group these organisms are capable of oxidizing a wide variety of reduced and partially reduced sulfur compounds such as hydrogen sulfide (H2S), elemental sulfur (S0), thiosulfate (S2O32-), and sulfite (SO32-). Sulfate (SO42-) is frequently a by-product of the oxidation. Often the oxidation occurs in a stepwise fashion with the help of the sulfite oxidase enzyme.
- Nitrogen oxidizers – the oxidation of ammonia (NH3) is performed as a two-step process by nitrifying microbes, where one group oxidizes ammonia to nitrite (NO2-) and the second group oxidizes the nitrite to nitrate (NO3-). The entire process is known as nitrification and is performed by small groups of aerobic bacteria and archaea, often found living together in soil or in water systems.
- Iron oxidizers – these organisms oxidize ferrous iron (Fe2+) to ferric iron (Fe3+). Since Fe2+ has such a positive standard reduction potential, the bioenergetics are not extremely favorable, even using oxygen as a final electron acceptor. The situation is made more

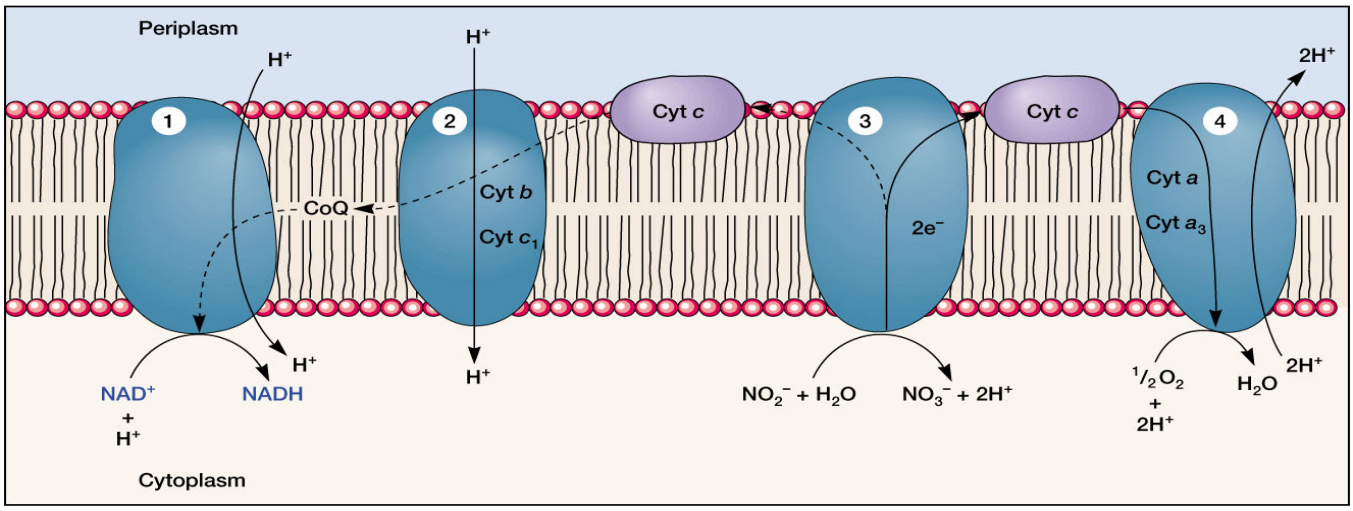
difficult for these organisms by the fact that Fe2+ spontaneously oxidizes to Fe3+ in the presence of oxygen; the organisms must use it for their own purposes before that happens.

- Electron acceptors - Chemolithotrophy can occur aerobically or anaerobically. Just as with either type of respiration, the best electron acceptor is oxygen, to create the biggest distance between the electron donor and the electron acceptor. Using a non-oxygen acceptor allows chemolithotrophs to have greater diversity and the ability to live in a wider variety of environments, although they sacrifice energy production.

**Amount of ATP generated**

Just as both the electron donors and acceptors can vary widely for this group of organisms, the amount of ATP generated for their efforts will vary widely as well. They will not make as much ATP as an organism using aerobic respiration, since the largest ΔE0' is found using glucose as an electron donor and oxygen as an electron acceptor. But how much less than 32 molecules of ATP greatly depends upon the actual donor and acceptor being used. The smaller the distance between the two, the less ATP that will be formed.

- Most can't directly reduce NAD+ to NADH (only $H_2$ oxidizers).
- NADH is needed to convert to NADPH for anabolic reactions.
- ETC must reverse electron flow from donors with more positive E'o than NADH; an energy source is needed for this "up-hill" reverse e- transfer.
- PMF is used for reversed electron flow, instead of making ATP.




**Chemolithoautotrophs vs chemolithoheterotrophs**

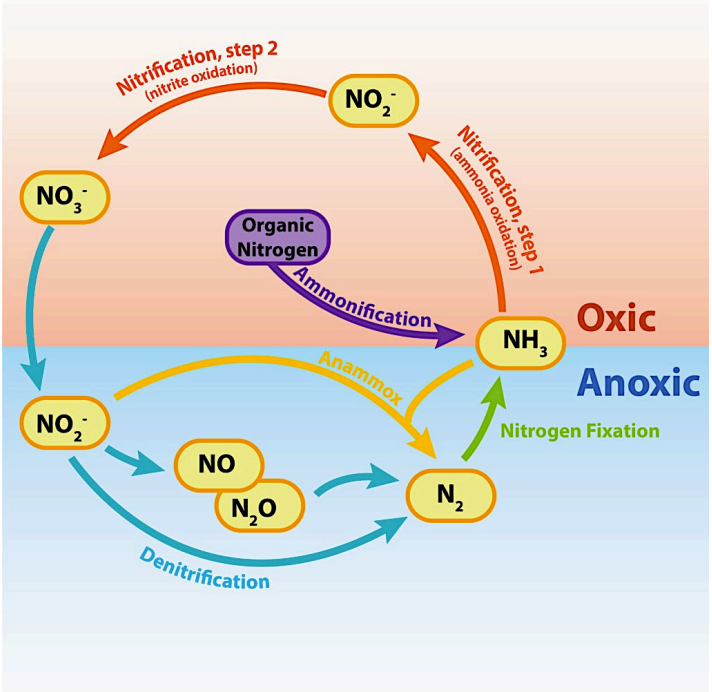
Most chemolithotrophs are autotrophs (chemolithoautotrophs), where they fix atmospheric carbon dioxide to assemble the organic compounds that they need. These organisms require both ATP and reducing power (i.e. NADH/NADPH) in order to ultimately convert the oxidized molecule CO2 into a greatly reduced organic compound, like glucose. If a chemolithoautotroph is using an electron donor with a higher redox potential than NAD+/NADP, they must use reverse electron flow to push electrons back up the electron tower. This is energetically unfavorable to the cell, consuming energy from the proton motive force to drive electrons in a reverse direction back through the ETC.

Some microbes are chemolithoheterotrophs, using an inorganic chemical for their energy and electron needs, but relying on organic chemicals in the environment for their carbon needs.

These organisms are also called mixotrophs, since they require both inorganic and chemical compounds for their growth and reproduction.

**Nitrogen Metabolism**

The nitrogen cycle depicts the different ways in which nitrogen, an essential element for life, is used and converted by organisms for various purposes. Much of the chemical conversions are performed by microbes as part of their metabolism, performing a valuable service in the process for other organisms in providing them with an alternate chemical form of the element.




**Nitrogen Cycle**

Nitrogen Fixation

Nitrogen fixation describes the conversion of the relatively inert dinitrogen gas (N2) into ammonia (NH3), a much more useable form of nitrogen for most life forms. The process is performed by diazotrophs, a limited number of bacteria and archaea that can grow without an external source of fixed nitrogen, because of their abilities. Nitrogen fixation is an essential process for Earth's organisms, since nitrogen is a required component of various organic molecules, such as amino acids and nucleotides. Plants, animals, and other organisms rely on bacteria and archaea to provide nitrogen in a fixed form, since no eukaryote is known that can fix nitrogen.

Nitrogen fixation is an extremely energy and electron intensive process, in order to break the triple bond in N2 and reduce it to NH3. It requires a particular enzyme known as nitrogenase, which is inactivated by O2. Thus, nitrogen fixation must take place in an anaerobic environment. Aerobic nitrogen-fixing organisms must devise special conditions or arrangements in order to protect their enzyme. Nitrogen-fixing organisms can either exist independently or pair up with a plant host:

1. Symbiotic nitrogen-fixing organisms: these bacteria partner up with a plant, to provide them with an environment appropriate for the functioning of their nitrogenase enzyme. The bacteria live in the plant's tissue, often in root nodules, fixing nitrogen and sharing the results. The plant provides both the location to fix nitrogen, as well as additional nutrients to support the energy-taxing process of nitrogen fixation. It has been shown that the bacteria and the host exchange chemical recognition signals that facilitate the relationship. One of the best known bacteria in this category is *Rhizobium*, which partners up with plants of the legume family (clover, soybeans, alfalfa, etc).
2. Free-living nitrogen-fixing organisms: these organisms, both bacteria and archaea, fix nitrogen for their own use that ends up being shared when the organisms dies or is ingested. Free-living nitrogen-fixing organisms that grow anaerobically do not have to worry about special adaptations for their nitrogenase enzyme. Aerobic organisms must make adaptations. Cyanobacteria, a multicellular bacterium, make specialized cells known as heterocysts in which nitrogen fixation occurs. Since Cyanobacteria produce oxygen as part of

their photosynthesis, an anoxygenic version occurs within the heterocyst, allowing the nitrogenase to remain active. The heterocysts share the fixed nitrogen with surrounding cells, while the surrounding cells provide additional nutrients to the heterocysts.

**Assimilation**

Assimilation is a reductive process by which an inorganic form of nitrogen is reduced to organic nitrogen compounds such as amino acids and nucleotides, allowing for cellular growth and reproduction. Only the amount needed by the cell is reduced. Ammonia assimilation occurs when the ammonia ($NH_3$)/ammonium ion ($NH_4^+$) formed during nitrogen fixation is incorporated into cellular nitrogen. Assimilative nitrate reduction is a reduction of nitrate to cellular nitrogen, in a multi-step process where nitrate is reduced to nitrite then ammonia and finally into organic nitrogen.

**Nitrification**

As mentioned above, nitrification is performed by chemolithotrophs using a reduced or partially reduced form of nitrogen as an electron donor to obtain energy. ATP is gained by the process of oxidative phosphorylation, using a ETC, PMF, and ATP synthase.

**Denitrification**

Denitrification refers to the reduction of $NO_3^-$ to gaseous nitrogen compounds, such as $N_2$. Denitrifying microbes perform anaerobic respiration, using $NO_3^-$ as an alternate final electron acceptor to $O_2$. This is a type of dissimilatory nitrate reduction where the nitrate is being reduced during energy conservation, not for the purposes of making organic compounds. This produces large amounts of excess byproducts, resulting in the loss of nitrogen from the local environment to the atmosphere.

**Anammox**

Anammox or *an*aerobic *amm*onia *ox*idation is performed by marine bacteria, relatively recently discovered, that utilize nitrogen compounds as both electron acceptor and electron donor. Ammonia is oxidized anaerobically as the electron donor while nitrite is utilized as the electron acceptor, with dinitrogen gas produced as a byproduct. The reactions occur within the anammoxosome, a specialized cytoplasmic structure which constitutes 50-70% of the total cell volume. Just like denitrification, the anammox reaction removes fixed nitrogen from a local environment, releasing it to the atmosphere.

**Eukaryote Photosynthesis**

Photosynthesis in plants and algae takes place in chloroplasts and entails two steps:

- Energy transferring (energy-transduction) reactions (commonly called the light-dependent or light reactions)
- Carbon fixation reactions (sometimes inappropriately called the dark reactions)

**Step one: Energy transfer**

The energy transferring reactions are photochemical processes that take place in two physically separate but chemically linked photosystems: Photosystem I (PsI) and Photosystem II (PsII). Photosystems are pigment molecules that capture energy from the sun and are arranged in the thylakoid membranes of the chloroplasts. The chlorophyll and other pigments of both

photosystems absorb light energy, most of which is stored temporarily in energy-rich chemical bonds of ATP (adenosine triphosphate) and the electron carrier NADPH (reduced nicotinamide adenine dinucleotide phosphate). ATP and NADPH supply the energy for the resultant carbon fixation reactions of step two. Oxygen ($O_2$) is a by-product of water molecules splitting in the initial energy exchanges of step one. The three products of the energy transfer phase are ATP, NADPH, and $O_2$.

**Step two: Carbon fixation**

The carbon fixation reactions of the second step of photosynthesis are biochemical and use the energy of ATP and the reducing power of NADPH to repackage the energy in a form that can be transported and stored, as the carbohydrates sugar and starch. Carbon fixation reactions do not require light; if cellular energy is available, the reactions occur.

Plants have developed three different pathways for photosynthetic carbon fixation, one basic procedure and two modifications of it.

- C3 Pathway (also called the Calvin cycle after its 1961 Nobel Prize–winning discoverer). This method is used by most common temperate zone species.
- C4 or Hatch-Slack Pathway. An additional step is added to the Calvin cycle, making it more efficient for plants structurally modified to do so. Many common grasses and tropical plants use this pathway; it is a necessary adaptation in areas of high light intensity, high temperatures or semi-aridity.
- CAM (crassulacean acid metabolism) Pathway. Another Calvin cycle modification is made by succulents and other plants growing in areas of high temperatures, high light, and low moisture (deserts especially). In this modification, carbon fixation takes place at night in a pathway similar to $C_4$ photosynthesis and, in addition, during the day carbon is fixed in the same cells using the $C_3$ pathway. This pathway is named for the family of plants, Crassulaceae, in which it was first discovered.

**Products**

The final products of carbon fixation are a disaccharide sugar, sucrose, and a polysaccharide, starch. The sucrose is formed from two monosaccharides (6-carbon or hexose sugars), glucose and fructose, joined together by an extra oxygen atom. Stored energy is transported from cell to cell in plants by the water-soluble sucrose. (In vertebrates, glucose is the transported sugar.)

Starch molecules are strings of glucose molecules too large to move through membranes, and, therefore, useful for storing energy. As energy is needed, the starch is converted to sucrose and transported. Plants build and fuel their bodies from these carbohydrates.

Two intermediate carbohydrates (manufactured before sucrose or starch) are the first detectable products in the $C_3$ and $C_4$ Pathways. In the $C_3$ Pathway the product is PGA (3-phosphoglycerate) (3 carbons), and in $C_4$ photosynthesis the first detectable product is oxaloacetate (4-carbons).

**Photosynthesis in Bacteria**

Photosynthetic bacteria have been around for longer than the Earth's atmosphere could sustain human life. It was only recently though that scientists began to unravel the mystery of how these micro-organisms execute the mechanisms of photosynthesis.

While scientists still have not been able to put all the pieces of the photosynthetic bacteria

puzzle in the right places, they are actively studying them and are gaining valuable knowledge about the way they photosynthesize and how they have evolved. In fact, they believe that these micro-organisms may have had a huge impact on why the world evolved the way it did, and may show potential for life in places deemed uninhabitable, including extreme climates like Antarctica and even other planets.

**What are photosynthetic bacteria?**

Much like the name suggests, these micro-organisms are special types of bacteria that contain light absorbing pigments and reaction centers which make them capable of converting light energy into chemical energy.

Cyanobacteria contain chlorophyll while other forms of bacteria contain bacteriochlorophyll. Although bacteriochlorophyll resembles chlorophyll, it absorbs light of a longer wavelength than chlorophyll. Bacteriochlorophyll a is the most common form of bacteriochlorophyll but other forms include b, c, d, e, f and g.

Bacteria that contain bacteriochlorophyll do not use water as an electron donor and therefore do not produce oxygen. This is known as anoxygenic photosynthesis. Cyanobacteria perform photosynthesis using water as an electron donor in a similar manner to plants. This results in the production of oxygen and is known as oxygenic photosynthesis.

**Classification of Photosynthetic Bacteria**

Oxygenic photosynthetic bacteria perform photosynthesis in a similar manner to plants. They contain light-harvesting pigments, absorb carbon dioxide, and release oxygen. Cyanobacteria or Cyanophyta are the only form of oxygenic photosynthetic bacteria known to date. There are, however, several species of Cyanobacteria. They are often blue-green in color and are thought to have contributed to the biodiversity on Earth by helping to convert the Earth's early oxygen-deficient atmosphere to an oxygen-rich environment. This transformation meant that most anaerobic organisms that thrived in the absence of oxygen eventually became extinct and new organisms that were dependent on oxygen began to emerge.

Cyanobacteria are mostly found in water but can survive on land, in rocks, and even in animal shells (or fur), and in coral. They are also known to be endosymbiont, which means they can live within the cells or body of another organism in a mutually beneficial way. Cyanobacteria also tend to live in extreme weather conditions, such as Antarctica, and are interesting to scientists because they may indicate a chance for life on other planets such as Mars.

Anoxygenic photosynthetic bacteria consume carbon dioxide but do not release oxygen. These include Green and Purple bacteria as well as Filamentous Anoxygenic Phototrophs (FAPs), Phototrophic Acidobacteria, and Phototrophic Heliobacteria. Let's look at the differences between these types of bacteria a little more closely.

Purple bacteria can be divided into two main types – the Chromatiaceae, which produce sulfur particles inside their cells, and the Ectothiorhodospiraceae, which produce sulphur particles outside their cells. They cannot photosynthesize in places that have an abundance of oxygen, so they are typically found in either stagnant water or hot sulfuric springs. Instead of using water to photosynthesize, like plants and cyanobacteria, purple sulfur bacteria use hydrogen sulfide as their reducing agent, which is why they give off sulfur rather than oxygen.

Purple bacteria are probably the most widely studied photosynthetic bacteria, being used for all sorts of scientific endeavors including theories on possible microbiological life on other planets. Purple non-sulfur bacteria do not release sulfur because instead of using hydrogen sulfide

as its reducing agent, they use hydrogen. While these bacteria can tolerate small amounts of sulfur, they tolerate much less than purple or green sulfur bacteria, and too much hydrogen sulfide is toxic to them.

Green sulfur bacteria generally do not move (non-motile), and can come in multiple shapes such as spheres, rods, and spirals. These bacteria have been found deep in the ocean near a black smoker in Mexico, where they survived off the light of a thermal vent. They have also been found underwater near Indonesia. These bacteria can survive in extreme conditions, like the other types of photosynthetic bacteria, suggesting an evolutionary potential for life in places otherwise thought uninhabitable.

Phototrophic Acidobacteria are found in a lot of soils and are fairly diverse. Some are acidophilic meaning they thrive under very acidic conditions. However, not much is known about this grouping of bacteria, because they are fairly new, the first being found in 1991.

Phototrophic Heliobacteria are also found in soils, especially water-saturated fields, like rice paddies. They use a particular type of bacteriochlorophyll, labelled g, which differentiates them from other types of photosynthetic bacteria. They are photoheterotroph, which means that they cannot use carbon dioxide as their primary source of carbon.

Green and red filamentous anoxygenic phototrophs (FAPs) were previously called green non-sulfur bacteria, until it was discovered that they could also use sulfur components to work through their processes. This type of bacteria uses filaments to move around. The color depends on the type of bacteriochlorophyll the particular organism uses. What is also unique about this form of bacteria is that it can either be photoautotrophic, meaning they create their own energy through the sun's energy; chemoorganotropic, which requires a source of carbon; or photoheterotrophic, which, as explained above, means they don't use carbon dioxide for their carbon source.

**Useful Applications for Photosynthetic Bacteria**

Photosynthetic bacteria are currently being used in various applications which include water purification, bio-fertilizers, animal feed and bioremediation of chemicals among many others. They are used in the treatment of polluted water since they can grow and utilize toxic substances such as H2S or H2S203.

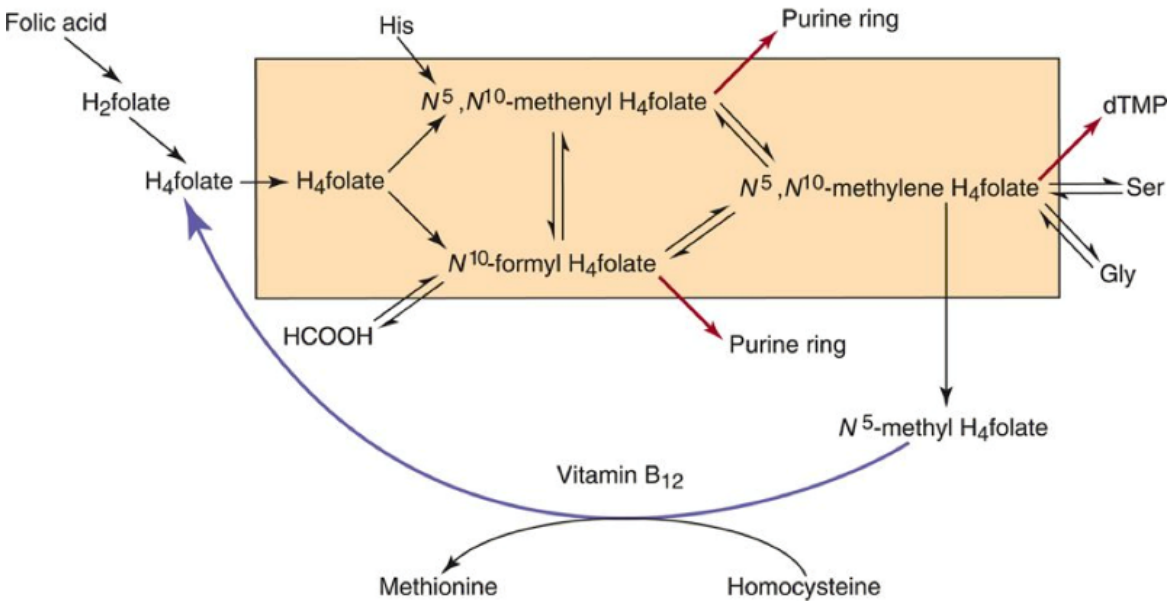
Researchers at Harvard's Wyss Institute have engineered photosynthetic bacteria to produce simple sugars and lactic acid. In the video below, Dr. Jeffrey Way explains the science behind the innovation and the potential benefits of this technology.

**One-carbon Metabolism: Basic Concepts**

There is a group of biochemical reactions that have a special set of enzymes and coenzymes. They are involved in amino acid metabolism and also play roles in nucleotide metabolism. This group of reactions is referred to as one-carbon metabolism because what they have in common is the transfer of one-carbon groups.

One-carbon metabolism exists because one-carbon groups are too volatile and need to be attached to something while being processed.

*A brief summary of the reactions involved in one-carbon metabolism is given in Devlin, Figure 26.17*




Essentially, there are three ways of moving groups of atoms containing a single carbon atom using the following molecules:

- Tetrahydrofolate (THF) as a cofactor in enzymatic reactions.
- S-adenosylmethionine (SAM) as a methyl (-$CH_3$) donor.
- Vitamin $B_{12}$ (Cobalamin) as a co-enzyme in methylation and rearrangement reactions.

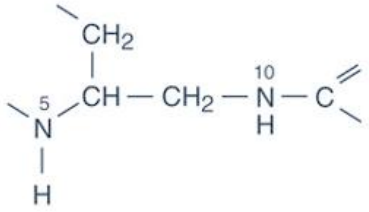
**Tetrahydrofolate (THF)**

THF is the most versatile one-carbon donor in biosynthetic reactions. THF is composed of three types of groups. THF is derived from the vitamin folic acid (folate). Folate is made by plants and microorganisms and we obtain it from our diets e.g., green leafy vegetables, beans, among others. We eat folate and use the enzyme dihydrofolate reductase to convert it into tetrahydrofolate, which is the active form that carries 1-carbon groups in a variety of reactions.

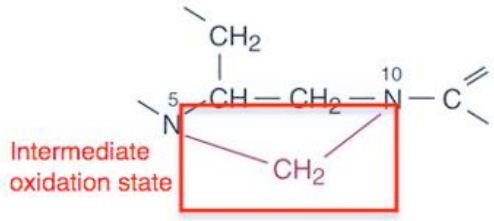
A key feature of THF is that it can carry a variety of 1-carbon groups.
These are listed in the table below:

| Oxidation State | Structure | Name |
|---|---|---|
| Most reduced | -$CH_3$ | Methyl |
| Intermediate | -$CH_2$- | Methylene |
| Most oxidized | -CHO | Formyl |
| | -CHNH | Formimino |
| | -CH= | Methenyl |

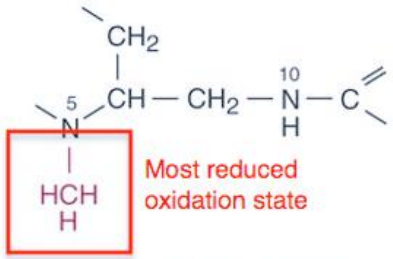
There are enzymes whose job it is to attach a 1-carbon group to THF, others to change the nature of that 1-carbon group, and others to transfer the 1-carbon group from THF onto a substrate.
One-carbon groups are being added to or removed from THF and are changing their nature while bound to THF.



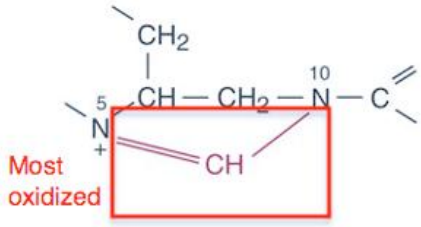
Tetrahydrofolate ($H_4$folate)



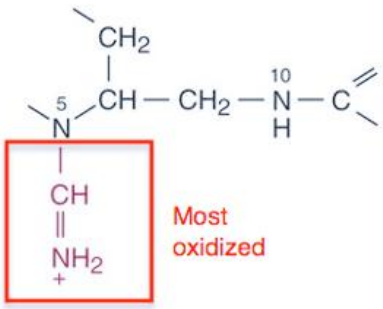

$N^5$,$N^{10}$-Methylene $H_4$folate



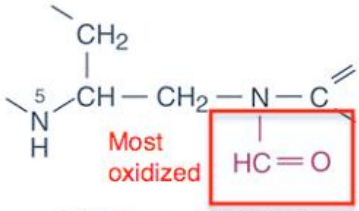

$N^5$-Methyl $H_4$folate




$N^5$,$N^{10}$-Methenyl $H_4$folate




$N^5$-Formimino $H_4$folate




$N^{10}$-Formyl $H_4$folate

**Cyanobacteria photosynthesis**

Photosynthesis

While contemporary cyanobacteria are linked to the plant kingdom as descendants of the progenitor of the endosymbiotic chloroplast, there are several features which are unique to this group.

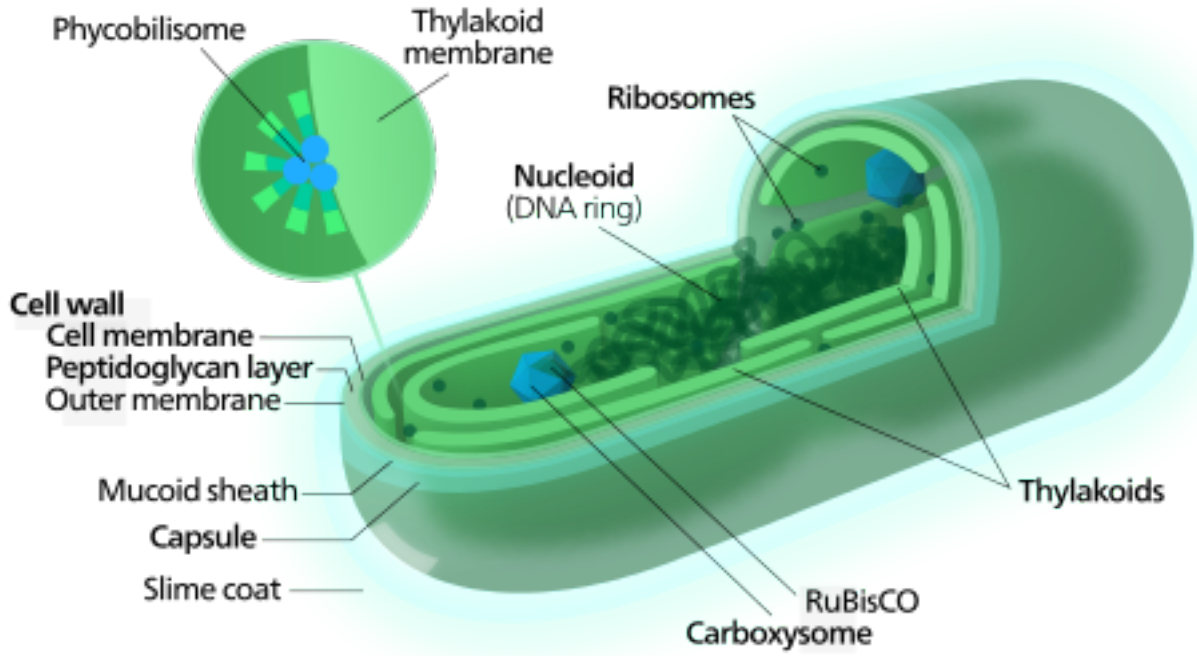
**Carbon fixation**

Cyanobacteria use the energy of sunlight to drive photosynthesis, a process where the energy of light is used to synthesize organic compounds from carbon dioxide. Because they are aquatic organisms, they typically employ several strategies which are collectively known as a "carbon concentrating mechanism" to aid in the acquisition of inorganic carbon ($CO_2$ or bicarbonate). Among the more specific strategies is the widespread prevalence of the bacterial microcompartments known as carboxysomes. These icosahedral structures are composed of hexameric shell proteins that assemble into cage-like structures that can be several hundreds of nanometers in diameter. It is believed that these structures tether the $CO_2$-fixing enzyme, RuBisCO, to the interior of the shell, as well as the enzyme carbonic anhydrase, using metabolic channeling to enhance the local $CO_2$ concentrations and thus increase the efficiency of the RuBisCO enzyme.

Electron transport.

In contrast to purple bacteria and other bacteria performing anoxygenic photosynthesis,

thylakoid membranes of cyanobacteria are not continuous with the plasma membrane but are separate compartments. The photosynthetic machinery is embedded in the thylakoid membranes, with phycobilisomes acting as light-harvesting antennae attached to the membrane, giving the green pigmentation observed (with wavelengths from 450 nm to 660 nm) in most cyanobacteria.




While most of the high-energy electrons derived from water are used by the cyanobacterial cells for their own needs, a fraction of these electrons may be donated to the external environment via electrogenic activity.

**Respiration**

Respiration in cyanobacteria can occur in the thylakoid membrane alongside photosynthesis, with their photosynthetic electron transport sharing the same compartment as the components of respiratory electron transport. While the goal of photosynthesis is to store energy by building carbohydrates from $CO_2$, respiration is the reverse of this, with carbohydrates turned back into $CO_2$ accompanying energy release.

Cyanobacteria appear to separate these two processes with their plasma membrane containing only components of the respiratory chain, while the thylakoid membrane hosts an interlinked respiratory and photosynthetic electron transport chain. Cyanobacteria use electrons from succinate dehydrogenase rather than from NADPH for respiration.

**Electron transport chain**

Many cyanobacteria are able to reduce nitrogen and carbon dioxide under aerobic conditions, a fact that may be responsible for their evolutionary and ecological success. The water-oxidizing photosynthesis is accomplished by coupling the activity of photosystem (PS) II and I (Z-scheme). In contrast to green sulfur bacteria which only use one photosystem, the use of water as an electron donor is energetically demanding, requiring two photosystems.

Attached to the thylakoid membrane, phycobilisomes act as light-harvesting antennae for the photosystems. The phycobilisome components (phycobiliproteins) are responsible for the blue-green pigmentation of most cyanobacteria. The variations on this theme are due mainly to carotenoids and phycoerythrins that give the cells their red-brownish coloration. In some

cyanobacteria, the color of light influences the composition of the phycobilisomes. In green light, the cells accumulate more phycoerythrin, whereas in red light they produce more phycocyanin. Thus, the bacteria appear green in red light and red in green light. This process of complementary chromatic adaptation is a way for the cells to maximize the use of available light for photosynthesis.

**Metabolism**

In general, photosynthesis in cyanobacteria uses water as an electron donor and produces oxygen as a byproduct, though some may also use hydrogen sulfide a process which occurs among other photosynthetic bacteria such as the purple sulfur bacteria.

Carbon dioxide is reduced to form carbohydrates via the Calvin cycle. The large amounts of oxygen in the atmosphere are considered to have been first created by the activities of ancient cyanobacteria. They are often found as symbionts with a number of other groups of organisms such as fungi (lichens), corals, pteridophytes (*Azolla*), angiosperms (*Gunnera*), etc.

There are some groups capable of heterotrophic growth, while others are parasitic, causing diseases in invertebrates or algae (e.g., the black band disease).

# UNIT –III

**Food poisoning**

Food poisoning, also called foodborne illness, is illness caused by eating contaminated food. Infectious organisms including bacteria, viruses and parasites or their toxins are the most common causes of food poisoning.

Infectious organisms or their toxins can contaminate food at any point of processing or production. Contamination can also occur at home if food is incorrectly handled or cooked.

**Symptoms**

Food poisoning symptoms vary with the source of contamination. Most types of food poisoning cause one or more of the following signs and symptoms:

- Nausea
- Vomiting
- Watery or bloody diarrhea
- Abdominal pain and cramps
- Fever

Signs and symptoms may start within hours after eating the contaminated food, or they may begin days or even weeks later. Sickness caused by food poisoning generally lasts from a few hours to several days.

**Foodborne Illnesses**

Foodborne illnesses are infections or irritations of the gastrointestinal (GI) tract caused by food or beverages that contain harmful bacteria, parasites, viruses, or chemicals.

**What causes foodborne illnesses?**

The majority of foodborne illnesses are caused by harmful bacteria and viruses. Some parasites and chemicals also cause foodborne illnesses.

**Bacteria**

Bacteria are tiny organisms that can cause infections of the GI tract. Not all bacteria are harmful to humans. Some harmful bacteria may already be present in foods when they are purchased. Raw foods including meat, poultry, fish and shellfish, eggs, unpasteurized milk and dairy products, and fresh produce often contain bacteria that cause foodborne illnesses.

**Many types of bacteria cause foodborne illnesses. Examples include**

- *Salmonella*, a bacterium found in many foods, including raw and undercooked meat, poultry, dairy products, and seafood. *Salmonella* may also be present on egg shells and inside eggs.
- *Campylobacter jejuni (C. jejuni),* found in raw or undercooked chicken and unpasteurized milk.
- *Shigella,* a bacterium spread from person to person. These bacteria are present in the stools of people who are infected. If people who are infected do not wash their hands thoroughly after using the bathroom, they can contaminate food that they handle or prepare. Water contaminated with infected stools can also contaminate produce in the field.
- *Escherichia coli (E. coli),* which includes several different strains, only a few of which cause illness in humans. *E. coli O157:H7* is the strain that causes the most severe illness. Common sources of *E. coli* include raw or undercooked hamburger, unpasteurized fruit juices and milk, and fresh produce.
- *Listeria monocytogenes (L. monocytogenes),* which has been found in raw and undercooked meats, unpasteurized milk, soft cheeses, and ready-to-eat deli meats and hot dogs.
- *Vibrio,* a bacterium that may contaminate fish or shellfish.
- *Clostridium botulinum (C. botulinum),* a bacterium that may contaminate improperly canned foods and smoked and salted fish.

**Viruses**

Viruses are tiny capsules, much smaller than bacteria that contain genetic material. Viruses cause infections that can lead to sickness. People can pass viruses to each other. Viruses are present in the stool

or vomit of people who are infected. People who are infected with a virus may contaminate food and drinks, especially if they do not wash their hands thoroughly after using the bathroom.

Common sources of foodborne viruses include

- Food prepared by a person infected with a virus
- Shellfish from contaminated water
- Produce irrigated with contaminated water

Common foodborne viruses include

- Norovirus, which causes inflammation of the stomach and intestines
- Hepatitis A, which causes inflammation of the liver

**Parasites**

Parasites are tiny organisms that live inside another organism. *Cryptosporidium parvum* and *Giardia intestinalis* are parasites that are spread through water contaminated with the stools of people or animals who are infected. Foods that come into contact with contaminated water during growth or preparation can become contaminated with these parasites. Food preparers who are infected with these parasites can also contaminate foods if they do not thoroughly wash their hands after using the bathroom and before handling food.

*Trichinella spiralis* is a type of roundworm parasite. People may be infected with this parasite by consuming raw or undercooked pork or wild game.

**Chemicals**

Harmful chemicals that cause illness may contaminate foods such as

- Fish or shellfish, which may feed on algae that produce toxins, leading to high concentrations of toxins in their bodies. Some types of fish, including tuna and mahi mahi, may be contaminated with bacteria that produce toxins if the fish are not properly refrigerated before they are cooked or served.
- Certain types of wild mushrooms.
- Unwashed fruits and vegetables that contain high concentrations of pesticides.

**Who gets foodborne illnesses?**

Anyone can get a foodborne illness. However, some people are more likely to develop foodborne illnesses than others, including

- Infants and children
- Pregnant women and their fetuses
- Older adults
- People with weak immune systems

These groups also have a greater risk of developing severe symptoms or complications of foodborne illnesses.

**Complications of foodborne illnesses**

Foodborne illnesses may lead to dehydration, hemolytic uremic syndrome (HUS), and other complications. Acute foodborne illnesses may also lead to chronic or long lasting health problems.

**Dehydration**

When someone does not drink enough fluids to replace those that are lost through vomiting and diarrhea, dehydration can result. When dehydrated, the body lacks enough fluid and electrolytes—minerals in salts, including sodium, potassium, and chloride—to function properly. Infants, children, older adults, and people with weak immune systems have the greatest risk of becoming dehydrated.

Signs of dehydration are

- Excessive thirst
- Infrequent urination
- Dark-colored urine

- Lethargy, dizziness, or faintness

Signs of dehydration in infants and young children are

- Dry mouth and tongue
- Lack of tears when crying
- No wet diapers for 3 hours or more
- High fever
- Unusually cranky or drowsy behavior
- Sunken eyes, cheeks, or soft spot in the skull

Severe dehydration may require intravenous fluids and hospitalization. Untreated severe dehydration can cause serious health problems such as organ damage, shock, or coma—a sleeplike state in which a person is not conscious.

**HUS**

Hemolytic uremic syndrome is a rare disease that mostly affects children younger than 10 years of age. HUS develops when *E. coli* bacteria lodged in the digestive tract make toxins that enter the bloodstream. The toxins start to destroy red blood cells, which help the blood to clot, and the lining of the blood vessels.

**Other Complications**

Some foodborne illnesses lead to other serious complications. For example, *C. botulinum* and certain chemicals in fish and seafood can paralyze the muscles that control breathing. *L. monocytogenes* can cause spontaneous abortion or stillbirth in pregnant women.

Research suggests that acute foodborne illnesses may lead to chronic disorders, including

- **Reactive arthritis,** a type of joint inflammation that usually affects the knees, ankles, or feet. Some people develop this disorder following foodborne illnesses caused by certain bacteria, including *C. jejuni* and *Salmonella*. Reactive arthritis usually lasts fewer than 6 months, but this condition may recur or become chronic arthritis.[4]
- **Irritable bowel syndrome (IBS),** a disorder of unknown cause that is associated with abdominal pain, bloating, and diarrhea or constipation or both. Foodborne illnesses caused by bacteria increase the risk of developing IBS.[5]
- **Guillain-Barré syndrome,** a disorder characterized by muscle weakness or paralysis that begins in the lower body and progresses to the upper body. This syndrome may occur after foodborne illnesses caused by bacteria, most commonly *C. jejuni.* Most people recover in 6 to 12 months.[6]

A recent study found that adults who had recovered from *E. coli O157:H7* infections had increased risks of high blood pressure, kidney problems, and cardiovascular disease.

**Microbiological Quality of Foods**

We all feel we know what is meant by quality and the difference between good quality and poor quality. One dictionary defines quality as the 'degree of excellence' possessed by a product, that is to say how good it is at serving its purpose.

**In terms of the microbiology of foods, quality comprises three aspects:**

**(1) Safety:** A food must not contain levels of a pathogen or its toxin likely to cause illness when the food is consumed.

**(2) Acceptability/Shelf-Life:** A food must not contain levels of microorganisms sufficient to render it organoleptically spoiled in an unacceptably short time.

**(3) Consistency:** A food must be of consistent quality both with respect to safety and to shelf life. The consumer will not accept products, which display large batch-to-batch variations in shelf life and is certainly not prepared to play Russian roulette with illness every time he or she eats a particular product.

**Three different types of microbiological criterion have been defined by The International Commission on Microbiological Specifications for Foods (ICMSF):**

(1) A microbiological standard is a criterion specified in a law or regulation. It is a legal requirement that foods must meet and is enforceable by the appropriate regulatory agency.

(2) A microbiological specification is a criterion applied in commerce. It is a contractual condition of acceptance that is applied by a purchaser attempting to define the microbiological quality of a product or ingredient. Failure of the supplier to meet the specification will result in rejection of the batch or a lower price.

(3) A microbiological guideline is used to monitor the microbiological acceptability of a product or process. It differs from the standard and specification in that it is more often advisory than mandatory.

**The ICMSF have also specified what should be included in a microbiological criterion as set out below:**

(1) A statement of the food to which the criterion applies. Clearly foods differ in their origin, composition, and processing; will present different microbial habitats; and will therefore pose different spoilage and public health problems.

(2) A statement of the micro-organisms or toxins of concern. These may cover both spoilage and health aspects, but decisions on what to include must be realistic and based on a sound understanding of the microbial ecology of the food in question.

(3) Details of the analytical methods to be used to detect and quantify the micro-organisms/toxins. Preferred methods for standards or specifications would be those elaborated by international bodies, although less sensitive or less reproducible methods may be used for simplicity and speed in confirming compliance with guidelines.

(4) The number and size of samples to be taken from a batch of food or from a source of concern such as a point in a processing line.

(5) The microbiological limits appropriate to the product and the number of sample results, which must conform, with these limits for the product to be acceptable.

(1) In this regard, it should be remembered that for certain foodborne pathogens such as Staphylococcus aureus or Clostridium perfringens, their mere presence does not necessarily indicate a hazard and specification of some numerical limits is necessary.

Micro-organisms are rarely distributed uniformly throughout a food, nor in fact are they usually distributed randomly. When micro-organisms are dispersed in a food material in the course of its production, some may die, some may be unable to grow and others may find themselves in micro-environments in which they can multiply. The resulting distribution, containing aggregates of cells, is described as a contagious distribution (Figure 11.1).

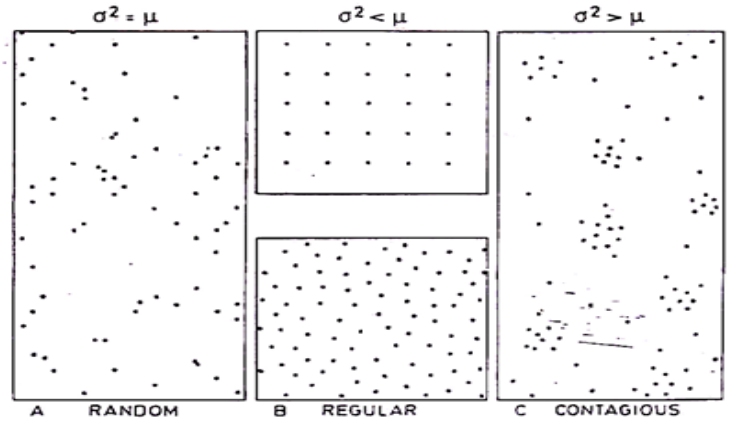



**Figure 11.1** *Possible types of spatial distribution of micro-organisms in food. $\sigma^2$, variance; μ, mean. Reproduced with permission from Jarvis (1989)*

**Codes of Good Manufacturing Practice:**

Good Manufacturing Practice (GMP) is defined as those procedures in a food-processing plant which consistently yield products of acceptable microbiological quality suitably monitored by laboratory and in-line tests.

A code of GMP must define details of the process that are necessary to achieve this goal such as times, temperatures etc., details of equipment, plant layout, disinfection (sanitation) and hygiene practices and laboratory tests.

Codes of GMP have been produced by a variety of organizations including national regulatory bodies, international organizations such as the Codex Alimentarius Commission as well as trade associations and professional bodies. They can be used by manufacturers as the basis for producing good quality product but may also be used by inspectors from regulatory bodies.

**The Hazard Analysis and Critical Control Point (HACCP) Concept:**

In the food industry today approaches based on Good Manufacturing Practice are being largely replaced by application of the Hazard Analysis Critical Control Point (HACCP) concept. This has improved on traditional practices by introducing a more systematic, rule-based approach for applying our knowledge of food microbiology to the control of microbiological quality.

The same system can also be adopted with physical and chemical factors affecting food safety or acceptability, but here we will confine ourselves to microbiological hazards. It should also be remembered that HACCP is primarily a preventative approach to quality assurance and as such it is not just a tool to control quality during processing but can be used to design quality into new products during their development.

HACCP was originally developed as part of the United States space programme by the Pillsbury Company, the National Aeronautics and Space Administration (NASA) and the US Army Natick Laboratories who used it to apply the same zero defects philosophy to food for astronauts as to other items of their equipment.

**Microbiological Examination of Foods**

The following points highlight top seven methods for the microbiological examination of foods. The methods are:-

1. Indicator Organisms
2. Direct Examination
3. Cultural Techniques
4. Enumeration Methods
5. Alternative Methods
6. Rapid Methods for the Detection of Specific Organisms and Toxins
7. Laboratory Accreditation.

**Indicator Organisms:**

It may be necessary to carry out a microbiological examination of a food for one or more of a number of reasons. The determination of the microbiological quality of a food or food constituent may be required in order to estimate its shelf-life or its suitability for human consumption.

Although an estimate of the total viable count may be desirable, it is often more useful to obtain an estimate of the numbers of a particular component of the total flora such as moulds in a cereal, psychrotrophic bacteria in a product to be stored at low temperature, anaerobes in a vacuum- packed food, or yeasts in a fruit beverage. Or it may be required to determine that a food meets established microbiological criteria.

The total mesophilic plate count is widely used as an indication of the microbiological quality of foods unless they are known to contain large numbers of bacteria as a natural consequence of their preparation such as fermented milk and meat products.

**Direct Examination:**

When examining foods, the possibility of detecting the presence of micro-organisms by looking at a sample directly under the microscope should not be missed.

A small amount of material can be mounted and teased out in a drop of water on a slide, covered with a cover slip, and examined, first with a low magnification, and then with a x 45 objective. The condenser should be set to optimize contrast even though that may result in some loss of resolution. Alternatively dark-field illumination or phase-contrast microscopy may be used. It is usually relatively easy to see yeasts and moulds and with care and patience it is possible to see bacteria in such a preparation.

**Cultural Techniques:**

Although there is clearly a place for the direct examination of a food for micro-organisms, the full microbiological examination usually requires that individual viable propagates are encouraged to multiply in liquid media or on the surface, or within the matrix, of a medium solidified with agar.

Agar is a polysaccharide with several remarkable properties which is produced by species of red algae. Although it is a complex and variable material, a major component of agar is agarose which is made of alternating units of 1,4-linked 3,6-anhydro-L-galactose (or L-galactose) and 1,3-linked D-galactose (or 6-0- methyl-D-galactose).

The properties of agar which make it so useful to microbiologists include the ability to form a gel at low concentrations (1.5-2%) which does not significantly influence the water potential of the medium. Such a gel is stable to quite high temperatures and requires a boiling water bath, or autoclave temperatures, to 'melt' it.

A very wide range of media are available to the microbiologist and details of their formulation, and how they are used, may be found in a number of readily available books and manuals. A selection of some commonly used media is listed in Table 10.2.

**Table 10.2** *A selection of media commonly used in food microbiology*

| *Medium* | *Use* |
|---|---|
| Plate Count Agar | Aerobic mesophilic count |
| MacConkey Broth | MPN of coliforms in water |
| Brilliant Green/Lactose/Bile Broth | MPN of coliforms in food |
| Violet Red/Bile/Glucose Agar | Enumeration of Enterobacteriaceae |
| Crystal Violet/Azide/Blood Agar | Enumeration of faecal streptococci |
| Baird–Parker Agar | Enumeration of *Staphylococcus aureus* |
| Rappaport–Vassiliadis Broth | Selective enrichment of *Salmonella* |
| Thiosulfate/Bile/Citrate/Sucrose Agar | Isolation of vibrios |
| Dichloran/18% Glycerol Agar | Enumeration of moulds |
| Rose Bengal/Chloramphenicol Agar | Enumeration of moulds and yeasts |

**Enumeration Methods:**

**Plate Counts:** It has already been suggested that to count micro-organisms in a food sample by direct microscopy has a limited sensitivity because of the very small sample size in the field of view at the magnification needed to see micro-organisms, especially bacteria. In a normal routine laboratory the most sensitive method of detecting the presence of a viable bacterium is to allow it to amplify itself to form a visible colony.

This forms the basis of the traditional pour plate, spread plate or Miles and Misra drop plate still widely used in microbiology laboratories. Table 10.3 compares the sample size examined and potential sensitivity of all these methods. In the pour plate method a sample (usually 1 ml) is pipetted directly into a sterile petri dish and mixed with an appropriate volume of molten agar.

**Table 10.3** *A comparison of the sensitivity of methods of enumeration*

| *Method* | *Volume of sample (ml)* | *Count (cfu $g^{-1}$) corresponding to a single organism or colony seen** |
|---|---|---|
| Direct microscopy | $5 \times 10^{-6}$ | $2 \times 10^{6}$ |
| Miles and Misra | 0.02 | $5 \times 10^{2}$ |
| Spread plate | 0.1 | $10^{2}$ |
| Pour plate | 1.0 | 10 |
| MPN | $3 \times 10.0$<br>$+ 3 \times 1$<br>$+ 3 \times 0.1$ | 0.36 |

* Based on a $10^{-1}$ dilution of a sample obtained by, for example, stomaching 1g (or ml) of food with 9 ml of diluent.

**Most Probable Number Counts:**

An alternative method of enumerating low numbers of viable micro-organisms is that referred to as the Most Probable Number (MPN) method. The method is usually based on inoculating replicate tubes of an appropriate liquid medium (usually 3, 4 or 5) with three different sample sizes or dilutions of the material to be studied (e.g. 10 g, 1.0 g and 0.1 g).

The medium used has to be designed to make it possible to decide whether growth or no growth has occurred and the number of positives at each sample size or dilution is determined after incubating the tubes. The MPN is obtained by referring to a table such as that shown in Table 10.4.

There are computer programmes for generating MPN values from different designs of the experiment and these programmes can also provide confidence limits for the MPN and suggest what the likelihood of particular combinations of positive results should be.

**Table 10.4** *A selection of MPN values**

| *Number of positive tubes* | *MPN* | *95% Confidence Limits* |
|---|---|---|
| 0 0 0 | < 0.30 | |
| 1 0 0 | 0.36 | 0.02 to 1.7 |
| 2 0 0 | 0.92 | 0.15 to 3.5 |
| 2 1 0 | 1.5 | 0.4 to 3.8 |
| 3 0 0 | 2.3 | 0.5 to 9.4 |
| 3 1 0 | 4.3 | 0.9 to 18.1 |
| 3 1 1 | 7.5 | 1.7 to 19.9 |
| 3 2 0 | 9.3 | 1.8 to 36 |
| 3 2 1 | 15 | 3.0 to 38 |
| 3 3 0 | 24 | 4.0 to 99 |
| 3 3 1 | 46 | 9.0 to 198 |
| 3 3 2 | 110 | 20.0 to 400 |
| 3 3 3 | > 110 | |

* Based on 3 × 1 g(ml) + 3 × 0.1 g(ml) + 3 × 0.01 g(ml) samples (expressed as organisms per 1 g)

A modern variation on the MPN theme is the use of the hydrophobic grid membrane filter (HGMF). A sample is filtered through the HGMF which is divided by a hydrophobic grid into a number (normally 1600) of small cells or growth compartments.

**Alternative Methods:**

Cultural methods are relatively labour intensive and require time for adequate growth to occur. Many food microbiologists also consider that the traditional enumeration methods are not only too slow but lead to an overdependence on the significance of numbers of colony-forming units.

Food manufacturers require information about the microbiological quality of commodities and raw materials rapidly and it could be argued that an assessment of microbial activity is as important as a knowledge of numbers. A number of methods have been developed which aim to give answers more quickly and hence are often referred to as 'Rapid Methods'.

**Rapid Methods for the Detection of Specific Organisms and Toxins:**

**Immunological Methods:**

Because of the potential specificity of immunoassays using polyclonal or monoclonal antibodies, there has been considerable effort devoted to developing their application in food microbiology. Commercial immunoassay kits are now available for detecting a variety of foodborne micro-organisms and their toxins, including mycotoxins.

Raising antibodies to specific surface antigens of micro-organisms, or to macromolecules such as staphylococcal or botulinum toxins, is relatively straightfor-ward and can be achieved directly. Mycotoxins, however, belong to a class of molecules known as haptens which can bind to an appropriate antibody but are of relatively low molecular weight and are not themselves immunogenic.

Haptens can be made immunogenic by binding them chemically to a carrier protein molecule, and antibodies have now been raised using this technique to a wide range of mycotoxins including the aflatoxins, trichothecenes, ochratoxin and fumonisins.

Although a number of different formats are used in immunoassays, their essential feature is the binding of antibody to antigen. A commonly used protocol is that of the sandwich ELISA (enzyme-linked immunosorbent assay) in which a capture antibody is immobilized on a solid surface of say a microtitre plate well.

**Laboratory Accreditation:**

From what has already been said it should be clear that there can be a number of different ways of detecting the same organism in a food matrix. The choice of method used can be governed by several factors and the relative merits of different methods is a topic of constant investigation and debate.

This can however lead to the situation where differences in a result reported by two laboratories simply reflect the different method used.

In addition to problems arising from intrinsic differences in the performance of different methods, the same method in different laboratories can be subject to variation introduced by factors such as differences in procedures, equipment and its calibration.

Some possible examples would include autoclave temperature profile when sterilizing media, time and temperature of incubation, sources of medium components and, of course, competence and experience of laboratory personnel.

A number of approaches are adopted to avoid such potential problems. Several national and international bodies approve standard methods for conducting certain analyses and one of these should be adopted for routine work and strictly adhered to wherever possible.

Testing laboratories also often participate in quality assurance schemes where a central body distributes standard samples for analysis, often specifying the precise time this should be conducted and the method to be used.

**Sampling**

**First decision** to make during the microbiological testing of foods is:

a. A surface sample.
b. A homogenized sample of the food.

Although most food samples will be homogenized, in some foods such as whole meat cuts where the interior is essentially sterile, a surface sample may be more significant because homogenization will include portions of food that contain no MO and dilute results.

Other applications where surface measurements are important in MO testing include food contact surfaces in a plant.

**A. SURFACE SAMPLING**

Although recovery of all microorganisms from a surface may not always be possible, the consistent monitoring of specific areas in a food plant by surface testing does provide valuable insight into the relative cleanliness of that area.

**1. Swabbing** – MO collected from a surface with sterile cotton or calcium alginate swabs (alginate swabs are the best since the alginate can be readily dissolved in hexametaphosphate), transferred to broth where they are dislodged, then diluted and used with further tests to determine total numbers. Sponges can be used to swab larger areas then placed in a buffer-filled bag.

- Advantages:
  - Easy to perform
  - Inexpensive
  - Well suited to flexible, uneven and heavily contaminated surfaces
- Disadvantage:
  - MO recovery may be poor (10% in some studies, but even that is still acceptable for many applications)

**2. Contact plates** (Rodac plates) – raised agar plate that is pressed against a surface and then incubated.

- Advantages:
  - Method of choice for smooth, firm and nonporous surfaces (e.g. vat in a cheese plant)
  - Any type of media can be used
- Disadvantages:
  - Colony overgrowth makes enumeration difficult on heavily contaminated surfaces
  - Only removes about 0.1% of contact flora - much less than swabs

A modified version of this technique is the agar syringe or "sausage." Tube full of agar, samples are pressed against a surface and then sliced off into a Petri plate for incubation.

**3. Excision method** – a plug of know surface area is taken from the food and then 1-3 mm is taken from the surface end, homogenized and plated to determine total numbers. Commonly used in whole meat cuts.

**Food Preservation**

Food preservation is the process of treating and handling food to stop or greatly slow down spoilage (loss of quality, edibility or nutritive value) caused or accelerated by micro-organisms. Preservation usually involves preventing the growth of bacteria, fungi, and other micro-organisms, as well as retarding the oxidation of fats which cause rancidity. It also includes processes to inhibit natural ageing and discolouration that can occur during food preparation such as the enzymatic browning reaction in apples after they are cut. Preservative for food may be defined as any chemical compound and/or process, when applied to food, retard alterations caused by the growth of microorganisms or enable the physical properties, chemical composition and nutritive value to remain unaffected by microbial growth.

**Principles of Food Preservation-Physical Methods**

1) ***Principle of Asepsis –*** As far as possible we should make sure that microorganism do not enter the food. The methods using this principle are: use of salt, sugar, oil, and vinegar as preservatives, use of nitrogen gas as a medium in canned foods.

2) ***Removal of Moisture –*** Microorganism as well as enzymes require presence of moisture for their growth and action. If the moisture content is reduced, the growth of microorganism and the activity of enzymes can be arrested to a great extent e.g. drying of fruits and vegetables, pulses cereals, milk etc.
3) ***Killing of Microorganisms and Destruction of Enzymes -***The food can be preserved by killing the microorganisms and the enzymes. The methods of food preservation using this principle are boiling, blanching, pasteurization and irradiation sterilization.
4) *Retarding the growth of microorganisms & the activity of enzymes* - The microorganisms grow rapidly at temperature from 10o – 60oC. The growth of microorganisms can be retarded at low temperature and is stopped at freezing temperature Methods using this principle are, refrigerators, freezing, use of preservatives eg. salt, vinegar, sugar and chemical preservatives.

**Advantages of Preservation**

1. ***Availability-*** Preservation of food helps in making the food available throughout the year.
2. ***Economical-*** It is economical as we preserve a particular food when it is seasonally available and cheap and use it when it is not available.
3. ***Avoid spoilage of food –*** With the help of different techniques the food is used when abundantly available and not wasted e.g. making of tomato ketchup when tomatoes are available making of mango syrup/pickle etc.
4. ***Variety in food-*** It brings variety in our food e.g. use of lime pickle, squashes, papaddum etc.
5. ***During Natural calamities -*** In natural calamities e.g. floods, draught etc. canned and tinned food are conveniently distributed and used as they are easy to carry.
6. ***Easy to Carry-*** When traveling some preserved foods are easy to carry as they cannot be spoiled e.g. chutney, pickles, packed foods, tinned foods etc.

Foods are mainly composed of biochemical compounds which are derived from plants and animals. Carbohydrates, proteins and fats are the major constituents of food. In addition, minor constituents such as minerals, vitamins, enzymes, acids, antioxidants, pigments, flavours are present. Foods are subject to physical, chemical, and biological deterioration. The major factors affecting food spoilage are

1) Growth and activities of microorganisms (bacteria, yeasts, and molds)
2) Activities of food enzymes and other chemical reactions within food itself
3) Infestation by insects, rodents
4) Inappropriate temperatures for a given food
5) Either the gain or loss of moisture
6) Reaction with oxygen
7) Light

The vast majority of instances of food spoilage can be attributed to one of two major causes: (1) the attack by microorganisms such as bacteria and molds, or (2) oxidation that causes the destruction of essential biochemical compounds and/or the destruction of plant and animal cells. Chemical and/or biochemical reactions results in decomposition of food- due to microbial growth. There is a adverse effect on appearance, flavour, texture, colour, consistence and/or nutritional quality of food.

**Principles of Food Preservation**

The principles of various methods for food preservation are as

1) Prevention or delay of microbial decomposition
   - By keeping out microorganisms (asepsis)
   - By removal of microorganisms

- By hindering the growth and activity of microorganisms (e.g. by low temperatures, drying, anaerobic conditions, or chemicals)
- By killing the microorganisms (e.g. by heat or radiation)

2) Prevention or delay of self decomposition of the food
   - By destruction or inactivation of food enzymes (by blanching)
   - By prevention or delay of chemical reactions (By using antioxidant)

**Methods of Food Preservation**

Preservation of food is achieved by application of physical, chemical and/or biological methods are as follows:

**Physical methods**

- Cooling to → Low temperature refrigeration (0 to 7°C ) - preserves for shorter period (days) → Freezing - preserves for several months
- Heating → pasteurization, cooking, sterilization etc
- Exposure to ionizing radiation → U.V., γ, etc
- Application of high pressure
- Drying → removal of water to a level which does not support the growth of microorganism

**Chemical methods**

- Quite often it is either impossible or undesirable to employ conventional physical methods of the preservation.
- In such situation one has to opt for chemical methods of preservation.
- It involves application of chemical additives which act as antimicrobial agents.

**Biological methods**: Souring (fermentation) lactic and acetic acid, e.g. cheese and cultured milk.

**Thermal treatment**

The term "thermal" refers to processes involving heat. Heating food is an effective way of preserving it because the great majority of harmful pathogens are killed at temperatures close to the boiling point of water. In this respect, heating foods is a form of food preservation comparable to that of freezing but much superior to it in its effectiveness. A preliminary step in many other forms of food preservation, especially forms that make use of packaging, is to heat the foods to temperatures sufficiently high to destroy pathogens.

In many cases, foods are actually cooked prior to their being packaged and stored. In other cases, cooking is neither appropriate nor necessary. The most familiar example of the latter situation is pasteurization. Conventional methods of pasteurization called for the heating of milk to a temperature between 145 and 149 °F (63 and 65 °C) for a period of about 30 minutes, and then cooling it to room temperature. In a more recent revision of that process, milk can also be "flash-pasteurized" by raising its temperature to about 160 °F (71 °C) for a minimum of 15 seconds, with equally successful results. A process known as ultra high pasteurization uses even higher temperatures of the order of 194 to 266 °F (90 to 130°C) for periods of a second or more.

**Low temperature**

The lower the temperature, the slower will be chemical reactions, enzyme action, and microbial growth. Each microorganism present has an optimal temperature for growth and a minimal temperature below which it cannot multiply. As the temperature drops from this optimal temperature toward the minimal, the rate of growth of the organism decreases and is slowest at the minimal temperature. Cooler temperatures will prevent growth, but slow metabolic activity may continue. Most bacteria, yeasts, and molds grow best in the temperature range 16-38$^{o}$C (except psychrotrophs). At temperatures below 10$^{o}$C,

growth is slow and becomes slower the colder it gets. The slowing of microbial activity with decreased temperatures is the principal behind refrigeration and freezing preservation.

**Drying**

One of the oldest methods of food preservation is by drying, which reduces water activity sufficiently to prevent or delay microbial growth. The term water activity is related to relative humidity. Relative humidity refers to the atmosphere surrounding a material or solution. Water activity is the ratio of vapour pressure of the solution to the vapour pressure of pure water at the same temperature. Under equilibrium conditions water activity equals RH/100. At the usual temperatures permitting microbial growth, most bacteria require a water activity as low as 0.90-1.00. Some yeasts and molds grow slowly at a water activity as low as 0.65. Food is dried either partially or completely to preserve it against microbial spoilage.

**Irradiation**

The lethal effects of radiation on pathogens has been known for many years. The radiation used for food preservation is normally gamma radiation from radioactive isotopes or machine-generated x rays or electron beams. One of the first applications of radiation for food preservation was in the treatment of various kinds of herbs and spices, an application approved by the United States Food and Drug Administration (FDA) in 1983. In 1985, the FDA extended its approval to the use of radiation for the treatment of pork as a means of destroying the pathogens that cause trichinosis. Experts predict that the ease and efficiency of food preservation by means of radiation will develop considerably in the future.

**Preservation of Food through Irradiation**

Radiation processing of food involves exposure of food to short wave radiation energy to achieve a specific purpose such as extension of shelf-life, insect disinfestation and elimination of food borne pathogens and parasites. In comparison with heat or chemical treatment, irradiation is considered a more effective and appropriate technology to destroy food borne pathogens. It offers a number of advantages to producers, processors, retailers and consumers. Radiation processing of food involves exposure of food to short wave radiation energy to achieve a specific purpose such as extension of shelf-life, insect disinfestation and elimination of food borne pathogens and parasites.

**Type of Radiation**

The type of radiation used in processing materials is limited to radiations from high energy gamma rays, X-rays and accelerated electrons. These radiations are also referred to as ionizing radiations because their energy is high enough to dislodge electrons from atoms and molecules and to convert them to electrically-charged particles called ions.

Gamma rays and X-rays, like radiowaves, microwaves, ultraviolet and visible light rays, form part of the electromagnetic spectrum and occur in the short-wavelength, high-energy region of the spectrum and have the greatest penetrating power. They have the same properties and effects on materials, their origin being the main difference between them. X-rays with varying energies are generated by machines. Gamma rays with specific energies come from the spontaneous disintegration of radionuclides.

Naturally occurring and man-made radionuclides, also called radioactive isotopes or radioisotopes, emit radiation as they spontaneously revert to a stable state. The time taken by a radionuclide to decay to half the level of radioactivity originally present is known as its half-life, and is specific for each radionuclide of a particular element. Only certain radiation sources can be used in food irradiation. These are the radionuclides cobalt-60 or cesium-137; X-ray machines having a maximum energy of five million electron volts (MeV) (an electron volt is the amount of energy gained by an electron when it is accelerated by a potential of one volt in a vacuum); or electron accelerators having a maximum energy of 10 MeV. Energies from these radiation sources are too low to induce radioactivity in any material, including food.

**Unit of Radiation Dose**

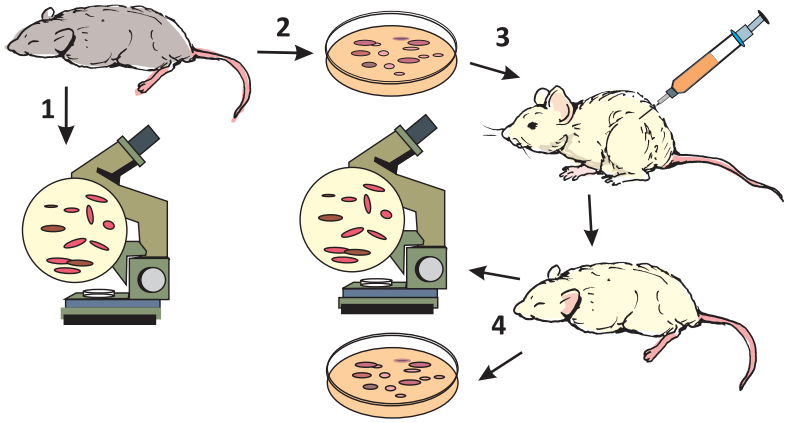
Radiation dose is the quantity of radiation energy absorbed by the food as it passes through the radiation field during processing. It is measured using a unit called the Gray (Gy). In early work the unit was the rad (1 Gy = 100 rads; 1 kGy =1000 Gy).

**Methods of food preservation on the basis of food preservation principles.**

| Physical method | Method |
|---|---|
| a) By removal of heat (Preservation by low temperature) | Refrigeration, Freezing preservation, dehydro-freezing, carbonation |
| b. By addition of heat (preservation by high temperature | Pasteurization (LTLT, HTST), sterilization, UHT Processing, microwave. |
| c. By removal of water | Drying (open sun, solar/poly tunnel solar), Dehydration (mechanical drying), Evaporation/concentration, Freeze concentration, reverse osmosis, freeze drying, foam mat drying and puff drying |
| d. By Irradiation | UV rays and gamma radiations |
| e. By non-thermal methods | High pressure processing, pulsed electric fields |
| **Chemical methods** | |
| a. By addition of acid (acetic or lactic) | Pickling (vegetable, olive, cucumber, fish, meat) |
| b. By addition of salt/brine | Salted mango/vegetable slices, salted and cured fish and meat<br>i. Dry salting<br>ii. Brining |
| c. By addition of sugar along with heating | Confectionary products like jams, jellies, preserves, candies, marmalades *etc*. |
| d. By addition of chemical preservatives. | i) Use of class II preservatives like Potassium meta-bi-sulphite, sodium benzoate, sorbic acid in food products.<br>ii) Use of permitted and harmless substances of microbial origin like tyrosine, resin, niacin as in dairy products. |
| iii. By fermentation | i. Alcoholic fermentation (wine, beer)<br>ii. Acetic acid fermentation (vinegar)<br>iii. Lactic acid fermentation (curd, cheese, pickling of vegetables). |
| iv. By combination method | i. Combination of one or more methods for synergistic preservation.<br>ii. Pasteurization combined with low temperature preservation.<br>iii. Canning: heating combined with packing in sealed container.<br>iv. Hurdle technology like low pH, salting, addition of acid, use of sugar, humectant and heating. |

*Chapter 4 - Lesson 4*

# *Host-Pathogen Interactions*




## *Introduction*

Microbes are found everywhere. On a daily basis we ingest, inhale, and transport thousands of organisms (i.e., bacterial, viral, protozoal, or parasitic). Most have no ill effects due to protective mechanisms in our body (i.e., coughing, urinating, sneezing, and defecating). Humans and animals have "friendly" organisms throughout their bodies that survive as normal flora and colonize a host but do not cause disease. However, if these organisms begin to cause any obvious damage to a host by invading tissues and/or producing toxins, they then become pathogens.

What is the significance of host-pathogen interactions? Host-pathogen interactions provide information that can help scientists and researchers understand disease pathogenesis, the biology of one or many pathogens, as well as the biology of the host. It is through these interactions that basic research discoveries are made.

## *Koch's Postulates: Experimental Steps to Determine Disease Causation*

Over a century ago, Robert Koch established that infectious diseases were caused by microbes. He was looking for the causative agent for anthrax. Koch's postulates are experimental criteria that are used to determine if a microbe caused a specific disease. The criteria include:

1. The microbe must be present in every case of the disease.
2. The organism must be grown in a pure culture from diseased hosts.
3. The same disease must be produced when a pure culture of the organism is introduced into a susceptible host.
4. The organism must be recovered from the experimentally infected hosts.

However, there are some exceptions to these criteria. These include:

1. Some organisms cannot be cultured in a lab and grown on artificial media.
2. Some pathogens can cause several disease conditions such as *M. tuberculosis,* which can cause lung disease and other diseases of the skin, bone, and internal organs.
3. There may be ethical reasons that do not allow testing, (i.e., human diseases with no animal model – smallpox, rubella).

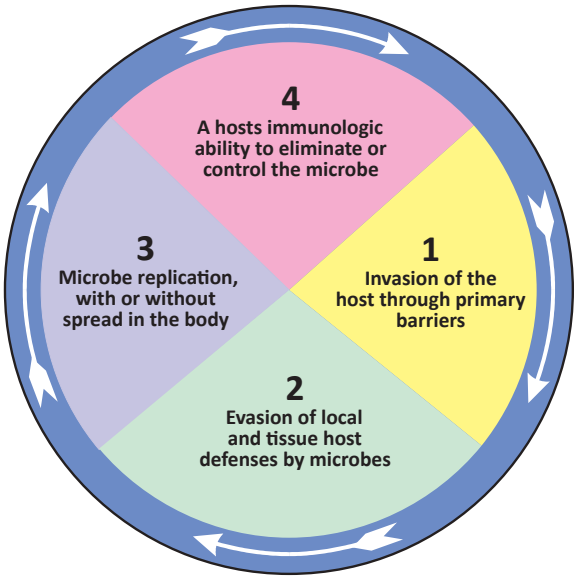
## *Host-Pathogen Interactions*

An individual's or animal's skin and mucous membranes generate an environment for microorganisms to interact with the body. This interaction between the host and the organism is referred to as symbiosis. There are three forms of symbiotic relationships that can occur at an anatomical level. In order for this to take place the following components will need to occur:

- **Mutualism:** In mutualism, both the microorganism and the body work together. An example of this relationship would be cows and the bacteria in their rumen. Bacterial cellulose facilitates digestion in the animal, while the bacteria benefit from nutrients in the rumen.
- **Commensalism:** In commensalism, either the body or the microorganism benefits, while the other is not affected by the interaction. Examples of this include microorganisms that make up the normal flora that inhabit the eyes. These organisms thrive on secretions and dead cells, but do not affect the host.
- **Parasitism:** In parasitism, one organism benefits at the expense of the other. For example, parasites use the gastrointestinal tract of a human or animal as an environment in which to reproduce.

### The Body's Normal Flora

The body contains two types of normal flora: 1) resident flora (survive for extended periods), and 2) transient flora (temporary). Normal flora help to provide defenses against invading pathogens by covering adherence sites, producing compounds toxic to other organisms, and preventing pathogens from consuming




*Components for the host pathogen interaction.*

available nutrients. For disease to occur there must be a change in the body's environment, which, in turn, allows the pathogen to overcome the normal flora. This can occur through a change in the pH of the body or elimination of normal flora due to antibiotics.

### Host Defenses

A microorganism will not be able to invade unless it overcomes an animal's or individual's host defenses. Specific host defenses may include:

- Skin and mucosal secretions
- Non-specific local responses (e.g., pH)
- Non-specific inflammatory responses
- Specific immune responses (e.g., lymphocytes)

The ability for a pathogen to overcome host defenses can be accomplished by two distinct components: a primary pathogen (causes disease in a healthy host) or opportunistic pathogen (causes disease if host is immunocompromised).

### Pathogen Defenses

Pathogens contain virulence factors that promote disease formation and provide the opportunity for a microbe to infect and cause disease. The greater the

virulence, the more likely disease will occur. Such factors include:

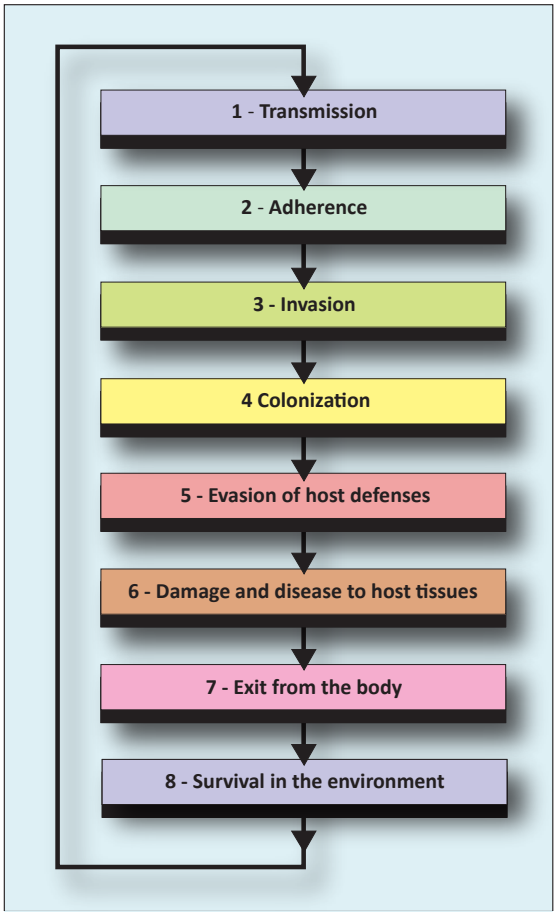
- Ability of a pathogen to adhere to a host
- Ability of a pathogen to colonize (overcome) a host
- Ability of a pathogen to evade host defenses

## *Mechanisms of Pathogenesis*

Pathogenesis is the method by which a disease can develop. This can occur through foodborne intoxication where the causative agent produces toxins in the body (e.g., botulism). Another route is the colonization of an invading pathogen on the host surface, which allows the pathogen to increase in numbers and produce toxins that are damaging to the host's cells (e.g., *Vibrio* and *Corynebacterium*).

Pathogenesis can also occur by pathogens invading and breaching the body's barrier in order to multiply. These organisms have mechanisms that will not allow macrophages (the body's defense against pathogens) to destroy them. They can also evade antibody detection (e.g., tuberculosis and plague). Finally, organisms can invade tissues within the body and produce toxins (e.g., *Shigella*).

The relationship between a host and pathogen is dynamic. Production of disease occurs through a process of steps. The first five mechanisms make up a pathogen's invasiveness (i.e., ability to invade tissues).




*Mechanisms of pathogenesis.*

**Transmission**

In order to begin infection and eventually cause disease, pathogens must find a transmission route. Transmission of an infectious agent can occur in many ways, but it is typically through exposed skin (e.g., a cut, abrasion, puncture, or wound) or mucous membranes (e.g., gastrointestinal tract, respiratory tract, or urogenital tract).

**Adherence**

Once the pathogen has gained access to the body, it must have some means of attaching itself to the host's tissues. This attachment is called adherence and is a necessary step in pathogenicity. Microbes contain ligands, which are projections that attach host receptors or surface proteins. Pathogens may have specific adherence mechanisms to attach to cells or tissue surfaces. Examples of this include: 1) tissue tropism (i.e., pathogens that prefer specific tissues over others), 2) species specificity (i.e., pathogens that only infect certain species), and 3) genetic specificity (i.e., surface mutations that occur so previous antibodies do not recognize the invading pathogen). If a microorganism cannot adhere to a host cell membrane, disease will not occur.

**Invasion**

At this point, microbes begin to invade the host and produce a bacteremia (i.e., presence of bacteria in the bloodstream) or viremia (presence of a virus in the bloodstream). Microorganisms are exposed to many barriers after introduction into the host. Some bacteria are able to cause disease while remaining on the

epithelial barriers, while many need to penetrate that barrier. Once this barrier has been penetrated, these pathogens can multiply without competition.

**Colonization**

Colonization is the multiplication of pathogenic organisms where toxins are produced and the normal flora are overcome. During this stage, pathogens compete with normal flora for space and nutrients. Pathogens usually colonize host tissues that are in contact with the external environment. During colonization, the host begins to show signs of septicemia (i.e., blood infection where bacteria are reproducing). For infection to proceed an infectious dose should be determined. This is the minimal number of microbes necessary to establish infection. Certain pathogens are less contagious and therefore require larger numbers of pathogens to cause disease (i.e., 10-100 for *Shigella* and 1,000,000 for *Salmonella*).

**Evasion of Host Defenses**

After colonization, pathogens circumvent the host's innate nd adapted defenses by phagocytosis. Multiple mechanisms are used by pathogens to evade a host's immune system. For the innate system this includes:

- Intracellular pathogens that live inside a host cell
- Avoid phagocyte recognition by producing capsules prevents phagocytosis
- Producing membrane damaging toxins which can kill phagocytes (e.g., leukocidins)
- Interfere with complement activation
- Survive in the phagocyte

Pathogens must also avoid adapted defenses. Pathogens can produce proteases (i.e., allow each pathogen to avoid antibodies) or catalases (i.e., prevents the digestion of an engulfed pathogen). They can also utilize antigenic variation to alter the antigen structure. In addition, pathogens can mimic host molecules, which can cause disease-related damage.

**Cause Damage or Disease to Host While Avoiding Host Defenses**

Damage can occur through direct or indirect pathways. Direct methods produce toxins, which are poisonous substances that produce toxemia within a host. Three types of toxins are produced to cause damage:

- **Exotoxins:** Proteins secreted by pathogens that cause damage to the host (botulinum toxin, tetanus toxin, hemolysin (ruptures red blood cells).
- **Endotoxins:** Toxic substances that are released when a cell is killed (Lipolysaccharides).
- **Exoenzymes:** Enzymes that function outside the host cells or tissues. These include coagulase (forms a fibrin clot that "hides" the microbe from phagocytosis), hyaluronidase (breaks connective tissues down), or fibrinase (breaks down blood clots to allow pathogens to continue spreading).

**Exiting the Host**

A pathogen must exit the body. This occurs through various routes. Examples include sneezing, coughing, diarrhea, coitus, pus, blood, or insect bites.

**Survival Outside the Host**

Finally, a pathogen must be able to survive in the environment long enough to be transmitted to another host. Some are hardy and can survive for several weeks before a new host is found. There are others that survive in animal reservoirs or require direct contact because they are fragile.

*Mosquito. Photo, James Gathany, CDC.*

*Pathogens may exit the body through a cough or sneeze. CDC.*

## *Infectious Diseases: The Basics*

An infectious disease is a clinically evident deviation from health. It occurs when there is a parasitic relationship between a host and a microorganism. Several different factors influence a microorganism's relationship to its host and level of severity. These include:

- **Pathogenicity:** The ability to produce disease in a host organism.
- **Virulence:** The degree of pathogenicity of a microorganism. Determinants of virulence for a pathogen include a pathogen's genetic, biochemical, or structural features. For example, one strain of influenza may only cause a fever and sore throat, while another may cause pneumonia or other serious respiratory condition.
- **Infectivity:** The level at which a microorganism is able to infect or invade a host.
- **Transmissibility:** The measure of a microorganism's ability to spread from one host to the next. This can include both distance and number of affected individuals.

## *Disease Stages*

Infections can be either subclicinical or inapparent. If clinical signs are seen, disease occurs. The incubation period is the time between infection and first appearance of signs or symptoms. This period depends on the dose level of microbes, microbe type, virulence, and host health.

The prodromal period of 1 to 2 days follows incubation in some diseases. This includes early, mild signs/symptoms such as, fatigue, muscle aches, and/or headache. Recovery occurs when signs/symptoms are subsiding but the host may remain more susceptible to secondary, opportunistic infections. During the convalescent period, the body returns to a pre-diseased state**.**

## *Conclusion*

Microorganisms are located everywhere, and there are many more microbes than hosts. The goal for both host and microbe is survival. Infection can be localized, focal, or occur systemically. Several factors affect the balance between health and infectious disease. Pathogenesis depends on genetics, the state of host health, age, and stress.

# UNIT –IV

**Infectious diseases**

Infectious diseases are caused by microorganisms, such as bacteria, viruses, fungi, and parasites. Doctors suspect an infection based on the person's symptoms, physical examination results, and risk factors. First, doctors confirm that the person has an infection rather than another type of illness. For example, a person with a cough and difficulty breathing may have pneumonia (a lung infection). However, the person may instead have asthma or heart failure, which are not caused by infection. In such a person, a chest x-ray can help doctors distinguish pneumonia from the other possible disorders.

Many different types of laboratory tests can identify microorganisms. Laboratory tests use a sample of blood, urine, sputum, or other fluid or tissue from the body. This sample may be

- Stained and examined under a microscope
- Cultured (placed in conditions that encourage the growth of microorganisms)
- Tested for antibodies (molecules produced by the person's immune system in response to the microorganism)
- Tested for a microorganism's antigens (molecules from the microorganism that can trigger an immune response in the body)
- Tested for genetic material (such as DNA or RNA) from the microorganism

No single test can identify every microorganism, and tests that work well for one microorganism often do not work well for another. Doctors must choose the test based on which microorganisms they think are most likely to cause a disorder. Sometimes several different tests are done, typically in a specific order, based on the results of the previous test. Each test further narrows the possibilities. If the right test is not done, doctors may not identify the cause of infection.

**Samples for Testing**

A sample is taken from an area of the person's body likely to contain the microorganism suspected of causing the infection. Samples may include

- Blood
- Sputum
- Urine
- Stool
- Tissue
- Cerebrospinal fluid
- Mucus from the nose, throat, or genital area

Some samples sent for testing, such as sputum, stool, and mucus from the nose or throat, normally contain many types of bacteria that do not cause disease. Doctors need to distinguish between these bacteria and those that could cause the person's illness.

Other samples come from areas that normally do not contain any microorganisms (that are sterile), such as urine, blood, or cerebrospinal fluid (the fluid that surrounds the brain and spinal cord). Finding any bacteria in such samples is abnormal as long as the area from which the sample was taken was first cleaned with an antiseptic to prevent contamination.

**Staining and Examination Using a Microscope**

Doctors sometimes can identify a microorganism simply by looking at it under a microscope.

Most samples are treated with stains. Stains are special dyes that color the microorganisms, causing them to stand out from the background. Some microorganisms have a distinctive size, shape, and stained color that enable doctors to recognize them.

However, many microorganisms look alike and cannot be distinguished using a microscope. Also, there must be enough of them, and they must be large enough to be seen with a microscope. For example, viruses cannot be identified using a microscope because they are too small.

For bacteria, doctors often first use Gram stain (a violet-colored stain). Bacteria are classified as follows:

- **Gram-positive** (they look blue because they retain the violet Gram stain)
- **Gram-negative** (they look red because they do not retain the stain)

Doctors can make some treatment decisions based on whether bacteria are gram-positive or gram-negative. In addition to Gram stain, other stains can be used depending on the microorganisms thought to be present.

**Culture of Microorganisms**

Many samples contain too few microorganisms to be seen using a microscope or to be identified using other tests. Thus, doctors usually try to grow the microorganism in a laboratory (called culture) until there are enough to identify.

The sample is placed on a sterile dish (plate) or in a test tube that contains specific nutrients to encourage growth of microorganisms. Different nutrients are used depending on which microorganism doctors suspect is causing the infection. Often, doctors add substances to the dish or test tube to stop the growth of microorganisms that do not cause the disease doctors suspect.

Many microorganisms, such as the bacteria that cause urinary tract infections or strep throat, can easily be grown in a culture. Some bacteria, such as the bacteria that cause syphilis, cannot be cultured at all. Other bacteria, such as those that cause tuberculosis, can be cultured but take weeks to grow. Some viruses can be cultured, but many cannot.

After the microorganisms are cultured, tests to identify them and to determine susceptibility and sensitivity to antimicrobial drugs are done.

T**esting of a Microorganism's Susceptibility and Sensitivity to Antimicrobial Drugs**

Although doctors know in general which antimicrobial drugs are effective against different microorganisms, microorganisms are constantly developing resistance to drugs that were previously effective. Thus, susceptibility testing is done to determine how effective various antimicrobial drugs are against the specific microorganism infecting the person. This testing helps doctors determine which drug to use for a particular person's infection.

Cultures are often used for susceptibility testing. Once a microorganism has been grown in a culture, doctors add different antimicrobial drugs to see which ones kill the microorganism. They also test how sensitive the microorganism is to a drug—that is, whether a small or a large amount of a drug is needed to kill the microorganism (sensitivity testing). If a large amount is needed to kill the microorganism in the laboratory, doctors usually do not use that drug.

Sometimes genetic testing can be used to detect genes in the microorganism that cause resistance to certain antimicrobial drugs. For example, methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria can be identified by testing for the *mecA* gene.

Because susceptibility testing occurs in the laboratory, the result does not always match what happens in the person's body when a drug is given. Factors related to the person receiving the drug can influence how effective a drug is (see also Overview of Response to Drugs). They include the following:

- How well the person's immune system is working
- How old the person is
- Whether the person has other disorders
- How the person's body absorbs and processes the drug

A**ntibiogram**

One of the tools available to infection preventionists, hospital epidemiologists and healthcare practitioners is the antibiogram. In simple terms, an antibiogram is a report that shows how susceptible strains of pathogens are to a variety of antibiotics.

As you may know, not every antibiotic works the same way. Some pathogens require one antibiotic, while others require a completely different antibiotic. However, some pathogens that used to

be susceptible to an antibiotic become gradually resistant to it, limiting the numbers of drugs that can be used to destroy it.
In today's post, we'll unpack this definition and see how these reports are used today.

**The One-Person Antibiogram**

At the patient level, a drug susceptibility report can be provided to the doctor to help choose the correct antibiotic. A sample from the patient is sent to the lab, where a technician tests it against a panel of antibiotics at various levels of concentration (to see how much of the drug is needed to kill the pathogen). Finally, the samples are observed for visible growth of the pathogen. They are looking for the **Minimum Inhibitory Concentration** (MIC), the lowest concentration of the drug that shows no pathogen growth.

Depending on the pathogen/antibiotic combination, there are predetermined levels of concentration (think of this as a "dose") required to have the pathogen labeled as "susceptible". These are called **breakpoints** and serve as a boundary between the four possible labels: Susceptible, Susceptible - Dose Dependent, Intermediate, and Resistant. The final report will give the healthcare team vital information to help them choose the best antibiotic for their patient.

The antibiogram isn't the final say on the choice, however. Physicians will still need to take into consideration the age, degree of illness, and antibiotic history for each patient before making the final decision.


**The Facility-Wide Antibiogram**

The antibiogram data does not only inform the team of one individual patient; all together, the collected antibiogram reports can be aggregated into one hospital-, network-, or even region-wide antibiogram. Studies show that antibiograms vary greatly between facilities, even those located one block apart, so the information from a local level to a regional level can provide great insight into trends and points for additional investigation.

While this report does not include the MIC, it provides the susceptibility rate of a variety of pathogens to a panel of antibiotics in the form of a percentage - the percentage of tested samples that were susceptible to that particular antibiotic.

This big-picture antibiogram helps in individual cases by bridging the gap between when the infection is sampled and when the results come back from the lab. Often, a patient will be sick enough that waiting for those results would put them at tremendous risk. The doctor needs to choose the best **empiric antibiotic therapy** - the best treatment *prior* to receiving the individual lab report. A doctor can refer to the hospital antibiogram to see the trends in how susceptible that strain is to the antibiotic choices. If the pathogen susceptibility rate to a particular antibiotic is around 80%, the doctor should select a different antibiotic. If the patient is critically ill, a susceptibility rate of 90-95% might be better. This

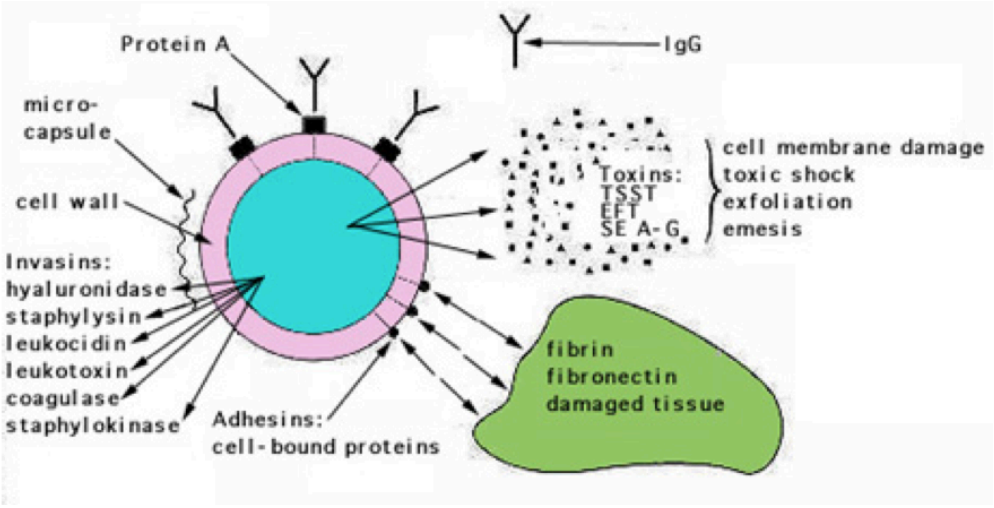
report helps the doctor make a more educated "guess" about which antibiotic will most likely eradicate their patient's infection.

In an era of growing antibiotic resistance, the use of antibiograms will surely become more prevalent. They are costly and time-consuming to create (and to update annually, as the Clinical and Laboratory Standards Institute recommends), but are becoming more and more prioritized by infection control teams. Does your facility use antibiograms? We'd love to hear how you and your team integrate this data into your daily work.

**Pathogenesis of *S Aureus* Infections**

*S aureus* expresses many cell surface-associated and extracellular proteins that are potential virulence factors. For the majority of diseases caused by this organism, pathogenesis is multifactorial. Thus it is difficult to determine precisely the role of any given factor. This also reflects the inadequacies of many animal models for staphylococcal diseases.

**Adherence:** In order to initiate infection the pathogen must gain access to the host and attach to host cells or tissues. *S aureus* cells express on their surface proteins that promote attachment to host proteins such as laminin and fibronectin that form part of the extracellular matrix (Figure 12-3). Fibronectin is present on epithelial and endothelial surfaces as well as being a component of blood clots. In addition, most strains express a fibrinogen/fibrin binding protein (the clumping factor) which promotes attachment to blood clots and traumatized tissue. Most strains of *S aureus* express fibronectin and fibrinogen-binding proteins.




**Adherence to Endothelial Cells**

*S aureus* can adhere to the surface of cultured human endothelial cells and become internalized by a phagocytosis-like process. It is not clear if attachment involves a novel receptor or a known surface protein of *S aureus*. Some researchers think that *S aureus* can initiate endocarditis by attaching to the undamaged endothelium. Others feel that trauma of even a very minor nature is required to promote attachment of bacteria.

**Avoidance of Host Defenses**

*S aureus* expresses a number of factors that have the potential to interfere with host defense mechanisms. However, strong evidence for a role in virulence of these factors is lacking.

**Capsular Polysaccharide**

The majority of clinical isolates of *S aureus* express a surface polysaccharide of either serotype 5 or 8. This has been called a microcapsule because it can be visualized only by electron microscopy after antibody labeling, unlike the copious capsules of other bacteria which are visualized by light microscopy. *S aureus* isolated from infections expresses high levels of polysaccharide but rapidly loses it upon

laboratory subculture. The function of the capsule is not clear. It may impede phagocytosis, but in in vitro tests this was only demonstrated in the absence of complement. Conversely, comparing wild-type and a capsule defective mutant strain in an endocarditis model suggested that polysaccharide expression actually impeded colonization of damaged heart valves, perhaps by masking adhesins.

**Protein A**

Protein A is a surface protein of *S aureus* which binds immunoglobulin G molecules by the Fc region. In serum, bacteria will bind IgG molecules the wrong way round by this non-immune mechanism. In principle this will disrupt opsonization and phagocytosis. Indeed mutants of *S aureus* lacking protein A are more efficiently phagocytozed in vitro, and studies with mutants in infection models suggest that protein A enhances virulence.

**Damage to the Host**

*S aureus* can express several different types of protein toxins which are probably responsible for symptoms during infections. Some damage the membranes of erythrocytes, causing hemolysis; but it is unlikely that hemolysis is relevant in vivo. The leukocidin causes membrane damage to leukocytes and is not hemolytic. Systemic release of α-toxin causes septic shock, while enterotoxins and TSST-1 cause toxic shock.

**Membrane Damaging Toxins**

**(a) α-toxin**

The best characterized and most potent membrane-damaging toxin of *S aureus* is α-toxin. It is expressed as a monomer that binds to the membrane of susceptible cells. Subunits then oligomerize to form hexameric rings with a central pore through which cellular contents leak.

Susceptible cells have a specific receptor for α-toxin which allows low concentrations of toxin to bind, causing small pores through which monovalent cations can pass. At higher concentrations, the toxin reacts non-specifically with membrane lipids, causing larger pores through which divalent cations and small molecules can pass. However, it is doubtful if this is relevant under normal physiological conditions.

**(b) β-toxin**

β-toxin is a sphingomyelinase which damages membranes rich in this lipid. The classical test for β-toxin is lysis of sheep erythrocytes. The majority of human isolates of *S aureus* do not express β-toxin. A lysogenic bacteriophage is inserted into the gene that encodes the toxin. This phenomenon is called negative phage conversion. Some of the phages that inactivate the β-toxin gene carry the determinant for an enterotoxin and staphylokinase.

In contrast the majority of isolates from bovine mastitis express β-toxin, suggesting that the toxin is important in the pathogenesis of mastitis. This is supported by the fact that β-toxin-deficient mutants have reduced virulence in a mouse model for mastitis.

**(c) δ-toxin**

The δ-toxin is a very small peptide toxin produced by most strains of *S aureus*. It is also produced by *S epidermidis* and *S lugdunensis*. The role of δ-toxin in disease is unknown.

**(d) γ-toxin and leukocidin**

The γ-toxin and the leukocidins are two-component protein toxins that damage membranes of susceptible cells. The proteins are expressed separately but act together to damage membranes. There is no evidence that they form multimers prior to insertion into membranes. The γ-toxin locus expresses three proteins. The B and C components form a leukotoxin with poor hemolytic activity, whereas the A and B components are hemolytic and weakly leukotoxic.

**E. coli**

Escherichia coli (E. coli) bacteria normally live in the intestines of healthy people and animals. Most varieties of E. coli are harmless or cause relatively brief diarrhea. But a few particularly nasty strains, such as E. coli O157:H7, can cause severe abdominal cramps, bloody diarrhea and vomiting.

**Symptoms**

Signs and symptoms of E. coli O157:H7 infection typically begin three or four days after exposure to the bacteria, though you may become ill as soon as one day after to more than a week later. Signs and symptoms include:

- Diarrhea, which may range from mild and watery to severe and bloody
- Abdominal cramping, pain or tenderness
- Nausea and vomiting, in some people

**Causes**

Among the many strains of E. coli, only a few trigger diarrhea. One group of E. coli — which includes O157:H7 — produces a powerful toxin that damages the lining of the small intestine, which can cause bloody diarrhea. You develop an E. coli infection when you ingest this strain of bacteria.

Unlike many other disease-causing bacteria, E. coli can cause an infection even if you ingest only small amounts. Because of this, you can be sickened by E. coli from eating a slightly undercooked hamburger or from swallowing a mouthful of contaminated pool water.

Potential sources of exposure include contaminated food or water and person-to-person contact.

**Contaminated food**

The most common way to acquire an E. coli infection is by eating contaminated food, such as:

- **Ground beef.** When cattle are slaughtered and processed, E. coli bacteria in their intestines can get on the meat. Ground beef combines meat from many different cattle, increasing the risk of contamination.
- **Unpasteurized milk.** E. coli bacteria on a cow's udder or on milking equipment can get into raw milk.
- **Fresh produce.** Runoff from cattle farms can contaminate fields where fresh produce is grown. Certain vegetables, such as spinach and lettuce, are particularly vulnerable to this type of contamination.

**Contaminated water**

Human and animal feces may pollute ground and surface water, including streams, rivers, lakes and water used to irrigate crops. Although public water systems use chlorine, ultraviolet light or ozone to kill E. coli, some outbreaks have been linked to contaminated municipal water supplies.

Private wells are a greater cause for concern because they don't often have any disinfecting system. Rural water supplies are the most likely to be contaminated. Some people also have been infected after swimming in pools or lakes contaminated with feces.

**Personal contact**

E. coli bacteria can easily travel from person to person, especially when infected adults and children don't wash their hands properly. Family members of young children with E. coli infection are especially likely to acquire it themselves. Outbreaks have also occurred among children visiting petting zoos and in animal barns at county fairs.

**Risk factors**

E. coli can affect anyone who is exposed to the bacteria. But some people are more likely to develop problems than are others. Risk factors include:

- **Age.** Young children and older adults are at higher risk of experiencing illness caused by E. coli and more-serious complications from the infection.
- **Weakened immune systems.** People who have weakened immune systems — from AIDS or drugs to treat cancer or prevent the rejection of organ transplants — are more likely to become ill from ingesting E. coli.

- **Eating certain types of food.** Riskier foods include undercooked hamburger; unpasteurized milk, apple juice or cider; and soft cheeses made from raw milk.
- **Time of year.** Though it's not clear why, the majority of E. coli infections in the U.S. occur from June through September.
- **Decreased stomach acid levels.** Stomach acid offers some protection against E. coli. If you take medications to reduce your levels of stomach acid, such as esomeprazole (Nexium), pantoprazole (Protonix), lansoprazole (Prevacid) and omeprazole (Prilosec), you may increase your risk of an E. coli infection.

**Complications**

Most healthy adults recover from E. coli illness within a week. Some people — particularly young children and older adults — may develop a life-threatening form of kidney failure called hemolytic uremic syndrome.

**Prevention**

No vaccine or medication can protect you from E. coli-based illness, though researchers are investigating potential vaccines. To reduce your chance of being exposed to E. coli, avoid risky foods and watch out for cross-contamination.

**Diagnosis**

To diagnose illness caused by E. coli infection, your doctor will send a sample of your stool to a laboratory to test for the presence of E. coli bacteria. The bacteria may be cultured to confirm the diagnosis and identify specific toxins, such as those produced by E. coli O157:H7.

**Treatment**

For illness caused by E. coli, no current treatments can cure the infection, relieve symptoms or prevent complications. For most people, treatment includes:

**Rest:** Fluids to help prevent dehydration and fatigue

Avoid taking an anti-diarrheal medication — this slows your digestive system down, preventing your body from getting rid of the toxins. Antibiotics generally aren't recommended because they can increase the risk of serious complications.

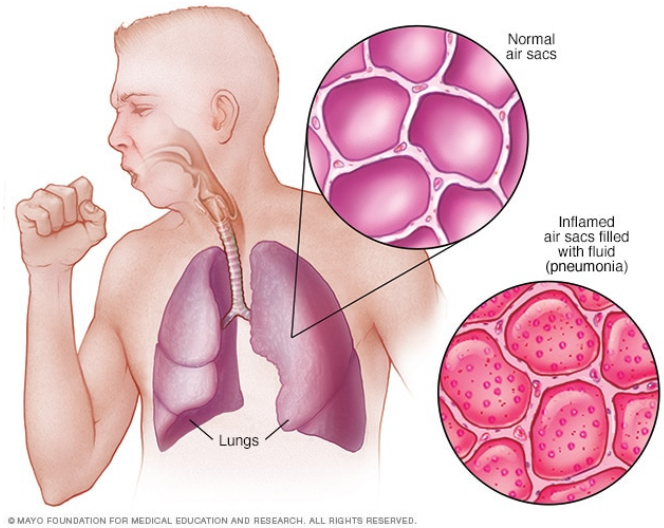
If you have a serious E. coli infection that has caused hemolytic uremic syndrome, you'll be hospitalized and given supportive care, including IV fluids, blood transfusions and kidney dialysis.

**Mycoplasma**

**Pneumonia and your lungs**

Pneumonia is an infection that inflames the air sacs in one or both lungs. The air sacs may fill with fluid or pus (purulent material), causing cough with phlegm or pus, fever, chills, and difficulty breathing. A variety of organisms, including bacteria, viruses and fungi, can cause pneumonia.

Pneumonia can range in seriousness from mild to life-threatening. It is most serious for infants and young children, people older than age 65, and people with health problems or weakened immune systems.





**Symptoms**

The signs and symptoms of pneumonia vary from mild to severe, depending on factors such as the type of germ causing the infection, and your age and overall health. Mild signs and symptoms often are similar to those of a cold or flu, but they last longer.

Signs and symptoms of pneumonia may include:

- Chest pain when you breathe or cough
- Confusion or changes in mental awareness (in adults age 65 and older)
- Cough, which may produce phlegm
- Fatigue
- Fever, sweating and shaking chills
- Lower than normal body temperature (in adults older than age 65 and people with weak immune systems)
- Nausea, vomiting or diarrhea
- Shortness of breath

Newborns and infants may not show any sign of the infection. Or they may vomit, have a fever and cough, appear restless or tired and without energy, or have difficulty breathing and eating.

**Causes**

Many germs can cause pneumonia. The most common are bacteria and viruses in the air we breathe. Your body usually prevents these germs from infecting your lungs. But sometimes these germs can overpower your immune system, even if your health is generally good.

Pneumonia is classified according to the types of germs that cause it and where you got the infection.

**Community-acquired pneumonia**

Community-acquired pneumonia is the most common type of pneumonia. It occurs outside of hospitals or other health care facilities. It may be caused by:

- **Bacteria.** The most common cause of bacterial pneumonia in the U.S. is Streptococcus pneumoniae. This type of pneumonia can occur on its own or after you've had a cold or the flu. It may affect one part (lobe) of the lung, a condition called lobar pneumonia.
- **Bacteria-like organisms.** Mycoplasma pneumoniae also can cause pneumonia. It typically produces milder symptoms than do other types of pneumonia. Walking pneumonia is an informal name given to this type of pneumonia, which typically isn't severe enough to require bed rest.
- **Fungi.** This type of pneumonia is most common in people with chronic health problems or weakened immune systems, and in people who have inhaled large doses of the organisms. The fungi that cause it can be found in soil or bird droppings and vary depending upon geographic location.
- **Viruses.** Some of the viruses that cause colds and the flu can cause pneumonia. Viruses are the most common cause of pneumonia in children younger than 5 years. Viral pneumonia is usually mild. But in some cases it can become very serious.

**Hospital-acquired pneumonia**

Some people catch pneumonia during a hospital stay for another illness. Hospital-acquired pneumonia can be serious because the bacteria causing it may be more resistant to antibiotics and because the people who get it are already sick. People who are on breathing machines (ventilators), often used in intensive care units, are at higher risk of this type of pneumonia.

**Health care-acquired pneumonia**

Health care-acquired pneumonia is a bacterial infection that occurs in people who live in long-term care facilities or who receive care in outpatient clinics, including kidney dialysis centers. Like hospital-acquired pneumonia, health care-acquired pneumonia can be caused by bacteria that are more resistant to antibiotics.

**Aspiration pneumonia**

Aspiration pneumonia occurs when you inhale food, drink, vomit or saliva into your lungs. Aspiration is more likely if something disturbs your normal gag reflex, such as a brain injury or swallowing problem, or excessive use of alcohol or drugs.

**Risk factors**

Pneumonia can affect anyone. But the two age groups at highest risk are:

- Children who are 2 years old or younger
- People who are age 65 or older

Other risk factors include:

**Prevention**

To help prevent pneumonia:

- **Get vaccinated.** Vaccines are available to prevent some types of pneumonia and the flu. Talk with your doctor about getting these shots. The vaccination guidelines have changed over time so make sure to review your vaccination status with your doctor even if you recall previously receiving a pneumonia vaccine.
- **Make sure children get vaccinated.** Doctors recommend a different pneumonia vaccine for children younger than age 2 and for children ages 2 to 5 years who are at particular risk of pneumococcal disease. Children who attend a group child care center should also get the vaccine. Doctors also recommend flu shots for children older than 6 months.
- **Practice good hygiene.** To protect yourself against respiratory infections that sometimes lead to pneumonia, wash your hands regularly or use an alcohol-based hand sanitizer.
- **Don't smoke.** Smoking damages your lungs' natural defenses against respiratory infections.
- **Keep your immune system strong.** Get enough sleep, exercise regularly and eat a healthy diet.

**Diagnosis**

If pneumonia is suspected, your doctor may recommend the following tests:

- **Blood tests.** Blood tests are used to confirm an infection and to try to identify the type of organism causing the infection. However, precise identification isn't always possible.
- **Chest X-ray.** This helps your doctor diagnose pneumonia and determine the extent and location of the infection. However, it can't tell your doctor what kind of germ is causing the pneumonia.
- **Pulse oximetry.** This measures the oxygen level in your blood. Pneumonia can prevent your lungs from moving enough oxygen into your bloodstream.
- **Sputum test.** A sample of fluid from your lungs (sputum) is taken after a deep cough and analyzed to help pinpoint the cause of the infection.

Your doctor might order additional tests if you're older than age 65, are in the hospital, or have serious symptoms or health conditions. These may include:

- **CT scan.** If your pneumonia isn't clearing as quickly as expected, your doctor may recommend a chest CT scan to obtain a more detailed image of your lungs.
- **Pleural fluid culture.** A fluid sample is taken by putting a needle between your ribs from the pleural area and analyzed to help determine the type of infection.

**Treatment**

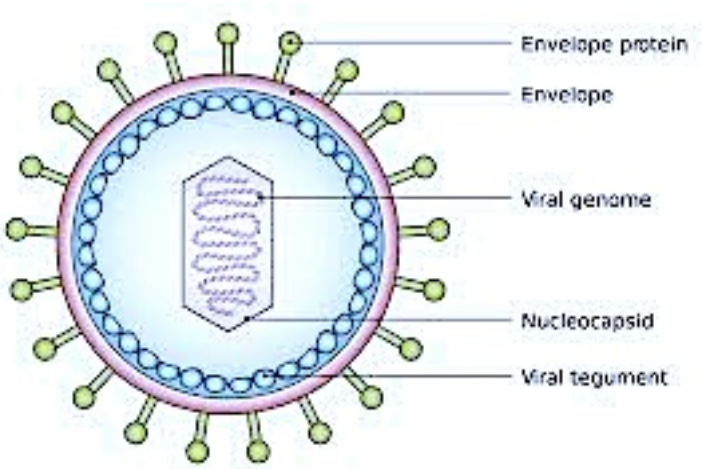
Specific treatments depend on the type and severity of your pneumonia, your age and your overall health. The options include:

- **Antibiotics.** These medicines are used to treat bacterial pneumonia. It may take time to identify the type of bacteria causing your pneumonia and to choose the best antibiotic to treat it. If your symptoms don't improve, your doctor may recommend a different antibiotic.
- **Cough medicine.** This medicine may be used to calm your cough so that you can rest. Because coughing helps loosen and move fluid from your lungs, it's a good idea not to eliminate your cough completely. In addition, you should know that very few studies have looked at whether over-the-counter cough medicines lessen coughing caused by pneumonia. If you want to try a cough suppressant, use the lowest dose that helps you rest.
- **Fever reducers/pain relievers.** You may take these as needed for fever and discomfort. These include

drugs such as aspirin, ibuprofen (Advil, Motrin IB, others) and acetaminophen (Tylenol, others).

**Viral Diseases**

A virus is a minute, contagious organism that replicates only when present inside the living cell of another organism. A very interesting character of viruses is that they can cause infections in all types of living being including humans, animals, and plants.When not inside a cell, virus exists as independent particles and these particles are mainly made of a genetic material such as RNA or DNA. This is why viruses have been defined to be at the edge of life.




**How are viruses different from bacteria?**

Even though they can both make us sick, bacteria and viruses are very different at the biological level. Bacteria are small and single-celled, but they are living organisms that do not depend on a host cell to reproduce. Because of these differences, bacterial and viral infections are treated very differently. For instance, antibiotics are only helpful against bacteria, not viruses.

**The structure of a virus**

There are a lot of different viruses in the world. So, viruses vary a ton in their sizes, shapes, and life cycles. If you're curious just how much, I recommend playing around with the ViralZone website. Click on a few virus names at random, and see what bizarre shapes and features you find!

Viruses do, however, have a few key features in common. These include:

- A protective protein shell, or **capsid**
- A nucleic acid genome made of DNA or RNA, tucked inside of the capsid
- A layer of membrane called the **envelope** (some but not all viruses)

The exterior layer is a membrane envelope. Inside the envelope is a protein capsid, which contains the nucleic acid genome.

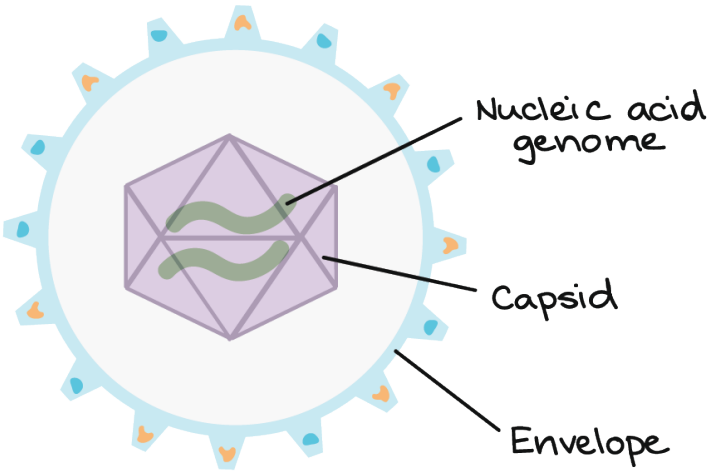
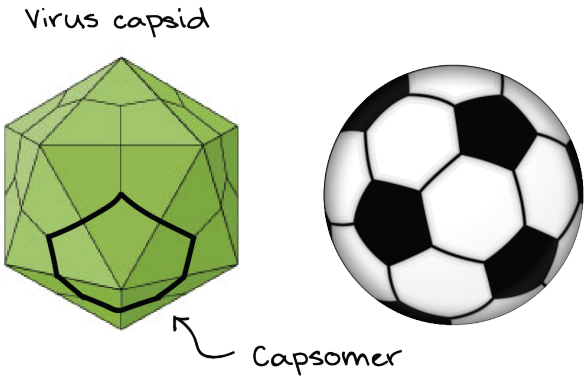


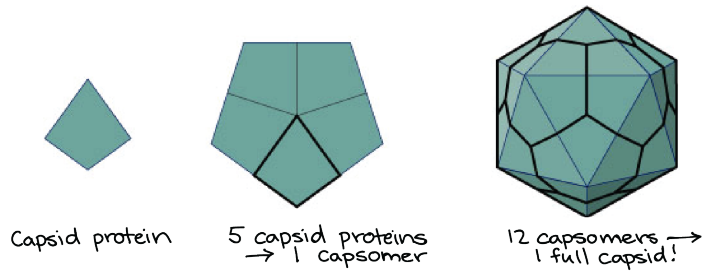

Image modified from "Scheme of a CMV virus." by Emmanuel Boutet, CC BY-SA 2.5. The modified image is licensed under a CC BY-SA 2.5 license.

**Virus capsids**

The **capsid**, or protein shell, of a virus is made up of many protein molecules (not just one big, hollow one). The proteins join to make units called**capsomers**, which together make up the capsid. Capsid proteins are always encoded by the virus genome, meaning that it's the virus (not the host cell) that provides instructions for making them.



Comparison of a soccer ball with a virus capsid. The hexagons are one type of capsomer while the pentagon are another type. Both types of capsomer are assembled from individual virus proteins.




**Capsid protein**

5 capsid proteins = 1 capsomer

12 capsomers = one full capsid

Capsids come in many forms, but they often take one of the following shapes (or a variation of these shapes):

1. **Icosahedral** – Icosahedral capsids have twenty faces, and are named after the twenty-sided shape called an icosahedron.
2. **Filamentous** – Filamentous capsids are named after their linear, thin, thread-like appearance. They may also be called rod-shaped or helical.
3. **Head-tail** –These capsids are kind of a hybrid between the filamentous and icosahedral shapes. They basically consist of an icosahedral head attached to a filamentous tail.

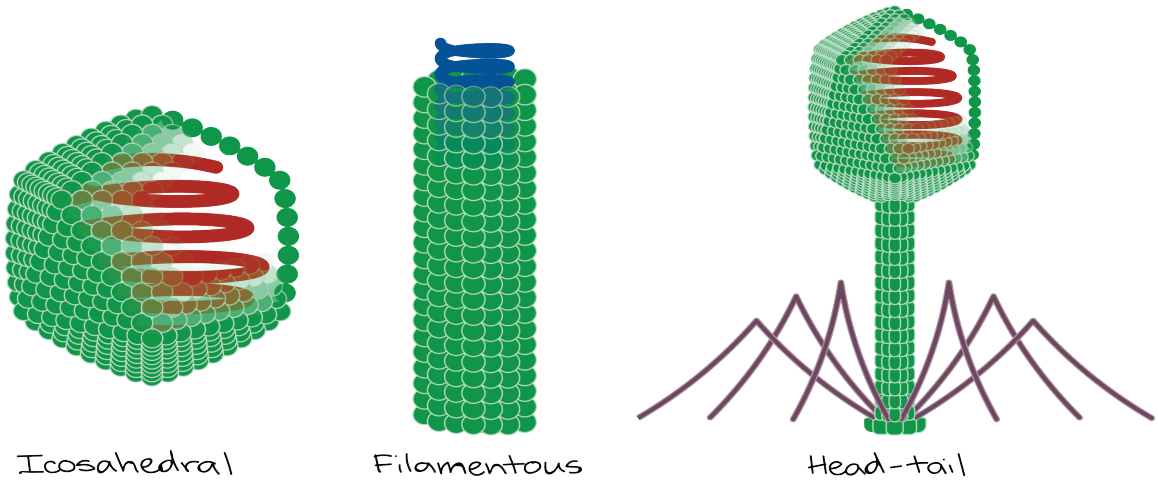
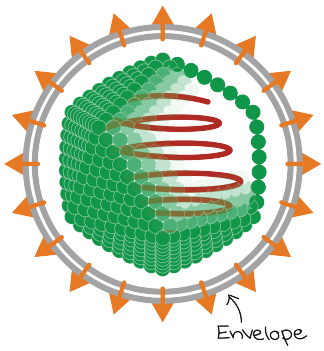



Diagram of icosahedral (roughly spherical), filamentous (rod-like), and head-tail (icosahedral head attached to filamentous tail) virus capsid shapes.

**Virus envelopes**

In addition to the capsid, some viruses also have an external lipid membrane known as an **envelope**, which surrounds the entire capsid.




Viruses with envelopes do not provide instructions for the envelope lipids. Instead, they "borrow" a patch from the host membranes on their way out of the cell. Envelopes do, however, contain proteins that are specified by the virus, which often help viral particles bind to host cells.

Although envelopes are common, especially among animal viruses, they are not found in every virus (i.e., are not a universal virus feature).

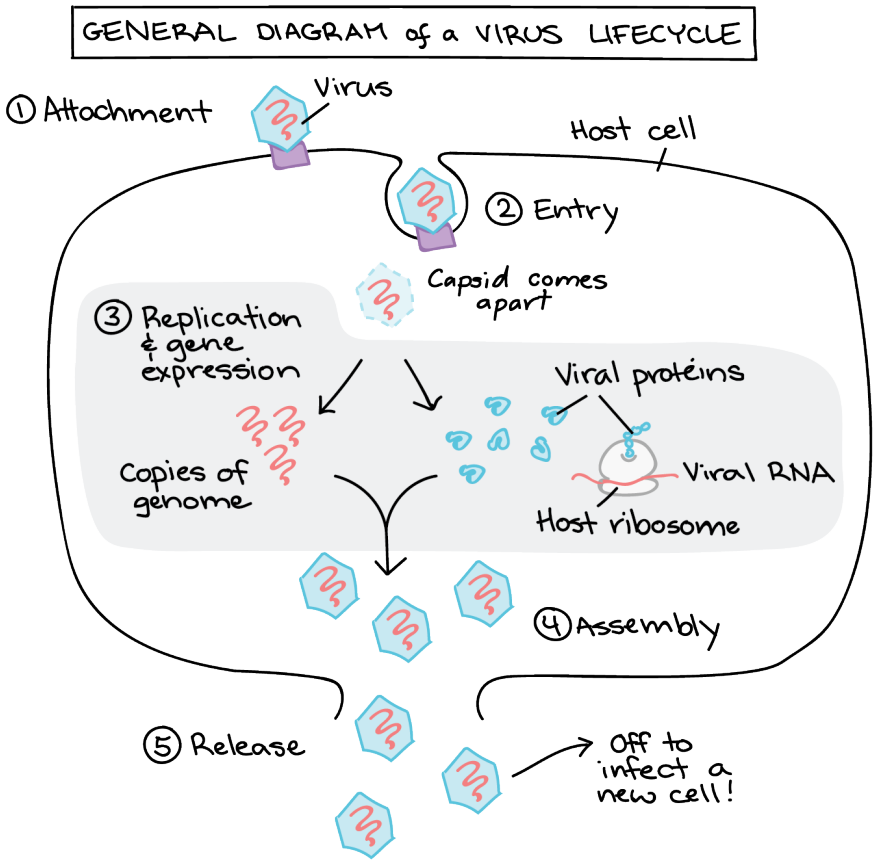
**What is a viral infection?**

In everyday life, we tend to think of a viral infection as the nasty collection of symptoms we get when catch a virus, such as the flu or the chicken pox. But what's actually happening in your body when you have a virus?

At the microscopic scale, a viral infection means that many viruses are using your cells to make more copies of themselves. The viral **lifecycle** is the set of steps in which a virus recognizes and enters a

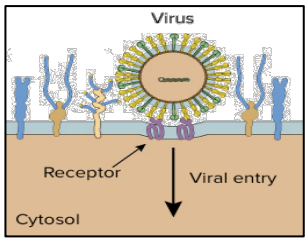
host cell, "reprograms" the host by providing instructions in the form of viral DNA or RNA, and uses the host's resources to make more virus particles (the output of the viral "program").

For a typical virus, the lifecycle can be divided into five broad steps (though the details of these steps will be different for each virus):

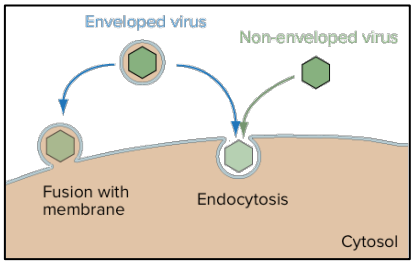


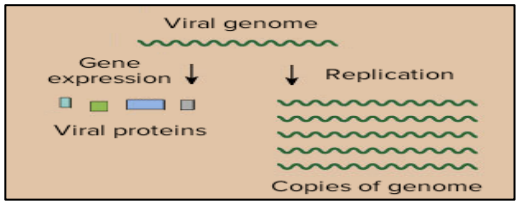

Steps of a viral infection, illustrated generically for a virus with a + sense RNA genome.

1. Attachment. Virus binds to receptor on cell surface.
2. Entry. Virus enters cell by endocytosis. In the cytoplasm, the capsid comes apart, releasing the RNA genome.
3. Replication and gene expression. The RNA genome is copied (this would be done by a viral enzyme, not shown) and translated into viral proteins using a host ribosome. The viral proteins produced include capsid proteins.
4. Assembly. Capsid proteins and RNA genomes come together to make new viral particles.
5. Release. The cell lyses (bursts), releasing the viral particles, which can then infect other host cells.



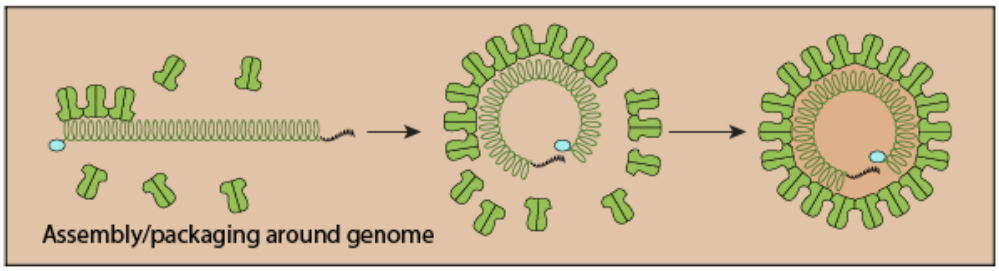

1. **Attachment.** The virus recognizes and binds to a host cell via a receptor molecule on the cell surface. Virus binding to its receptor on the cell surface.
2. **Entry**. The virus or its genetic material enters the cell. Routes of entry include endocytosis (in which the membrane folds inward to bring the virus into the cell in a bubble) and direct fusion of the viral particle with the membrane, releasing its contents into the cell.
3. **Genome replication and gene expression.** The viral genome is copied and its genes are expressed to make viral proteins.



The viral genome is copied, and its genes are also expressed to make viral proteins.

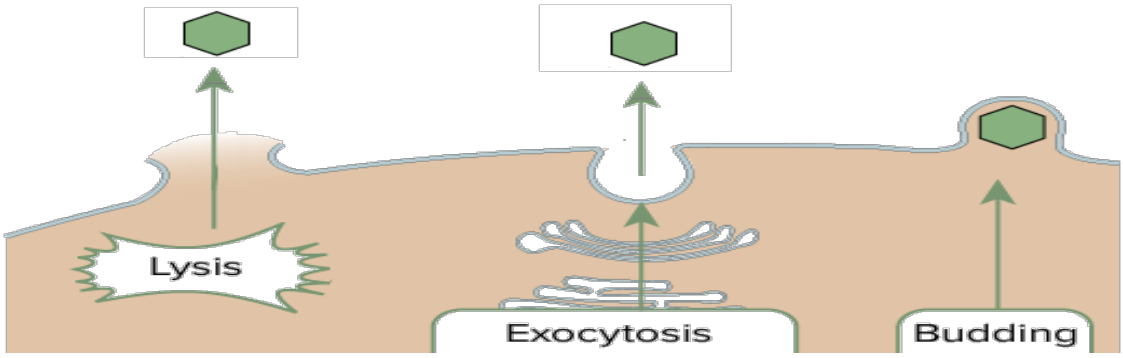
4. **Assembly.** New viral particles are assembled from the genome copies and viral proteins.




Proteins of the capsid assemble around the viral genome, forming a new viral particle with the genome on the inside (encased by the capsid).

5. **Release.** Completed viral particles exit the cell and can infect other cells.

[More about release]




Viruses may exit through lysis of the cell, exocytosis, or budding at the plasma membrane.

The diagram above shows how these steps might occur for a virus with a single-stranded RNA genome. You can see real examples of viral lifecycles in the articles on bacteriophages (bacteria-infecting viruses) and animal viruses.

**Laboratory diagnosis of viral infections**

In the diagnostic laboratory virus infections can be confirmed by a multitude of methods. Diagnostic virology has changed rapidly due to the advent of molecular techniques and increased clinical sensitivity of serological assays.

A wide variety of samples can be used for virological testing. The type of sample sent to the laboratory often depends on the type of viral infection being diagnosed and the test required. Proper sampling technique is essential to avoid potential pre-analytical errors. For example, different types of samples must be collected in appropriate tubes to maintain the integrity of the sample and stored at appropriate temperatures (usually 4°C) to preserve the virus and prevent bacterial or fungal growth. Sometimes multiple sites may also be sampled.

Types of samples include:

- Blood
- Skin
- Sputum, gargles and bronchial washings
- Urine
- Semen
- Faeces
- Cerebrospinal fluid
- Tissues (biopsies or post-mortem)
- Dried blood spots

**Virus isolation**

Viruses are often isolated from the initial patient sample. This allows the virus sample to be grown into larger quantities and allows a larger number to tests to be run on them. This is particularly important for samples that contain new or rare viruses for which diagnostic tests are not yet developed.

Many viruses can be grown in cell culture in the lab. To do this, the virus sample is mixed with cells, a process called adsorption, after which the cells become infected and produce more copies of the virus. Although different viruses often only grow in certain types of cells, there are cells that support growth of a large variety of viruses and are a good starting point, for example the African monkey kidney cell line (Vero cells), human lung fibroblasts (MRC-5), and human epidermoid carcinoma cells (HEp-2). One sign of knowing whether the cells are successfully replicating the virus is to check for a change in cell morphology or for the presence of cell deathusing a microscope.

Other viruses may require alternative methods for growth such as the inoculation of embryonated chicken eggs (e.g. avian influenza viruses) or the intracranial inoculation of virus using newborn mice (e.g. lyssaviruses).

**Nucleic acid based methods**

Molecular techniques are the most specific and sensitive diagnostic tests. They are capable of detecting either the whole viral genome or parts of the viral genome. In the past nucleic acid tests have mainly been used as a secondary test to confirm positive serological results. However, as they become cheaper and more automated, they are increasingly becoming the primary tool for diagnostics.

**Polymerase chain reaction**

Detection of viral RNA and DNA genomes can be performed using polymerase chain reaction. This technique makes many copies of the virus genome using virus-specific probes. Variations of PCR such as nested reverse transcriptase PCR and real time PCR can also be used to determine viral loads in patient serum. This is often used to monitor treatment success in HIV cases.

**Sequencing**

Sequencing is the only diagnostic method that will provide the full sequence of a virus genome. Hence, it provides the most information about very small differences between two viruses that would look the same using other diagnostic tests. Currently it is only used when this depth of information is required. For example, sequencing is useful when specific mutations in the patient are tested for in order to determine antiviral therapy and susceptibility to infection. However, as the tests are getting cheaper, faster and more automated, sequencing will likely become the primary diagnostic tool in the future.

**Microscopy based methods**

Immunofluorescence or immunoperoxidase assays are commonly used to detect whether a virus is present in a tissue sample. These tests are based on the principle that if the tissue is infected with a virus, an antibody specific to that virus will be able to bind to it. To do this, antibodies that are specific to different types of viruses are mixed with the tissue sample. After the tissue is exposed to a specific wavelength of light or a chemical that allows the antibody to be visualized.

These tests require specialized antibodies that are produced and purchased from commercial companies. These commercial antibodies are usually well characterized and are known to bind to only one specific type of virus. They are also conjugated to a special kind of tag that allows the antibody to be visualized in the lab, i.e.so that it will emit fluorescence or a color. Hence, immunofluorescence refers to the detection of a fluorescent antibody (immuno) and immunoperoxidase refers to the detection of a colored antibody (peroxidase produces a dark brown color).

**Electron microscopy**

Electron microscopy is a method that can take a picture of a whole virus and can reveal its shape and structure. It is not typically used as a routine diagnostic test as it requires a highly specialized type of sample preparation, microscope and technical expertise. However, electron microscopy is highly versatile due to its ability to analyze any type of sample and identify any type of virus. Therefore, it remains the

gold standard for identifying viruses that do not show up on routine diagnostic tests or for which routine tests present conflicting results.

**Hemagglutination assay**

Some viruses attach to molecules present on the surface of red blood cells, for example, influenza virus. A consequence of this is that – at certain concentrations – a viral suspension may bind together (agglutinate) the red blood cells thus preventing them from settling out of suspension.

**Hepatitis**

Hepatitis is an inflammation of the liver. The condition can be self-limiting or can progress to fibrosis (scarring), cirrhosis or liver cancer. Hepatitis viruses are the most common cause of hepatitis in the world but other infections, toxic substances (e.g. alcohol, certain drugs), and autoimmune diseases can also cause hepatitis.

There are 5 main hepatitis viruses, referred to as types A, B, C, D and E. These 5 types are of greatest concern because of the burden of illness and death they cause and the potential for outbreaks and epidemic spread. In particular, types B and C lead to chronic disease in hundreds of millions of people and, together, are the most common cause of liver cirrhosis and cancer.

Hepatitis A and E are typically caused by ingestion of contaminated food or water. Hepatitis B, C and D usually occur as a result of parenteral contact with infected body fluids. Common modes of transmission for these viruses include receipt of contaminated blood or blood products, invasive medical procedures using contaminated equipment and for hepatitis B transmission from mother to baby at birth, from family member to child, and also by sexual contact.

**Means of potential viral hepatitis transmission**

| Means | HAV | HBV | HCV | HDV | HEV |
|---|---|---|---|---|---|
| Food and water contamination | most common | no | no | HDV infection is linked to HBV infection. | most common |
| Blood e.g. through transfusion; contaminated instruments in healthcare settings; sharing needles among drug users | no | yes | yes | | rare |
| Sex with an infected person | rare | yes | yes | | rare |
| Mother-to-child transmission | rare | yes | yes | | rare |

**Diagnosis**

Hepatitis can be **difficult to diagnose** because it can be asymptomatic or symptoms may be non-specific.

- **Non-specific or absent symptoms (90% of cases):**
  - Pain in right hypochondrium - fever
  - Nausea and vomiting - arthralgia
  - Urticarial
- **Specific symptom (≤ 10% of cases):**
  - Icterus (jaundice)
- **Severe form: fulminant hepatitis**
  - Clinical signs: hepatic encephalopathy
  - Biological signs: prothrombin level (< 50%) ; transaminase level not correlated with the severity of fulminant hepatitis

**Viral Hepatitis: Quick Guide to Serologic Markers**

| Situation | Hepatitis | Serologic markers |
|---|---|---|
| Acute hepatitis | A, B, C, | Anti-HAV IgMHBsAg / Anti-HBc IgM /Anti-HBc Total |

| | | |
|---|---|---|
| | D, E | /HBeAg/Anti-HBe/Anti-HCV/HDV IgM, Ag Delta HEV IgM / HEV IgG |
| Chronic hepatitis | B, C | HBsAg / Anti-HBc, then HBe Ag / Anti-HBe/Anti-HCV |
| Prenatal HBV screening | B | HBsAg: if HBsAg + :<br>• Mother: monitored for HBe Ag / Anti-HBe and<br>• Newborn: quantitative anti-HBs after vaccination |
| At-risk groups | B, C | Anti-HBc<br>• if - → vaccinate<br>• if + → quantitative HBsAg / Anti-HBs<br>Anti-HCV* |
| HBV vaccination | B | Pre-vaccination: Quantitative Anti-HBs Total<br>• if - → vaccinate<br>• if + → quantitative HBsAg / Anti-HBc Total Post-vaccination: quantitative anti-HBs Total |
| HAV vaccination | A | Patient > 30 years: Total anti-HAV<br>• if - → vaccinate |

**Prevention**

The incidence of viral hepatitis is not distributed equally around the world. This is mainly due to **lack of access to prevention measures in low-resource populations and countries**. For this reason, the WHO global policy for the prevention and control of viral hepatitis aims to tailor responses to the specific national or regional context[3]. In general, this falls into alignment with essential global health strategies. For hepatitis, the most important prevention strategies are **universal access to childhood vaccination for hepatitis B**; **improved hygiene** and practices in and out of the healthcare setting; and **screening**.

**Vaccination:** Effective vaccines are available for hepatitis A and B[2,3]. The vaccine for HBV also protects against HDV, since HDV only affects populations already infected with HBV.

- Universal vaccination in childhood is showing very good results in containing the number ofHBV infections.
- Vaccination is helping to reduce mother-to-child transmissions (principal transmission for HBV)
- Awareness and vaccination programs are also part of reducing the spread among adults, in particular at-risk groups. Vaccination is compulsory for some at-risk groups in some countries

**Hygiene:** Proper hygiene is an important prevention method for all types of viral hepatitis. This includes:

- Clean drinking water and hygienic food handling
- Proper hand washing
- Safer sex
- Injection drug users: not sharing needles and needle exchange programs
- Health-care settings: fundamental infection control measures; aseptic techniques; no reuse of needles and syringes; safe injection practices

**Treatment**

Treatment for the various types of hepatitis varies greatly, as does treatment of acute versus chronic illness.

**Hepatitis A**: Usually resolves on its own. Treatment of symptoms includes rest and elimination of alcohol.

**Hepatitis B:** Acute HBV infection is not usually treated in immunocompetent adults, as it should resolve naturally.

**Current treatments for chronic HBV[5]:**

- ❖ Pegylated Interferon alpha (Peg-IFN)
- ❖ Nucleotides analogues (NAs):
- ➢ First line: Entecavir (ETV), Tenofovir( TDF)
- ➢ Second line: Adefovir, Telbivudine, Lamivudine
- ❖ Liver transplant can be considered for decompensated cirrhosis
- ❖ Therapeutic monitoring recommended during, at the end of treatment, and at a period after end of treatment

**Hepatitis C:** No specific treatment for acute HCV.

**Hepatitis D:** No specific treatment for acute HDV. See prevention and treatment of its helper virus, HBV.

**Hepatitis E:** Generally, no specific treatment for acute HEV, although treatment with Ribavirin may be effective. For immunocompromised patient, immunosuppressive treatment may be lowered. Otherwise, treatment is usually focused on relieving signs and symptoms.

**Polio Virus**

Polio, also known as poliomyelitis and infantile paralysis, is a highly contagious viral infection that can lead to paralysis, breathing problems, or even death. Polio can be classified as occurring with or without symptoms. About 95 percent of all cases are asymptomatic, and between 4 and 8 percent of cases are symptomatic.

Here are some key points about polio. More detail and supporting information is in the main article.

- Polio is caused by the poliovirus.
- The vast majority of polio infections present no symptoms.
- Polio has been eradicated in every country of the world except for Nigeria, Pakistan and Afghanistan.
- Pregnant women are more susceptible to polio.
- Around half of the people who have had polio go on to develop post-polio syndrome.

**Symptoms**

Polio, in its most severe forms, can cause paralysis and death. However, most people with polio do not display any symptoms or become noticeably sick. When symptoms do appear, they differ depending on the type of polio.

Symptomatic polio can be broken down further into a mild form, called non-paralytic or abortive polio, and a severe form called paralytic polio that occurs in around 1 percent of cases.

Many people with non-paralytic polio make a full recovery. Unfortunately, those with paralytic polio generally develop permanent paralysis.

**Non-paralytic polio symptoms**

Non-paralytic polio, also called abortive poliomyelitis, leads to flu-like symptoms that last for a few days or weeks. These include:

- ❖ Fever
- ❖ Sore throat
- ❖ Headache
- ❖ Vomiting
- ❖ Fatigue
- ❖ Back and neck pain
- ❖ Arm and leg stiffness
- ❖ Muscle tenderness and spasms

- Meningitis, an infection of the membranes surrounding the brain

**Paralytic polio symptoms**

Paralytic polio affects only a small percentage of those invaded by the polio virus. In these cases, the virus enters motor neurons where it replicates and destroys the cells. These cells are in the spinal cord, brain stem, or motor cortex, which is an area of the brain important in controlling movements.

Symptoms of paralytic polio often start in a similar way to non-paralytic polio, but later progress to more serious symptoms such as:

- A loss of muscle reflexes
- Severe muscle pain and spasms
- Loose or floppy limbs that are often worse on one side of the body
- Paralytic polio may also be classified as:
- **Spinal polio**: the virus attacks motor neurons in the spinal cord that causes paralysis in the arms and legs, and breathing problems.
- **Bulbar polio**: the virus affects the neurons responsible for sight, taste, swallowing, and breathing.
- **Bulbospinal polio**: the virus causes symptoms of both spinal and bulbar polio.
- Complications and post-polio syndrome
- Post-polio syndrome describes a cluster of symptoms that affect up to 64 percent of all polio patients. It occurs several years after polio has passed. On average, post-polio syndrome occurs 35 years after the infection.

**Polio diagnosis**

Polio is often recognized because of symptoms, such as neck and back stiffness, abnormal reflexes, and trouble with swallowing and breathing. A doctor who suspects polio will perform laboratory tests that check for poliovirus by examining throat secretions, stool samples, or cerebrospinal fluid.

**Vaccine**

There are two vaccines available to fight polio:

- Inactivated poliovirus (IPV)
- Oral polio vaccine (OPV)

**Causes**

Polio is caused by the poliovirus. The polio virus usually enters the environment in the feces of someone who is infected. In areas with poor sanitation, the virus easily spreads from feces into the water supply, or, by touch, into food.

In addition, because polio is so contagious, direct contact with a person infected with the virus can cause polio.

Individuals who carry the poliovirus can spread it via their feces for weeks, even if they have shown no symptoms themselves.

Once the virus has entered an individual, it infects the cells of the throat and intestine.

The virus stays within the intestines, before spreading to other areas of the body. Eventually, the virus moves into the bloodstream where it can spread to the entire body.

**Risk factors**

As is the case with many other infectious diseases, people who get polio tend to be some of the most vulnerable members of the population. This includes the very young, pregnant women, and those with immune systems that are substantially weakened by other medical conditions.

Anyone who has not been immunized against polio is especially susceptible to contracting the infection.

Additional risk factors for polio include:

- Traveling to places where polio is endemic or widespread, especially Pakistan and Afghanistan
- Living with someone infected with polio
- Having a weak immune system
- Pregnant women are more susceptible to polio, but it does not appear to affect the unborn child
- Working in a laboratory where live poliovirus is kept

**Prevention**

- Vaccines are the main way to prevent polio.
- However, other methods of limiting the spread of this potentially fatal disease include:
- Avoiding food or beverages that may have been contaminated by a person with poliovirus
- Checking with a medical professional that your vaccinations are current
- Being sure to receive any required booster doses of the vaccine
- Washing your hands frequently
- Using hand sanitizer when soap is not available
- Making sure you only touch the eyes, nose, or mouth with clean hands
- Covering the mouth while sneezing or coughing
- Avoiding close contact with people who are sick, including kissing, hugging, and sharing utensils
- Be sure to receive a vaccination before traveling to an area that is prone to polio breakouts. You can check for up-to-date information about these areas on the Centers for Disease Prevention and Control (CDC) website.

**Fungal Infections**

Disease caused by fungal species is called fungal infection. Fungal infections are often classified as either

- Opportunistic
- Primary

Opportunistic infections are those that develop mainly in immunocompromised hosts; primary infections can develop in immunocompetent hosts.

Fungal infections can be

- Systemic
- Local

Local fungal infections typically involve the skin (see Overview of Dermatophytoses), mouth (causing stomatitis), and/or vagina (causing candidal vaginitis) and may occur in normal or immunocompromised hosts.

(See also Antifungal Drugs and Fungal Skin Infections.)

**Opportunistic fungal infections**

Many fungi are opportunists and are usually not pathogenic except in an immunocompromised host. Causes of immunocompromise include AIDS, azotemia, diabetes mellitus, lymphoma, leukemia, other hematologic cancers, burns, and therapy with corticosteroids, immunosuppressants, or antimetabolites. Patients who spend more than several days in an ICU can become compromised because of medical procedures, underlying disorders, and/or undernutrition.

Typical opportunistic systemic fungal infections (mycoses) include

- Candidiasis
- Aspergillosis
- Mucormycosis (zygomycosis)
- Fusariosis

Systemic mycoses affecting severely immunocompromised patients often manifest acutely with rapidly progressive pneumonia, fungemia, or manifestations of extrapulmonary dissemination.

**Primary fungal infections**

Primary fungal infections usually result from inhalation of fungal spores, which can cause a localized pneumonia as the primary manifestation of infection.

In immunocompetent patients, systemic mycoses typically have a chronic course; disseminated mycoses with pneumonia and septicemia are rare and, if lung lesions develop, usually progress slowly. Months may elapse before medical attention is sought or a diagnosis is made. Symptoms are rarely intense in such chronic mycoses, but fever, chills, night sweats, anorexia, weight loss, malaise, and depression may occur. Various organs may be infected, causing symptoms and dysfunction.

Primary fungal infections may have a characteristic geographic distribution, which is especially true for the endemic mycoses caused by certain dimorphic fungi. For example,

- Coccidioidomycosis: Confined primarily to the southwestern US, Washington, northern Mexico, and Central and South America
- Histoplasmosis: Occurring primarily in the eastern and Midwestern US and parts of Central and South America, Africa, Asia, and Australia
- Blastomycosis: Confined to North America and Africa
- Paracoccidioidomycosis (formerly, South American blastomycosis): Confined to that continent

However, travelers can manifest disease any time after returning from endemic areas.

**Diagnosis**

- Cultures and stains
- Histopathology
- Serologic tests

(mainly for *Aspergillus*, *Blastomyces*, *Candida*, *Coccidioides*, *Cryptococcus,* and *Histoplasma*)

If clinicians suspect an acute or a chronic primary fungal infection, they should obtain a detailed travel and residential history to determine whether patients may have been exposed to certain endemic mycoses, perhaps years previously.

Pulmonary fungal infections must be distinguished from tumors and chronic pneumonias caused by nonfungal organisms such as mycobacteria (including TB). Specimens are obtained for fungal and mycobacterial culture and histopathology. Sputum samples may be adequate, but occasionally bronchoalveolar lavage, transthoracic needle biopsy, or even surgery may be required to obtain an acceptable specimen.

Fungi that cause primary systemic infections are readily recognized by their histopathologic appearance. However, identifying the specific fungus may be difficult and usually requires fungal culture. The clinical significance of positive sputum cultures may be unclear if they show commensal organisms (eg, *Candida albicans*) or fungi ubiquitous in the environment (eg, *Aspergillus* sp). Therefore, other evidence (eg, host factors such as immunosuppression, serologic evidence, tissue invasion) may be required to help establish a diagnosis.

**Serologic tests** may be used to check for many systemic mycoses if culture and histopathology are unavailable or unrevealing, although few provide definitive diagnoses. Particularly useful tests include the following:

- Measurement of organism-specific antigens, most notably from *Cryptococcus neoformans*, *Histoplasma capsulatum*, and *Aspergillus* sp (occasional cross-reactivity with other fungi has been noted with each of these serologic tests)
- Serum beta-glucan, which is often positive in invasive candidiasis as well as *Pneumocystis jirovecii* infections

- Complement fixation assays and newer enzyme immunoassays for anticoccidioidal antibodies, which are satisfactorily specific and do not require proof of rising levels (high titers confirm the diagnosis and indicate high risk of extrapulmonary dissemination)

Most other tests for antifungal antibodies have low sensitivity, specificity, or both and, because measurement of acute and convalescent titers is required, cannot be used to guide initial therapy.

**Treatment**

- Antifungal drugs
- Measures to prevent moisture

Fungal infections are typically treated with antifungal drugs, usually with antifungal drugs that are applied directly to the affected area (called topical drugs). Topical drugs may include creams, gels, lotions, solutions, or shampoos. Antifungal drugs may also be taken by mouth.

In addition to drugs, people may use measures to keep the affected areas dry, such as applying powders or wearing open-toed shoes. For some infections, doctors give corticosteroids to relieve inflammation and itching.

**Mycosis**

**Mycosis** is a fungal infection of animals, including humans. Mycoses are common and a variety of environmental and physiological conditions can contribute to the development of fungal diseases. Inhalation of fungal spores or localized colonization of the skin may initiate persistent infections; therefore, mycoses often start in the lungs or on the skin.

**Causes**

Individuals being treated with antibiotics are at higher risk of fungal infections. Individuals with weakened immune systems are also at risk of developing fungal infections. This is the case of people with HIV/AIDS, people under steroid treatments, and people taking chemotherapy. People with diabetes also tend to develop fungal infections. Very young and very old people, also, are groups at risk. Although all are at risk of developing fungal infections, the likelihood is higher in these groups.

***Candida albicans***

*Candida albicans* is part of our natural microflora or the microorganisms that commonly live in or on our bodies. It can be found in the GI tract, the mouth, and the vagina. Most of the time it causes no issues, but it's possible for overgrowths and infections to happen.

*Candida albicans* is the most prevalent cause of fungal infections in people. Its species name,*albicans*, comes from the Latin word for "white." The yeast appears white when cultured on a plate. And in the case of certain infections, like thrush, it can create white patches.

**Types of *Candida albicans* infections**

**1. Urinary Yeast Infection**

*Candida* species are the most common cause of fungal urinary tract infections (UTIs). *Candida* UTIs can occur in the lower portion of the urinary tract or in some cases can ascend up to the kidneys.

The following can put you at risk of developing a *Candida* UTI:

- Having taken a course of antibiotics
- Having a medical device inserted, such as a urinary catheter
- diabetes
- A weakened immune system

**Symptoms**

Many people with a *Candida* UTI don't have symptoms. If symptoms are present, they can include:

- An increased need to urinate

- A painful or burning sensation when urinating
- Abdominal or pelvic pain
- Blood in your urine

**Treatment**

Treatment is only recommended for symptomatic individuals. The antifungal drug fluconazole can be used in many cases.

If a catheter is in place, it should be removed.

**2. Genital Yeast Infection**

*Candida albicans* is the most common cause of genital yeast infections. Normally, a type of bacteria called *Lactobacillus* keeps the amount of *Candida* in the genital area under control. However, when *Lactobacillus* levels are disrupted in some way, *Candida* can overgrow and cause an infection.

You can also develop a *Candida* genital infection after participating in certain sexual activities, particularly those that involve oral-genital contact.

Although otherwise healthy individuals can get genital *Candida* infections, the following groups are at an increased risk:

- People that have taken antibiotics recently
- People with uncontrolled diabetes
- Immunosuppressed individuals
- Pregnant women
- People that are taking oral contraceptives or who are on hormone therapy

**Symptoms**

Symptoms of a genital *candida* infection can include:

- A burning feeling while having sex or while urinating
- An itchy or painful feeling in or around the vagina
- Redness, irritation, or swelling around the vagina
- Abnormal vaginal discharge that can be either watery, or thick and white
- A rash around the vagina
- A rash on the penis

*Candida* species can also infect the male genitals, often if their partner has a vaginal *Candida*infection. The infection may be asymptomatic, but can cause an itchy or burning rash around the head of the penis.

**Treatment**

Mild or moderate genital *Candida* infections can be treated with a short course of an over-the-counter (OTC) or prescription antifungal cream, pill, or suppository. You could also be prescribed a single dose of an oral antifungal medication, such as fluconazole.

For more complicated infections, you may be prescribed a longer course of medication, either in the form of a cream, a pill, or an ointment.

**3. Oral Thrush**

Despite being a normal part of the microflora of your mouth, *Candida albicans* can cause infectionsif it overgrows. The infection may not be limited to just your mouth. It can spread to your tonsils and the back of your throat as well.

Severe infections may spread to the esophagus.

People that are at an increased risk for developing oral thrush include:

- Those taking antibiotics or corticosteroid drugs
- Someone with undiagnosed or uncontrolled diabetes
- Immunosuppressed individuals
- Those who wear dentures, particularly upper dentures

**Symptoms**

Some of the common symptoms of oral thrush include:

- White spots in your mouth that have the appearance of cottage cheese and may bleed when touched
- A burning or painful sensation in your mouth
- Redness inside your mouth or at the corners of your mouth
- Difficulty with eating or swallowing
- Loss of taste
- A cotton-like feeling inside your mouth

If an oral thrush infection is left untreated, it can lead to a systemic *Candida* infection, particularly in people with a weakened immune system.

**Treatment**

Oral thrush is treated with an antifungal medication that can come in the form of a pill, liquid, or lozenge. Examples of drugs that are used include nystatin or clotrimazole.

An oral course of fluconazole can be given for more severe cases.

**4. Mucocutaneous Candidiasis**

Candida albicans is the most common cause of fungal skin infections. *Candida* species can also infect your skin and mucus membranes. *Candida albicans* is most often the cause of a fungal skin infection, although other *Candida*strains can also cause it.

Areas that are warm, moist, or sweaty provide good environments for yeast to thrive. Examples of such areas include the armpits, groin, the skin between your fingers and toes, the corners of your mouth, and the area under your breasts.

Other risk factors for developing a *Candida* skin infection include:

- Wearing tight or synthetic undergarments
- Having poor hygiene or changing undergarments infrequently, including infrequent diaper changes for infants
- Taking antibiotics or corticosteroid drugs
- Having diabetes
- Having a weakened immune system

***Candida* infections diagnosed**

In order to diagnose candidiasis, your doctor will first take your medical history and ask you about your symptoms. They may also ask if you have any conditions or medications that could lead to a weakened immune system, or if you've taken a course of antibiotics recently.

Many common cases of candidiasis can often be diagnosed through a physical examination.

If your doctor is uncertain if your symptoms are due to a *Candida* infection, they may take a sample from the affected area. This sample can then be used to culture the organism and to identify what species it is. For example, if candidemia is suspected, your doctor will collect a blood sample for testing.

Identifying the species of *Candida* that's causing your infection is also helpful because your doctor will be able to prescribe an antifungal medication that will be effective in treating that particular species.

**Parasitic Infection**

A **parasitic disease**, also known as parasitosis, is an infectious disease caused or transmitted by a parasite. Many parasites do not cause diseases as it may eventually lead to death of both organism and host. Parasitic diseases can affect practically all living organisms, including plants and mammals. The study of parasitic diseases is calledparasitology.

Some parasites like *Toxoplasma gondii* and *Plasmodium* spp. can cause disease directly, but other organisms can cause disease by the toxins that they produce.

**Signs and Symptoms**

Symptoms of parasites may not always be obvious. However, such symptoms may mimic anemia or a hormone deficiency. Some of the symptoms caused by several worm infestations can include itching affecting the anus or the vaginal area, abdominal pain, weight loss, increased appetite, bowel obstructions, diarrhea, andvomiting eventually leading to dehydration, sleeping problems, worms present in the vomit or stools, anemia, aching muscles or joints, general malaise, allergies, fatigue, nervousness. Symptoms may also be confused with pneumonia or food poisoning.

The effects caused by parasitic diseases range from mild discomfort to death. The nematode parasites *Necator americanus* and *Ancylostoma duodenale* cause humanhookworm infection, which leads to anaemia, protein malnutrition and, in severely malnourished people, shortness of breath and weakness. This infection affects approximately 740 million people in the developing countries, including children and adults, of the tropics specifically in poor rural areas located in sub-Saharan Africa,Latin America, South-East Asia and China. Chronic hookworm in children leads to impaired physical and intellectual development, school performance and attendance are reduced. Pregnant women affected by a hookworm infection can also develop anemia, which results in negative outcomes both for the mother and the infant. Some of them are: low birth weight, impaired milk production, as well as increased risk of death for the mother and the baby.

**Causes**

Mammals can get parasites from contaminated food or water, bug bites, or sexual contact. Ingestion of contaminated water can produce Giardia infections.[6]

Parasites normally enter the body through the skin or mouth. Close contact with pets can lead to parasite infestation as dogs and cats are host to many parasites.

Other risks that can lead people to acquire parasites are walking with barefeet, inadequate disposal of feces, lack of hygiene, close contact with someone carrying specific parasites, and eating undercooked foods, unwashed fruits and vegetables or foods from contaminated regions.

Parasites can also be transferred to their host by the bite of an insect vector, i.e. mosquito, bed bug, fleas.

**Diagnosis**

Laboratory analysis of samples of blood, stool, urine, or phlegm. Laboratory analysis of specimens, including special tests to identify proteins released by the parasite (antigen testing) or genetic material (DNA) from the parasite, may be needed. Samples of blood, stool, urine, or phlegm (sputum) may be taken.

Doctors may test blood samples for antibodies to the parasite. Antibodies are proteins produced by the immune system to help defend the body against a particular attack, including that by parasites.

Doctors may also take a sample of tissue that may contain the parasite. For example, a biopsy may be done to obtain a sample of intestinal or other infected tissue. A sample of skin may be snipped. Several samples and repeated examinations may be necessary to find the parasite.

**Identifying parasites in the intestinal tract**

If parasites live in the intestinal tract, the parasite or its eggs or cysts (a dormant and hardy form of the parasite) may be found in the person's stool when a sample is examined under a microscope. Or parasites may be identified by testing the stool for proteins released by the parasite or genetic materials from the parasite. Antibiotics, laxatives, and antacids should not be used until after the stool sample has been collected. These drugs can reduce the number of parasites enough to make seeing the parasites in a stool sample difficult or impossible.

**Entamoeba histolytica:**

Amebiasis is a disease caused by infection with a parasitic amoeba that, when symptomatic, can cause dysentery and invasive extraintestinal problems. The cause of amebiasis is mainly the protozoan parasite *Entamoeba histolytica*. Some risk factors for amebiasis include consuming contaminated food or water, association with food handlers whose hands are contaminated, contact with contaminated medical devices such as colonic irrigation devices, and being pregnant.

Amebiasis is contagious and maybe contagious for weeks to many years if untreated. Only about 10%-20% of infected individuals show symptoms and signs. The symptoms and signs include

- Loose stools,
- Mild abdominal cramping,
- Frequent, watery, and/or bloody stools with severe abdominal cramping (termed amoebic dysentery) may occur,
- Flatulence,
- Appetite loss, and
- Fatigue.

**Life Cycle, Diseases and laboratory diagnosis**

*Entamoeba histolytica* is an enteric protozoan parasite with worldwide distribution. It is responsible for amoebic dysentery (bloody diarrhea) and invasive extraintestinal amebiasis (such as liver abscess, peritonitis, pleuropulmonary abscess). Other species of Entamoeba-*E.hartmanii, E.coli* and *E.dispar* does not cause diseases but their trophozoite is difficult to distinguish from those of *E.histolytica* by light microscopy.

**Mode of transmission:** Feco-oral route, via the ingestion of contaminated food or water containing mature **quadrinucleate cyst** of *Entamoeba histolytica*. Trophozoites if ingested **would not survive**exposure to the gastric environment.

***Mneomonic**: **EntAmoebaHistoLytica i.e. (Remember:***

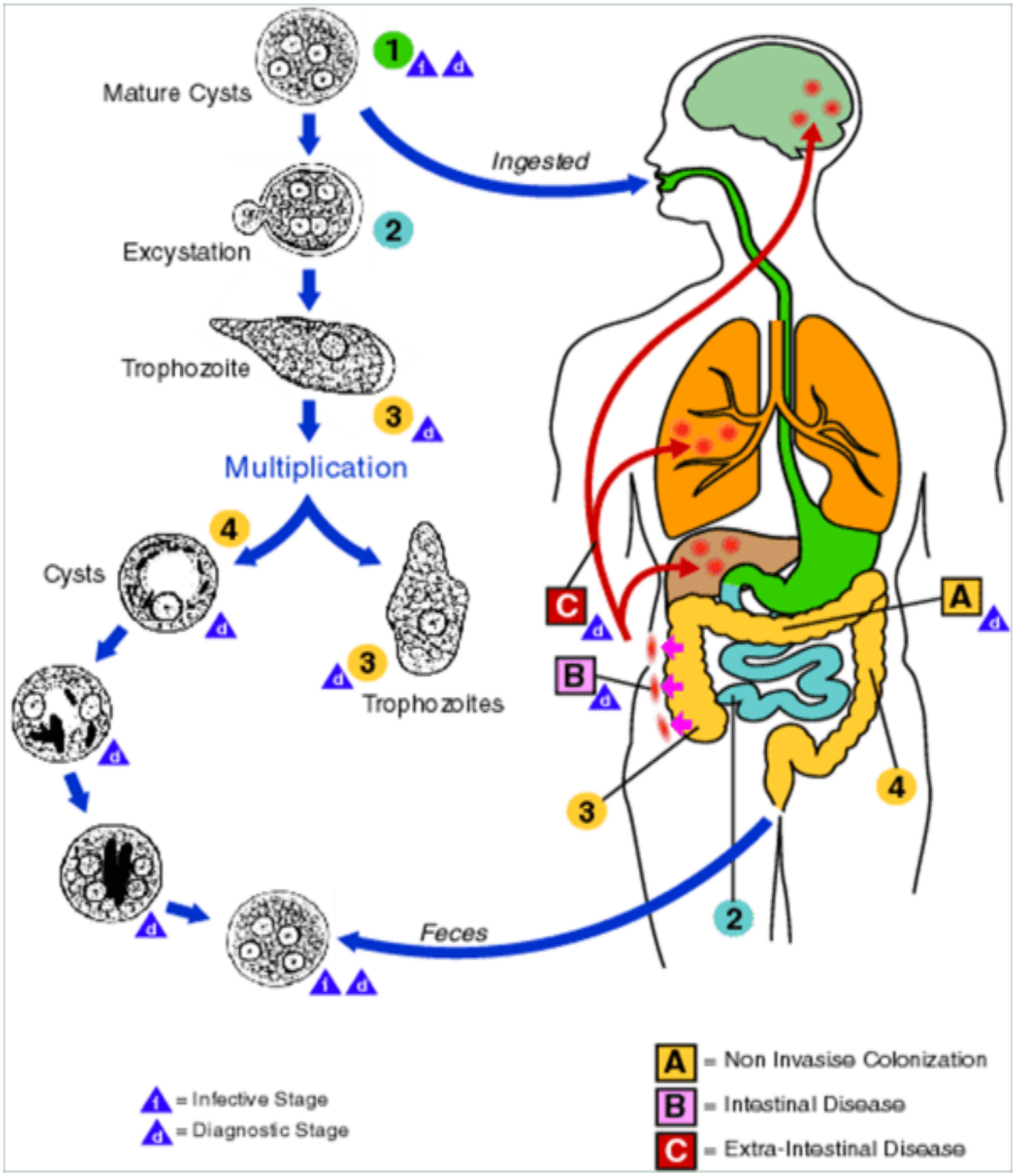
- *Ent: Enterocytes (a cell of the intestinal lining);*
- *Amoeba: Protozoa;*
- *Histo: Tissue;*
- *Lytica: lysis.**)***

*As this protozoan parasite lyse the cells of intestinal tract; there will be bleeding; so the stool contains blood and mucus (Amoebic dysentry).*

**Infective form:** Mature quadrinucleate cyst; it is **spherical in shape** with refractile wall (Note: ***Giardia lamblia*** cyst also has four nuclei, but the cyst is oval in shape).

**Life cycle of *Entamoeba histolytica***

Infection by *Entamoeba histolytica* occurs by ingestion of **mature quadrinucleate cysts** in fecally contaminated food, water, or hands.The quadrinucleate cyst is **resistant to the gastric environment**and passes unaltered through the stomach




**Life Cycle of *Entamoeba histolytica***

**Clinical Findings**

- Acute intestinal amebiasis
  - dysentery (i.e. bloody, mucus containing diarrhea)
  - lower abdominal discomfort,
  - flatulence and
- Chronic amebiasis : low grade symptoms such as occasional diarrhea, weight loss and fatigue also occurs.
- Roughly 90% of infected individuals have asymptomatic infection but they may be carriers,
- Ameboma, a granulomatous lesion may form in the cecal or rectosigmoid areas of the colon in some patients. These lesions resemble an adenocarcinoma of the colon and must be distinguished from them.

**Laboratory diagnosis**

Diagnosis of intestinal amebiasis rests on**finding either trophozoites in diarrheal stools or cysts in formed stools.** Diarrheal stools should be examined **within one hour of collection** to see the ameboid motility of the trophozoite. The trophozoite**characteristically contain ingested red blood cells.**

**Characteristics of Stool**

- **Macroscopic appearance of stool**: Offensive dark brown semisolid stool, acid in reaction, admixed with blood, mucus and much fecal matter.
- **General microscopic examination**:
  - Presence of charcot-Leyden crystals. Scanty cellular exudates, and consists of only the nuclear masses (“pyknotic bodies).
  - *E. histolytica* infection is distinguished from bacillary dysentery by the lack of high fever and absence PMN leukocytosis

**Laboratory diagnosis methods:**

**A. Microscopy:**

*E. histolytica* can be distinguished from other amoebas by two major criteria

1. **Nature of the nucleus of the trophozoite.**

   The *E. histolytica* nucleus has a small central nucleolus and fine chromatin granules along the border of the nuclear membrane. The nuclei of other amebas are quite different. **Note:** The trophozoites of *Entamobea dispar*, a nonpathogenic species of Entamoeba, are morphologically indistinguishable from those of *E. histolytica*

2. **Cyst size and number of its nuclei.**

   Mature cysts of *E. histolytica* are smaller than those of *Entamoeba coli* and contain four nuclei, where as *E. coli* cysts have eight nuclei.

B. Antigen detection: detection of *E.histolytica* antigen in the stool

C. Serologic testing is useful for the diagnosis of invasive amebiasis.

C. Detection of nucleic acid of this protozoan parasite by **PCR based assay.**

# UNIT -V

**Waste Management Resources**

Waste management is the precise name for the collection, transportation, disposal or recycling and monitoring of waste. This term is assigned to the material, waste material that is produced through human being activity. This material is managed to avoid its adverse effect over human health and environment. Most of the time, waste is managed to get resources from it. The waste to be managed includes all forms of matter i.e. gaseous, liquid, solid and radioactive matter.

The methods for the management of waste may differ for developed and developing nations. For urban and rural populations, industrial and residential areas it does differ as well. The management of waste in metropolitan and rural areas is general responsibility of the local government. While the waste that is produced by the industries is managed by the industry itself, incase it is non-hazardous.

**Methods for dumping off waste:**

**Landfill:** this method involves burying off the waste and this is the most common practice for the disposal of waste around the Globe. These landfills are quite often conventional with deserted and vacant locations around the cities. In case, landfills or borrow pits are designed carefully they can serve as economical and quite sanitized method for waste dumping. However, not much effectively designed and older landfills can cost a big amount to the government not just in terms of money but also in the environmental and health issues. Apart from the general poorly designed landfill's common problems like wind-blown debris and generation of liquid, it can also cause production of gas, which is extremely hazardous. This gas can be a reason for production of odor, killing surface vegetation and greenhouse effects.

The characteristic, which is must for an up to date landfill, is inclusion of clay or leachate lining. The waste that is deposited is generally compressed for increasing the density and stability and later it is covered to have it prevented from vermin. One thing, which is addition to modern landfills, is the "gas extraction system" installation. This system is included to have the gas extracted from the borrow pit.

**Incineration:**

This is the dumping off method, which involves combustion for waste materials. This sort of dumping off for waste materials through incineration and temperature is known as "thermal treatment". This method is utilized to convert waste materials in to gas, heat, ash and steam.

Incineration is conducted on both individual and industrial scale. This method is used for disposing off all sorts of matters. This generally is the most recognized practical method for disposing off perilous material. This however, is the conflict-ridden method for it causes the emission of perilous gases. Incineration is a common practice in Japan because of scarcity of land, which facilitates through not requiring landfill for waste dumping. Two widely used terms, which are facilitating burning of waste material in furnace and boiler for generation of heat, electricity and steam, are (Waste-to-energy) WtW and (energy-from-waste)EfW.

The burning procedure in this method for waste disposal is never perfect so, fear for gas pollutants is mounting. Special concerns have been focused over some extremely importunate organics as dioxins. These organic products are created with the incinerator and they are causations for serious consequences affecting environment.

**Methods for recycling:**

Products like PVC, LDEP, PP and PS are recyclable though they are not collected for recycling. The material, which is composed of a single type, is recyclables and is much easy to work with. However, complex products are difficult to treat and so are complex for recycling.

**Biological reprocessing:**

Waste materials, which come in organic nature are treated through biological reprocessing. The waste materials with organic nature are plant, food and paper products. This reprocessing or recycling of this organic matter is put to biological decomposition which later if recycled in form of mulch or compost

for landscaping and agricultural purposes. Additionally, the waste gas, which is collected from the process, is used for the production of electricity. The goal behind biological reprocessing is to control and speed up the natural decomposition for organic matter.

A numerous sort of composting techniques and methods for digestion are employed depending upon the requirement as if digestion is required for household heaps or industrial materials. There are diverse methods for biological reprocessing like anaerobic and aerobic techniques.

**What is Wastewater Treatment?**

Wastewater treatment is the process of converting wastewater – water that is no longer needed or is no longer suitable for use – into bilge water that can be discharged back into the environment. It's formed by a number of activities including bathing, washing, using the toilet, and rainwater runoff. Wastewater is full of contaminants including bacteria, chemicals and other toxins. Its treatment aims at reducing the contaminants to acceptable levels to make the water safe for discharge back into the environment.

There are two wastewater treatment plants namely chemical or physical treatment plant, and biological wastewater treatment plant. Biological waste treatment plants use biological matter and bacteria to break down waste matter. Physical waste treatment plants use chemical reactions as well as physical processes to treat wastewater. Biological treatment systems are ideal for treating wastewater from households and business premises. Physical wastewater treatment plants are mostly used to treat wastewater from industries, factories and manufacturing firms. This is because most of the wastewater from these industries contains chemicals and other toxins that can largely harm the environment.

**Step by Step Wastewater Treatment Process**

**1. Wastewater Collection**

This is the first step in waste water treatment process. Collection systems are put in place by municipal administration, home owners as well as business owners to ensure that all the wastewater is collected and directed to a central point. This water is then directed to a treatment plant using underground drainage systems or by exhauster tracks owned and operated by business people. The transportation of wastewater should however be done under hygienic conditions. The pipes or tracks should be leak proof and the people offering the exhausting services should wear protective clothing.

**2. Odor Control**

At the treatment plant, odor control is very important. Wastewater contains a lot of dirty substances that cause a foul smell over time. To ensure that the surrounding areas are free of the foul smell, odor treatment processes are initiated at the treatment plant. All odor sources are contained and treated using chemicals to neutralize the foul smell producing elements. It is the first wastewater treatment plant process and it's very important.

**3. Screening**

This is the next step in wastewater treatment process. Screening involves the removal of large objects for example nappies, cotton buds, plastics, diapers, rags, sanitary items, nappies, face wipes, broken bottles or bottle tops that in one way or another may damage the equipment. Failure to observe this step, results in constant machine and equipment problems. Specially designed equipment is used to get rid of grit that is usually washed down into the sewer lines by rainwater. The solid wastes removed from the wastewater are then transported and disposed off in landfills.

**4. Primary Treatment**

This process involves the separation of macrobiotic solid matter from the wastewater. Primary treatment is done by pouring the wastewater into big tanks for the solid matter to settle at the surface of the tanks. The sludge, the solid waste that settles at the surface of the tanks, is removed by large scrappers

and is pushed to the center of the cylindrical tanks and later pumped out of the tanks for further treatment. The remaining water is then pumped for secondary treatment.

**5. Secondary Treatment**

Also known as the activated sludge process, the secondary treatment stage involves adding seed sludge to the wastewater to ensure that is broken down further. Air is first pumped into huge aeration tanks which mix the wastewater with the seed sludge which is basically small amount of sludge, which fuels the growth of bacteria that uses oxygenand the growth of other small microorganisms that consume the remaining organic matter. This process leads to the production of large particles that settle down at the bottom of the huge tanks. The wastewater passes through the large tanks for a period of 3-6 hours.

**6. Bio-solids handling**

The solid matter that settle out after the primary and secondary treatment stages are directed to digesters. The digesters are heated at room temperature. The solid wastes are then treated for a month where they undergo anaerobic digestion. During this process, methane gases are produced and there is a formation of nutrient rich bio-solids which are recycled and dewatered into local firms. The methane gas formed is usually used as a source of energy at the treatment plants. It can be used to produce electricity in engines or to simply drive plant equipment. This gas can also be used in boilers to generate heat for digesters.

**7. Tertiary treatment**

This stage is similar to the one used by drinking water treatment plants which clean raw water for drinking purposes. The tertiary treatment stage has the ability to remove up to 99 percent of the impurities from the wastewater. This produces effluent water that is close to drinking water quality. Unfortunately, this process tends to be a bit expensive as it requires special equipment, well trained and highly skilled equipment operators, chemicals and a steady energy supply. All these are not readily available.

**8. Disinfection**

After the primary treatment stage and the secondary treatment process, there are still some diseases causing organisms in the remaining treated wastewater. To eliminate them, the wastewater must be disinfected for at least 20-25 minutes in tanks that contain a mixture of chlorine and sodium hypochlorite. The disinfection process is an integral part of the treatment process because it guards the health of the animals and the local people who use the water for other purposes. The effluent (treated waste water) is later released into the environment through the local water ways.

**9. Sludge Treatment**

The sludge that is produced and collected during the primary and secondary treatment processes requires concentration and thickening to enable further processing. It is put into thickening tanks that allow it to settle down and later separates from the water. This process can take up to 24 hours. The remaining water is collected and sent back to the huge aeration tanks for further treatment. The sludge is then treated and sent back into the environment and can be used for agricultural use.

Wastewater treatment has a number of benefits. For example, wastewater treatment ensures that the environment is kept clean, there is no water pollution, makes use of the most important natural resource; water, the treated water can be used for cooling machines in factories and industries, prevents the outbreak of waterborne diseases and most importantly, it ensures that there is adequate water for other purposes like irrigation.

**Conclusion**

In summary, wastewater treatment process is one of the most important environmental conservation processes that should be encouraged worldwide. Most wastewater treatment plants treat wastewater from homes and business places. Industrial plant, refineries and manufacturing plants wastewater is usually treated at the onsite facilities. These facilities are designed to ensure that the wastewater is treated before it can be released to the local environment. Some of the water is used for

cooling the machines within the plants and treated again. They try to ensure that nothing is lost. It illegal for disposing untreated wastewater into rivers, lakes, oceans or into the environment and if found culpable one can be prosecuted.

**Composting**

Composting is nature's way of recycling. Composting biodegrades organic waste. i.e. food waste, manure, leaves, grass trimmings, paper, wood, feathers, crop residue etc., and turns it into a valuable organic fertilizer.

Composting is a natural biological process, carried out under controlled aerobic conditions (requires oxygen). In this process, various microorganisms, including bacteria and fungi, break down organic matter into simpler substances. The effectiveness of the composting process is dependent upon the environmental conditions present within the composting system i.e. oxygen, temperature, moisture, material disturbance, organic matter and the size and activity of microbial populations.

Composting is not a mysterious or complicated process. Natural recycling (composting) occurs on a continuous basis in the natural environment. Organic matter is metabolized by microorganisms and consumed by invertebrates. The resulting nutrients are returned to the soil to support plant growth.

Composting is relatively simple to manage and can be carried out on a wide range of scales in almost any indoor or outdoor environment and in almost any geographic location. It has the potential to manage most of the organic material in the waste stream including restaurant waste, leaves and yard wastes, farm waste, animal manure, animal carcasses, paper products, sewage sludge, wood etc. and can be easily incorporated into any waste management plan.

Since approximately 45 - 55% of the waste stream is organic matter, composting can play a significant role in diverting waste from landfills thereby conserving landfill space and reducing the production of leachate and methane gas. In addition, an effective composting program can produce a high quality soil amendment with a variety of end uses.

The essential elements required by the composting microorganisms are carbon, nitrogen, oxygen and moisture. If any of these elements are lacking, or if they are not provided in the proper proportion, the microorganisms will not flourish and will not provide adequate heat. A composting process that operates at optimum performance will convert organic matter into stable compost that is odor and pathogen free, and a poor breeding substrate for flies and other insects. In addition, it will significantly reduce the volume and weight of organic waste as the composting process converts much of the biodegradable component to gaseous carbon dioxide.

**The composting process is carried out by three classes of microbes -**

- • Psychrophiles - low temperature microbes
- • Mesophiles -medium temperature microbes
- • Thermophiles - high temperature microbes

Generally, composting begins at mesophilic temperatures and progresses into the thermophilic range. In later stages other organisms including Actinomycetes, Centipedes, Millipedes, Fungi, Sowbugs, Spiders and Earthworms assist in the process.

**Temperature**

Temperature is directly proportional to the biological activity within the composting system. As the metabolic rate of the microbes accelerates, the temperature within the system increases. Conversely, as the metabolic rate of the microbes decreases, the system temperature decreases. Maintaining a temperature of 130°F or more for 3 to 4 days favors the destruction of weed seeds, fly larvae and plant pathogens.

At a temperature of 155 degrees F, organic matter will decompose about twice as fast as at 130 degrees F. Temperatures above 155 degrees F may result in the destruction of certain microbe

populations. In this case temperature may rapidly decline. Temperature will slowly rise again as the microbe population regenerates.

Moisture content, oxygen availability, and microbial activity all influence temperature. When the pile temperature is increasing, it is operating at optimum performance and should be left alone. As the temperature peaks, and begins to decrease, the pile should be turned to incorporate oxygen into the compost. Subsequently , the pile should respond to the turning and incorporation of oxygen, and temperature should again cycle upwards. The turning process should be continued until the pile fails to re-heat. This indicates that the compost material is biologically stable.

Composting microorganisms thrive in moist conditions. For optimum performance, moisture content within the composting environment should be maintained at 45 percent. Too much water can cause the compost pile to go anaerobic and emit obnoxious odors. Too little will prevent the microorganisms from propagating.

**Particle Size**

The ideal particle size is around 2 to 3 inches. In some cases, such as in the composting of grass clippings, the raw material may be too dense to permit adequate air flow or may be too moist. A common solution to this problem is to add a bulking agent (straw, dry leaves, paper, cardboard) to allow for proper air flow. Mixing materials of different sizes and textures also helps aerate the compost pile.

**Turning**

During the composting process oxygen is used up quickly by the microbes as they metabolize the organic matter. As the oxygen becomes depleted the composting process slows and temperatures decline. Aerating the compost by turning should ensure an adequate supply of oxygen to the microbes.

**Composting Period**

The composting period is governed by a number of factors including, temperature, moisture, oxygen, particle size, the carbon-to-nitrogen ratio and the degree of turning involved. Generally, effective management of these factors will accelerate the composting process.

**Carbon to Nitrogen Ratio**

The microbes in compost use carbon for energy and nitrogen for protein synthesis. The proportion of these two elements required by the microbes averages about 30 parts carbon to 1 part nitrogen. Accordingly, the ideal ratio of Carbon to Nitrogen (C:N) is 30 to 1 (measured on a dry weight basis). This ratio governs the speed at which the microbes decompose organic waste.

Most organic materials do not have this ratio and, to accelerate the composting process, it may be necessary to balance the numbers.

The C:N ratio of materials can be calculated by using table 1 below.Example, if you have two bags of cow manure (C:N = 20:1) and one bag of corn stalks (C:N = 60:1) then combined you have a C:N ration of (20:1 + 20:1 + 60:1)/3 = (100:1)/3 = 33:1

**Table 1 lists the Carbon/Nitrogen Ratios of Some Common Organic Materials**

| Material | C:N ratio |
|---|---|
| **Vegetable wastes** | **12-20:1** |
| **Alfalfa hay** | **13:1** |
| **Cow manure** | **20:1** |
| **Apple pomace** | **21:1** |
| **Leaves** | **40-80:1** |
| **Corn stalks** | **60:1** |
| **Oat straw** | **74:1** |
| **Wheat straw** | **80:1** |
| **Paper** | **150-200:1** |
| **Sawdust** | **100-500:1** |

| | |
|---|---|
| **Grass clippings** | **12-25:1** |
| **Coffee grounds** | **20:1** |
| **Bark** | **100-130:1** |
| **Fruit wastes** | **35:1** |
| **Poultry manure (fresh)** | **10:1** |
| **Horse manure** | **25:1** |
| **Newspaper** | **50-200:1** |
| **Pine needles** | **60-110:1** |
| **Rotted manure** | **20:1** |

**Composting Methods**

**Hot Composting**

Hot composting is the most efficient method for producing quality compost in a relatively short time. In addition, it favors the destruction of weed seeds, fly larvae and pathogens. While hot composting, using the windrow or bin method, requires a high degree of management, hot composting, using the in-vessel method, requires a lesser degree of management.

**Cold Composting**

This method is ideal for adding organic matter around trees, in garden plots, in eroded areas etc. The time required to decompose organic matter using this method is governed, to a large extent, by environmental conditions and could take two years or more.

**Sheet Composting**

Sheet composting is carried out by spreading organic material on the surface of the soil or untilled ground and allowing it to decompose naturally. Over time, the material will decompose and filter into the soil. This method is ideally suited for forage land, no-till applications, erosion control, roadside landscaping etc. The process does not favor the destruction of weed seeds, fly larvae, pathogens etc. and composting materials should be limited to plant residue and manure. Again, decomposition time is governed by environmental conditions and can be quite lengthy.

**Trench Composting**

Trench composting is relatively simple. Simply dig a trench 6 - 8 inches deep, fill with 3 - 4 inches of organic material and cover with soil. Wait a few weeks and plant directly above the trench. This method does not favor the destruction of weed seeds, fly larvae and pathogens and the composting process can be relatively slow.

**Loading the Bin / Windrow**

Place the raw materials in layers using a balance of high carbon (moist) and low carbon (dry) materials. Each layer should be no more than four to six inches in depth. Spray each layer with a light mist of CBCT Stock Solution (Mix CBCT Concentrate and water at a rate of 1:200). This will initiate and accelerate the composting process and eliminate odors).

**Procedure:**

Step 1. Start with a 4 to 6 inch layer of coarse material set on the bottom of the composter or on top of the soil.

Step 2. Add a 3 to 4 inch layer of low carbon material.

Step 3. Add a 4 to 6 inch layer of high carbon material.

Step 4. Add a 1 inch layer of garden soil or finished compost.

Step 5. Mix the layers of high carbon material, low carbon material, and soil or compost.

Repeat steps 2 through 5 until the composting bin is filled (maximum 4 feet in height). Cap with dry material.

**Loading the Vessel (in-vessel composting)**

To accelerate the composting process, simply mix the high carbon and low carbon materials together before placing them in the composter. Add the mixture to the composter in small batches, spraying each batch with a light mist of water or CBCT stock solution.

**Adding material during the composting process**

Ideally, new materials should be added to the composting system during turning or mixing. Generally, the addition of moist materials accelerates the composting process while the addition of dry materials slows the process.

**About Compost**

Finished compost can be classified as a 100% organic fertilizer containing primary nutrients as well as trace minerals, humus and humic acids, in a slow release form. Compost improves soil porosity, drainage and aeration and moisture holding capacity and reduces compaction. Compost can retain up to ten times it's weight in water. In addition, compost helps buffer soils against extreme chemical imbalances; aids in unlocking soil minerals; releases nutrients over a wide time window; acts as a buffer against the absorption of chemicals and heavy metals; promotes the development of healthy root zones; suppresses diseases associated with certain fungi; and helps plants tolerate drought conditions.

**Applications**

Compost can be used in a variety of applications. High quality compost can be used in agriculture, horticulture, landscaping and home gardening. Medium quality compost can be used in applications such as erosion control and roadside landscaping. Low quality compost can be used as a landfill cover or in land reclamation projects.

**What is biogas?**

Biogas consists mainly of methane ($CH_4$) and carbon dioxide ($CO_2$). Methane is a valuable form of gas, as it is an efficient energy carrier with a wide range of uses. The amount of $CO_2$ that is produced corresponds to the amount of $CO_2$ captured when the biomass was created.
This means that biogas is a $CO_2$ neutral and renewable source of energy.

**How is biogas produced?**

Biogas is produced through the processing of various types of organic waste. It is a renewable and environmentally friendly fuel made from 100% local feedstocks that is suitable for a diversity of uses including road vehicle fuel and industrial uses. The circular-economy impact of biogas production is further enhanced by the organic nutrients recovered in the production process.

Biogas can be produced from a vast variety of raw materials (feedstocks). The biggest role in the biogas production process is played by microbes feeding on the biomass.

Digestion carried out by these microorganisms creates methane, which can be used as it is locally or upgraded to biogas equivalent to natural gas quality, enabling the transport of the biogas over longer distances. Material containing organic nutrients is also produced in the process, and this can be utilized for purposes such as agriculture.

**Stages in biogas production**

Biogas is produced using well-established technology in a process involving several stages:

1. Biowaste is crushed into smaller pieces and slurrified to prepare it for the anaerobic digestion process. Slurrifying means adding liquid to the biowaste to make it easier to process.
2. Microbes need warm conditions, so the biowaste is heated to around 37 °C.
3. The actual biogas production takes place through anaerobic digestion in large tanks for about three weeks.
4. In the final stage, the gas is purified (upgraded) by removing impurities and carbon dioxide.

After this, the biogas is ready for use by enterprises andconsumers, for example in a liquefied form or following injection into the gas pipeline network.

**Turning diverse range of materials into gas**

Biogas production starts from the arrival of feedstocks at the biogas plant. A diverse range of solid as well as sludge-like feedstocks can be used.

Materials suitable for biogas production include:

- Biodegradable waste from enterprises and industrial facilities, such as surplus lactose from the production of lactose-free dairy products
- Spoiled food from shops
- Biowaste generated by consumers
- Sludge from wastewater treatment plants
- Manure and field biomass from agriculture

The material is typically delivered to the biogas plant's reception pit by lorry or waste management vehicle.

**Biomass is turned into gas by microbes**

In the biogas reactor, microbial action begins and the biomass enters a gradual process of fermentation. In practice this means that microbes feed on the organic matter, such as proteins, carbohydrates and lipids, and their digestion turns these into methane and carbon dioxide.

Most of the organic matter is broken down into biogas – a mixture of methane and carbon dioxide – in approximately three weeks. The biogas is collected in a spherical gas holder from the top of the biogas reactors.

**Digestate utilized as fertilizers or gardening soil**

The residual solids and liquids created in biogas production are referred to as digestate. This digestate goes into a post-digester reactor and from there further into storage tanks. Digestates are well suited for uses such as fertilization of fields.

Digestates can also be centrifuged to separate the solid and liquid parts.

Solid digestates have uses such as fertilizers in agriculture or in landscaping and can also be turned into gardening soil through a process of maturation involving composting.

Digestates are centrifuged to yield enough process water for the slurrification of biowaste at the beginning of the process. This helps reduce the use of clean water. The centrifuged liquid is rich in nutrients, particularly nitrogen, that can be separated further using methods such as stripping technology and used as fertilizers or nutrient sources in industrial processes.

**Clean biogas helps move towards low-carbon society**

Gas would already be ready for several uses straight from the biogas plant gas holder. However, before being injected into the gas pipeline network or used to fuel vehicles, it will still undergo purification. In this upgrading process, gas is filtered and flown into columns where it is scrubbed by cascading water at a very specific pressure and temperature. Water efficiently absorbs carbon dioxide and sulfur compounds contained by the gas.

Biogas can also be purified using other methods, such as passing it through activated carbon filters to remove impurities.

The final upgraded biogas injected into the gas network is at least 95% and usually around 98% methane. Upgraded biogas still contains a couple of per cent of carbon dioxide as its further separation from methane is not cost-effective let alone sensible as regards the usability of the gas. Biogas is dried carefully before injection into the gas network to prevent condensation in winter subzero conditions.

The biogas produced can be used for purposes such as fuelling municipal waste management vehicles, urban buses or private cars. At the same time, gas serves as evidence of those practical actions that are taking us towards the low-carbon society of the future.

**Production of Bacterial Bio-Fertilizer:**

With day-by-day increasing the population, especially in developing countries like India, the stress on agriculture is also increasing continuously. With the development, the land area under farming is not increasing but is further decreasing, this has posed extra burden on the agriculture. Therefore, the land available for agriculture should be economically utilized and maximum results be obtained.

Most of our agricultural lands are deprived of either one mineral or the other. These minerals are essential for the growth and development of plants. One of the nutrients for any type of plant is nitrogen. Nitrogen is a major element required by the plant for growth and development. The nitrogen is provided in the form of chemical fertilizer.

Such chemical fertilizers pose health hazards and pollution problem in soil besides these are quite expensive, bringing the cost of production much higher. Therefore, bio-fertilizers are being recommended in place of chemical fertilizers. Bio-fertilizers are the formulations of living microorganisms which are able to fix atmospheric nitrogen in the available form for plants (nitrate form) either by living freely in the soil or associated symbiotically with plants.

Although nitrogen fixers are present in the soil, enrichment of soil with effective microbial strains is much beneficial for the crop yields. Use of composite bio-fertilizers can increase soil fertility.

**It has been proved that bio-fertilizers are cost effective, cheap and renewable source to supplement the chemical fertilizers:**

***(i) History:***

In 1895, Nobbe and Hiltner applied for patents in England and the United States for a legume inoculant that was later marketed as Nitragin. Nitragin was produced on gelatin and agar nutrient media. However, agar based inoculants were soon replaced by peat-based ones because in agar based inoculants, mortality was very high during the dry phase. In India, the production of bio-fertilizers on commercial scale was started only during late 1960's when yellow seeded soybean was introduced for the first time. Recognition of Indian peat as suitable carrier for production of bio-fertilizer in 1969 further augmented, the growth of bio-fertilizer industry in India.

The performance of Indian peat-based bio-fertilizer at Indian Agricultural Research Institute, New Delhi, was found to be comparable to that obtained with imported 'Nitragin' bio-fertilizer from the U.S.A. Since then, the process of development of bio-fertilizer, specially of rhizobial bio-fertilizer for various crops in India has made a tremendous success.

***(ii) Production of Bio-Fertilizer:***

In order to meet the food requirements of ever increasing population, the nitrogen fertilizer requirement for crop production by 2000 A.D. was estimated to be about $11.4 \times 10^6$ tonnes. Biological nitrogen fixation can be the key to fill up this gap because of high cost and several other demerits of chemical fertilizers.

For production of a good and efficient bio-fertilizer, first of all an efficient nitrogen fixing strain is required, then its inoculum (the form in which the strain is to be applied in fields) is produced.

Packing, storing and maintenance are other aspects of bio-fertilizer production. While producing bio-fertilizers the standards laid down by BIS have also to be kept in mind for making the product authentic. Commercial production of bacteria, involved in the production of bio-fertilizer is shown in Fig. 34.1.

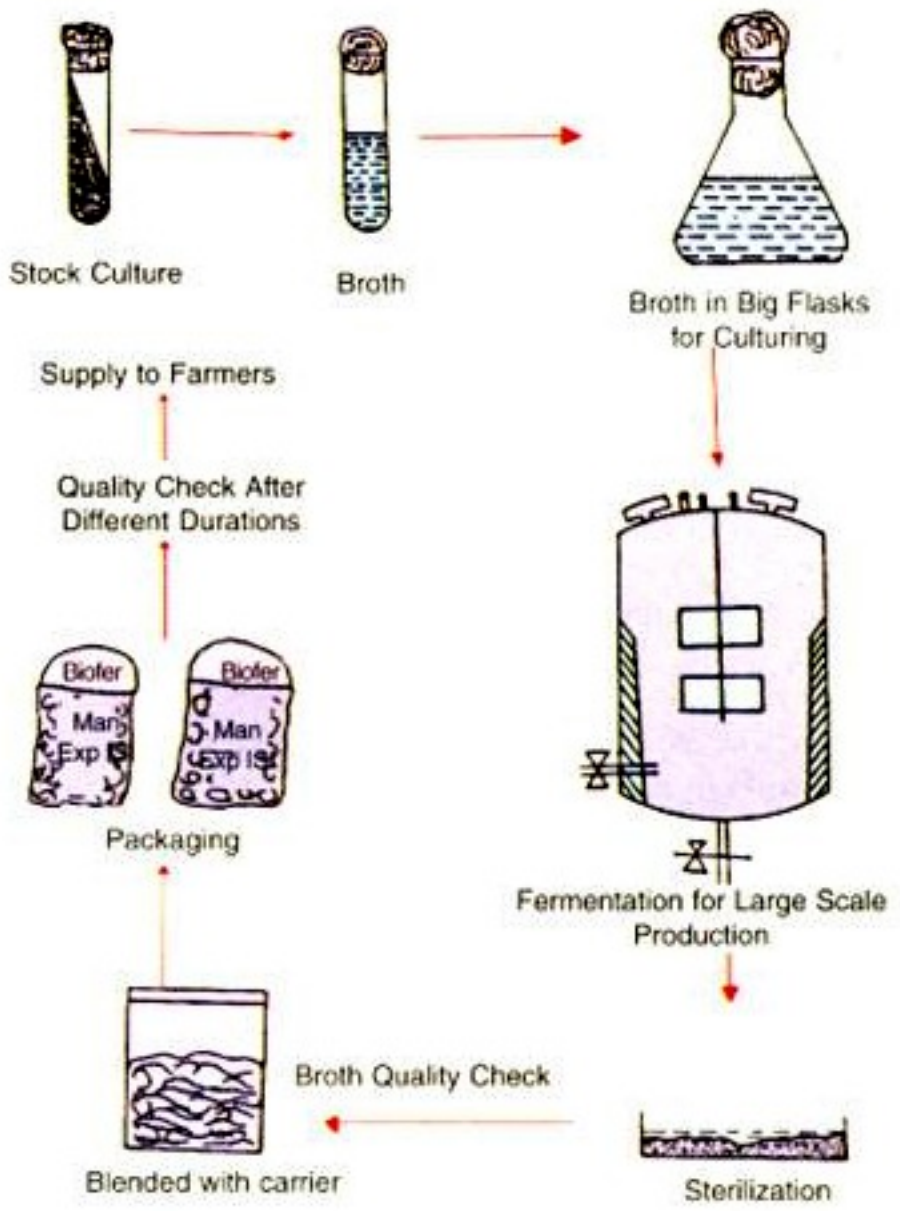



**Fig. 34.1** : Commercial production of biofertilizer.

***(iii) Criteria for Strain Selection:***

The efficient nitrogen fixing strain is evolved or selected in laboratory, maintained and multiplied on nutritionally rich artificial medium before inoculating the seed or soil. In soil, the strain has to survive and multiply to compete for infection site on roots against hostile environment in soil.

***(iv) Steps for Preparing Bio-Fertilizer:***

The isolated strain is inoculated in small flasks containing suitable medium for inoculum production. The volume of the starter culture should be a minimum of 1% to obtain atleast $1\times10^9$ cells/ml. Now the culture obtained is added to the carrier for inoculant (bio-fertilizer) preparation.

Carriers carry the nitrogen fixing organisms to the fields. In some cases carrier is first sterilised and then inoculated, while in other cases it is first inoculated and then sterilised by UV irradiation. The inoculum is now packed with $10^9$-$10^{10}$ viable cells per gram. Final moisture content should be around 40-60%. For large scale production of inoculum, culture fermenters are used.

**(a) Seed Pelleting:**

Direct seed coating with the gum arable or sugary syrup and useful nitrogen fixing strains especially the coating of rhizobia over specific host legume seeds are another method for obtaining fruitful results. As before, first of all the inoculum is prepared of the desired strain and then the seeds are

inoculated by using either direct coating method or slurry method. Immediately after seed coating, $CaCO_3$ is added to sticky seeds.

The practice of seed inoculation dates back to 1896 when Voecher used this technique. In many soils the nodule bacteria are absent or are not adequate in either number or quality to meet the nitrogen requirements of the plants. Under these conditions, it is necessary to inoculate seeds or seedlings with highly effective rhizobia.

**(b) Inoculant Carriers:**

Most inoculants are the mixture of the broth culture and a finely milled, neutralized carrier material. Carrier is a substance having properties such as, non-toxicity, good moisture absorption capacity, free of lump forming material, easy to sterilize, inexpensive, easily available and good buffering capacity, so that it can prolong and maintain the growth of nitrogen fixing microorganisms which it is carrying.

The most frequently used carrier for inoculant production is peat. However, peat is not available in certain countries such as India. A wide range of substitutes e.g. lignite, coal, charcoal, bagasse, filter mud, vermiculite, polyacrylamide, mineral soils, vegetable oils, etc. have been tested as alternative carriers.

Carrier processing e.g. mining, drying and milling are the most capital intensive aspects of inoculant (bio-fertiliser) production. First of all the carrier like peat is mined, drained and cleared off stones, roots, etc. Then, it is shredded and dried.

The peat is then passed through heavy mills. Material with a particle size of 10-40 μm is collected for seed coating. Peat with particle size of 500-1500 μm is used for soil implant inoculant. Carriers have to be neutralised by adding precipitated calcium carbonate (pH 6.5-7.0). After this, the carriers are sterilized for use as inoculants.

**(c) Quality Standards for Inoculants:**

Like every product, the bio-fertilizers should also follow certain standards. The inoculant should be carrier-based and should contain a minimum of $10^8$ viable cells per gram of carrier on dry mass basis within 15 days of manufacture, and $10^7$ within 15 days before the expiry date marked on the packet when the inoculant is stored at 25-30°C. The inoculant should have a maximum expiry period of 12 months from date of manufacture. The inoculant should not have any contaminant.

The contamination is one of the biggest problems faced by the bio-fertilizer industry. The pH of inoculant should be between 6.0 and 7.5. Each packet containing the bio-fertiliser should be marked with the information's e.g, name of product, leguminous crop for which intended, name and address of manufacture, type of carrier, batch or code number, date of manufacture, date of expiry, net quantity meant for net area and storage instructions. Each packet should also be marked with ISI (BIS) certification mark.

The inoculant (bio-fertilizer) should be stored in a cool place away from direct heat preferably at a temperature of 15°C. The bio inoculant should be packed in 50-75 μ low density polyethylene packets.

Two main methods of inoculation are currently being used (a) seed inoculation and (b) soil inoculation. The soil inoculation is done by delivering the inoculant directly into the sowing furrow with the seeds. Seed inoculation by pelleting or coating the seed with inoculant is the most popular methods.

***(v) Green Manuring:***

**Green manuring is defined as a** "farming practice where a leguminous plant which has derived enough benefits from its association with appropriate species of Rhizobium is ploughed into the field soil and then a non -legume is sown and allowed to get benefitted from the already present nitrogen fixer".

The practice of green manuring began from time immemorial from several century B.C. in India and China. During the course of time, availability of chemical fertilizers decreased the significance of green

manuring. In recent years, due to hike in price of chemical fertilizers, the practice of green manuring is reemphasized.

Some of the cultivated legumes and annual legumes such as Crotolaria juncea, C. striata, Cassia mimosoides, Cyamopsis pamas, Glycine wightii, Indigofera linifolia, Sesbania rostrata, Leucaena leucocephala, etc. contribute nitrogen.

In addition to nitrogen, green manuring provides organic matter, phosphorus, potassium besides minimising the pathogenic organisms in soil. The reclamation of "usar lands" can also be done by green manuring.

In India besides a large number of private and semi-Government organisations, the National Bio-fertilizer Development Centre sponsored by the Ministry of Agriculture and the establishment of National Centres for blue green algal collections at IARI, New Delhi, the Department of Biotechnology, Govt. of India, Ministry of Science & Technology are the major developments that reflect our concern to harness bio-fertilizers in our agricultural economy.

**2. Algal and Other Bio-Fertilizers:**

Biological nitrogen fertilizers play a vital role to solve the problems of soil fertility, soil productivity and environmental quality. Anabaena azollae, a cyanobacterium lives in symbiotic association with the free floating water fern Azolla.

The symbiotic system Azolla-Anabaena complex is known to contribute 40-60 kg N $ha^{-1}$ per rice crop. Anabaena azolle can grow photo autotrophically and fixes atmospheric nitrogen. The nitrogen fixing cyanobacteria such as A. azollae and variabilis when immobilized in polyurethane foam and sugar cane waste have significantly increased the nitrogen fixing activity and ammonia secretion.

The inoculation of cyanobacteria in nee crop significantly influenced the growth of rice crop by secretion of ammonia in flood water. The use of neem cake coupled with the inculation of Azolla greatly increased the nitrogen utilization efficiency in rice crop. Besides Anabaena, other nitrogen fixing cyanobacteria like Aulosira, Calothrix, Hapalosiphon, Scytonema, Tolypothrix and Westiellopsis have been held responsible for the spontaneous fertility of the tropic rice fields.

In addition to contributing N, the cyanobacteria add organic matter, secrete growth promoting substance like auxins and vitamins, mobilise insoluble phosphate and improve physical and chemical nature of the soil. Algalization has been shown to ameliorate the saline- alkali soils, help in the formation of soil aggregates, reduce soil compaction, and narrow C: N ratio.

These organisms enable the crop to utilize more of the applied nutrients leading to increased fertilizer utilising efficiency of crop plant. Most of the cyanobacteria act as supplements to fertilizer N contributing up to 30 kg N $ha^{-1}$ $season^{-1}$. The increase in the crop yield varies between 5-25 percent.

***(i) Mass Production of Cyanobacterial Biofertilizers:***

For outdoor cultivation of cyanobacterial biofertilizers, the regional specific strain should be used. In such practices, a mixture of 5 or 6 regionally acclimatized strains of cyanobacteria e.g. species of Anabaena, Aulosira, Cylindrospermum, Gloeotrichia, Nostoc, Plectonema, Tolypothrix etc. are generally used as starter inoculum.

**The following methods are used for mass cultivation:**

a. Cemented tank method,
b. Shallow metal troughs method,
c. Polythene lined pit method and
d. Field method.

The polythene lined method is most suitable for small and marginal farmers for the preparation of bio-fertilizer. In this method, small pits are prepared in field and lined with thick polythene sheets.

**The mass cultivation of cyanobacteria is done by using any of the above four methods; the steps are given below:**

a. Prepare the cemented tank, shallow trays of iron sheets or polythene lined pits in an open area. Width of tanks or pits should not be more than 1.5 m. This will facilitate the proper handling of culture.
b. Transfer 2-3 kg soil and add 100 g superphosphate. Water the pit to about 10 cm height. Mix lime to adjust the pH. Add 2 ml of insecticide to protect the culture from mosquitoes. Mix well and allow to settle down soil particles.
c. When water becomes clear, sprinkle 100 g starter culture on the surface of water.
d. When temperature remains around 35-40°C during summer, optimum growth of cyanobacteria is achieved. The water level is always maintained about 10 cm during the period.
e. After drying, the algal mass (mat) is separated from the soil that forms flakes. During summer about 1 kg pure algal mat per $m^2$ area is produced. It is collected, powdered, and packed in polythene bag and supplied to the farmers after sealing the packets.
f. The algal flakes can be used as starter inoculum again.

***(ii) Mass Cultivation of Azolla:***

The aquatic heterosporus fern contains endophytic cyanobacterium, Anabaena azollae in its leaf cavity. There are number of species of Azolla, namely A. caroliniana, A, filiculoides, A. maxicana, A. nilotica, A. pinnata and A. rubra which are used as biofertilizer especially for paddy. For mass cultivation of Azolla, microplots (20 $m^2$) are prepared in nurseries in which sufficient water (5-10 cm) is added.

For profuse growth of Azolla, 4-20 kg $P_2O_5$/ha is also amended. Optimum pH (8.0) and temperature (14-30°C) should be maintained. Finally, microplots are inoculated with fresh Azolla (0.5 to 0.4 kg/$m^2$). An insecticide (Furadon) is used to check the insect's attack. After 3 weeks, the mat of Azolla is ready for harvest and the same microplot is inoculated with fresh Azolla to repeat the cultivation.
Azolla mat is harvested and dried to use as green manure.

**There are two methods for its application in field:**

a. Incorporation of Azolla in soil prior to rice cultivation, and
b. Transplantation of rice followed by water draining and incorporation of Azolla.

However, reports from the IRRI, Manila (Philippines) revealed that growing of Azolla in rice field before rice transplantation increased the yield equivalent to that obtained from 30 kg/ha nitrogen as urea or ammonium phosphate.

**3. Endophytic Nitrogen Fixers:**

Recently, several non-leguminous and particularly graminaceous species such as rice, wheat and forage grasses have registered tremendous interest in nitrogen fixation. Isolation of a number of diazotrophic bacteria such as Azospirillum, Herbaspirillum and Acetobacter is reported.

The term endophyte refers to microorganisms (bacteria and fungi) that colonize root interior of plants and live most of their life inside the plant tissue. Splitting the term endophyte into facultative and obligate was suggested to distinguish, respectively, strains that are able to colonize both the surface and root interior and to survive well in soil from those that do not survive well in soil but colonize the root interior and aerial parts.

***(i) Facultative Endophytic Diazotrophs:***

This group is composed of Azospirillum spp. and considered important with non-legume plants. Although A. lipoferum was the first species of the genus isolated by Tarrand (1978). A. brasilense among all the seven known species is the best characterized at physiological and molecular levels.

***(ii) Obligate Endophytic Diazotrophs:***

This group includes Acetobacter diazotrophicus (syn. Gluconacetobacter diazotrophicus) a nitrogen fixing bacterium clustered in the alpha sub-class of the proteobacteria, Azoarcus spp., Herbaspirillum spp. and a partially identified Burkholderia sp. are clustered in the beta sub-class of the proteobacteria.

***(iii) Other Bacteria:***

Alcaligens, a diazotrophic member of this genus has been consistently isolated from the rhizosphere of wet rice land. Burkholderia, the other bacterium appears to have potential as rice inoculant. In the case of Klebsiella, substituted nitrogen fixation has been observed in rice inoculated with K. oxytoca or any other Klebsiella spp. that are considered as endophytes.

The diazotrophic nature of some members of the genus Pseudomonas is still a matter of debate. Nevertheless, several bacteria within it are clearly diazotrophic such as Pseudomonas diazotrophicus, P. flurorescens, P. saccharophila and P. stutzeri. Recently, several researchers have attempted to construct an artificial association between rhizobia and rice particularly with Azorhizobium caulinodans.

**(a) Isolation and Identification of Endophytes:**

isolation and identification of natural diazotrophs from plant samples, root or stem, washed with sterile water, surface sterilized with 70% ethanol for 5 minutes and with sodium hypochlorite (2-5%) for 30 second, washed several times using sterile water. Sterilization of root and stem will be verified by rolling them on BMS agar plates.

Then homogenize the sample in a mortar and pestle in sterile phosphate buffer, saline 1% sugar solution and serially diluted and 0.1 ml sample transfer into vials containing 5-8 ml of respective semisolid media for the targeted bacterium with respective C sources with an initial pH of 6.0.

The number of diazotrophic populations is determined by the most probable number methods using a McCrady table. Vials with veil pellicles reaching the surface after incubation at 30°C with or without gas production and with positive reaction for acetylene reduction activity, show the presence of good endophytes.

**(b) Applications in Agriculture:**

Obligate endophytes have an enormous potential for use because of their ability to colonize the entire plant interior and establish themselves niches protected from oxygen or other inhibitory factors; thus their potential to fix nitrogen can be expressed maximally.

Recent studies in Brazil showed that the sugarcane varieties fix up to 80% nitrogen. It has been reported that wetland rice receives some nitrogen by endodiazotrophs. Tropical pasture grasses such as Brachiaria, Digitaria, Panicum and Paspalum spp. fix nitrogen.

**4. Bio-Fertilizers aiding Phosphorus Nutrition:**

Tropical soils are deficient in phosphorus. Further most of the microorganisms solubilize P and thus make it available for plant growth. It is estimated that in most tropical soils, 75% super phosphate applied is fixed and only 25% is available for plant growth.

There are some fungi such as Aspergillus awamori, Penicillium digitatum, etc. and bacteria like Bacillus polymyxa, Pseudomonas striata, etc. that solubilize unavailable form of P to available form. India has 250 mt of rock phosphate deposits. The cheaper source of rock phosphate like Mussoorie rock phosphate and Udaipur rock phosphate available in our country can be used along with phosphate solubilising microorganisms (Table 34.1).

Vesicular-arbuscular mycorrhizal (VAM) fungi colonize roots of several crop plants. They are zygomycetous fungi belonging to the genera Glomus, Gigaspora, Acaulospora, Sclercystis, etc.

These are obligate symbionts and cannot be cultured on synthetic media. They help plant growth through improved phosphorus nutrition and protect the roots against pathogens. Nearly 25-30% of phosphate fertilizer can be saved through inoculation with efficient VAM fungi as reported by Bagyaraj (1992).

**5. Production of Mycorrhizal Bio-Fertilizer:**

Methods of inoculum production of mycorrhizal fungi differ with respects to their nature, depending upon types i.e., ectomycorrhizal or endomycorrhizal.

***(i) Ectomycorrhizal Fungi:***

In this case, the basidiospores, chopped sporocarps, sclerotia, pure mycelial culture, fragmented mycorrhizal roots or soil from mycorhizosphere region can be used as inoculum. The inoculum is mixed with nursery soil and seeds are sown thereafter. Institute for mycorrhizal Research and Development, USA and Abbot Laboratories, USA have developed a mycelial inoculum of Pisolithus tinctorius in a mycelial vermiculite-peat moss substrate with trade name 'MycoRhiz' which is commercially available on large quantities (Table 34.2).

***(ii) VA Mycorrhizal Fungi:***

VA mycorrhiza can be produced on a large scale by pot culture technique. This requires the host plant mycorrhizal fungi and natural soil. The host plants which support large scale production of inoculum are sudan grass, strawberry, sorghum, maize, onion, citrus, etc.

The starter inoculum of VAM can be isolated from soil by wet sieving and decantation technique. VAM spores are surface sterilised and brought to the pot culture. Commonly used pot substrates are sand: soil (1:1, w/w) with a little amount of moisture.

**There are two methods of using the inoculum:**

- Using a dried spore-root-soil to plants by placing the inoculum several centimetres below the seeds or seedlings,
- Using a mixture of soil- roots, and spores in soil pellets and spores are adhered to seed surface with adhesive.

Commercially available pot culture of VA mycorrhizal hosts grown under aseptic conditions can provide effective inoculum. Various types of VAM inocula are currently produced by Native Plants, Inc (NPI), Salt Lake City.

In India, Tata Energy Research Institute (TERI), New Delhi and Forest Research Institute, Dehradun have established mycorrhizae banks. Inocula of these can be procured as needed and used in horticulture and forestry programmes.

**Biopesticides**

Biopesticides are certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals. For example, canola oil and baking soda have pesticidal applications and are considered biopesticides. As of April 2016, there are 299 registered biopesticide active ingredients and 1401 active biopesticide product registrations.

**Classes of Biopesticides**

Biopesticides fall into three major classes:

1. **Biochemical pesticides** are naturally occurring substances that control pests by non-toxic mechanisms. Conventional pesticides, by contrast, are generally synthetic materials that directly kill or inactivate the pest. Biochemical pesticides include substances that interfere with mating, such as insect sex pheromones, as well as various scented plant extracts that attract insect pests to traps. Because it is sometimes difficult to determine whether a substance meets the criteria for classification as a biochemical pesticide, EPA has established a special committee to make such decisions.
2. **Microbial pesticides** consist of a microorganism (e.g., a bacterium, fungus, virus or protozoan) as the active ingredient. Microbial pesticides can control many different kinds of pests, although each separate active ingredient is relatively specific for its target pest[s]. For example, there are fungi that control certain weeds and other fungi that kill specific insects. The most widely used microbial

pesticides are subspecies and strains of *Bacillus thuringiensis*, or Bt. Each strain of this bacterium produces a different mix of proteins and specifically kills one or a few related species of insect larvae. While some Bt ingredients control moth larvae found on plants, other Bt ingredients are specific for larvae of flies and mosquitoes. The target insect species are determined by whether the particular Bt produces a protein that can bind to a larval gut receptor, thereby causing the insect larvae to starve.

3. **Plant-Incorporated-Protectants** (PIPs) are pesticidal substances that plants produce from genetic material that has been added to the plant. For example, scientists can take the gene for the Bt pesticidal protein and introduce the gene into the plant's own genetic material. Then the plant, instead of the Bt bacterium, manufactures the substance that destroys the pest. The protein and its genetic material, but not the plant itself, are regulated by EPA.

**Advantages of using biopesticides**

- Biopesticides are usually inherently less toxic than conventional pesticides.
- Biopesticides generally affect only the target pest and closely related organisms, in contrast to broad spectrum, conventional pesticides that may affect organisms as different as birds, insects and mammals.
- Biopesticides often are effective in very small quantities and often decompose quickly, resulting in lower exposures and largely avoiding the pollution problems caused by conventional pesticides.
- When used as a component of Integrated Pest Management (IPM) programs, biopesticides can greatly reduce the use of conventional pesticides, while crop yields remain high.

To use biopesticides effectively (and safely), however, users need to know a great deal about managing pests and must carefully follow all label directions.

**Microbial Leaching (Bioleaching, Biomining)**

Microbial leaching is the process by which metals are dissolved from ore bearing rocks using microorganisms. For the last 10 centuries, microorganisms have assisted in the recovery of copper dissolved in drainage from water. Thus biomining has emerge as an important branch of biotechnology in recent years. Microbial technology renders helps in case of recovery of ores which cannot be economically processed with chemical methods, because they contain low grade metals. Therefore, large quantity of low grade ores are produced during the separation of high grade ores. The low grade ores are discarded in waste heaps which enter in the environment. The low grade ores contain significant amount of nickel, lead, and zinc ores which could be processed by microbial leaching. Bioleaching of uranium and copper has been widely commercialized. But large scale leaching process may cause environmental problems when dump is not managed properly. This results in seepage of leach fluids containing large quantity of metals and low pH into nearby natural water supplies and ground water.

Thus, biomining is, economically sound hydrometallurgical process with lesser environmental problem than conventional commercial application. However, it is an inter-disciplinary field involving metallurgy, chemical engineering, microbiology and molecular biology. It has tremendous practical application. In a country like India biomining has great national significance where there is vast unexploited mineral potential (Mogal and Desai, 1992).

**Miroorganisms used for Leaching**

The most commonly used microorganisms for bioleaching are *Thiobacillus thiooxidans* and*T.ferrooxidans.* The other microorganisms may also be used in bioleaching viz., *Bacillus licheniformis, B. luteus, B. megaterium, B. polymyxa, Leptospirillum ferrooxidans, Pseudomonas fluorescens, Sulfolobus acidocaldarius, Thermothrix thioparus, Thiobacillus thermophilica,* etc.

**Chemistry of Microbial Leaching**

*T. thiooxidans* and *T. ferrooxidans* have always been found to be present in mixture on leaching dumps. *Thiobacillus* is the most extensively studied Gram-negative bacillus bacterium which derives energy from oxidation of $Fe^{2+}$ or insoluble sulphur. In bioleaching there are two following reaction mechanisms:

**Direct Bacterial Leaching**

In direct bacterial leaching a physical contact exists between bacteria and ores and oxidation of minerals takes place through several enzymatically catalyzed steps. For example, pyrite is oxidized to ferric sulphate as below:

$$2FeS_2 + 7O_2 + 2H_2O \xrightarrow{\text{T. ferrooxidans}} 2FeSO_4 + 2H_2SO_4$$

**Leaching Process**

There are three commercial methods used in leaching:

*(i)* Slope Leaching. About 10,000 tonnes of ores are ground first to get fine pieces. It is dumped in large piles down a mountain side leaching dump. Water containing inoculum of*Thiobacillus* is continuously sprinkled over the pile. Water is collected at bottom. It is used to extract metals and generate bacteria in an oxidation pond.

*(ii)* Heap Leaching. The ore is dumped in large heaps called leach dump. Further steps of treatment are as described for slope leaching.

*(iii) In situ* Leaching. In this process ores remain in its original position in earth. Surface blasting of rock is done just to increase permeability of water. Thereafter, water containing*Thiobacillus* is pumped through drilled passage to the ores. Acidic water seeps through the rock and collects at bottom. Again from bottom water is pumped, mineral is extracted and water is reused after generation of bacteria.

**Examples of Bioleaching**

**Copper Leaching**

Throughout the world copper leaching plants have been widely used for many years. It is operated as simple heap leaching process or combination of both heap leaching and in situleaching process. Dilute sulphuric acid (pH 2) is percolated down through the pile. The liquid coming out of the bottom of pile reach in mineral. It is collected and transported to precipitation plant, metal is reprecipitated and purified. Liquid is pumped back to top of pile and cycle is repeated. For removal of copper the ores commonly used are chalcocite (Cu2S), chalcopyrite (CuFeS2) or covellite (CuS). Several other metals are also associated with these ores. Chalcocite is oxidized to soluble form of copper (Cu2+) and covellite by T. ferrooxidans.

Cu2S + O2 ---------->CuS + Cu2+ + H2O

Covellite is oxidized to copper sulphate chemically or by bacteria.

2CuFeS2 + 8½ O2 + H2SO4 ---------->2CuSO4 + Fe2(SO4)3 + H20

Thereafter, strictly chemical reaction occurs which is the most important reaction in copper leaching.

CuS + 8Fe3+ + 4H2O ---------->Cu2+ + SFe2+ + SO42- + 8H+

**Uranium Leaching**

Uranium leaching is more important than copper, although less amount of uranium is obtained than copper. For getting one tonne of uranium, a thousand tonne of uranium ore must be handled. In situ uranium leaching is gaining vast acceptance. However, uranium leaching from ore on a large scale is widely practiced in the USA, South Africa, Canada and India.

Insoluble tetravalent uranium is oxidized with a hot H2SO4/Fe3+ solution to make soluble hexavalent uranium sulfate at pH 1.5-3.5 and temperature 35°C (Crueger and Crueger, 1984).

$$UO_2 + Fe_2(SO_4)_3 \text{ ----------> } UO_2SO_4 + 2FeSO_4$$

Uranium leaching is indirect process. *T. ferrooxidans* does not directly attack on uranium ore, but on the iron oxidant. The pyrite reaction is used for the initial production of Fe3+ leach solution.

$$2FeS + H_2O + 7½\ O_2 \xrightarrow{\text{T. ferrooxidans}} Fe_2(SO_4)_3 + H_2SO_4$$

**Gold and Silver Leaching**

Today's microbial leaching of refractory precious metal ores to enhancegold and silver recovery is one of the most promising applications. Gold is obtained through bioleaching of arsenopyrite/pyrite ore and its cyanidation process. Silver is more readily solubilized than gold during microbial leaching of iron sulfide.

**Silica Leaching**

Magnesite, bauxite, dolomite and basalt are the ores of silica. Mohanty et al (1990) isolated *Bacillus Ucheniformis* from magnesite ore deposits. Later it was shown to be associated with bioleaching, concomitant mineralysis and silican uptake by the bacterium. It was concluded that silican uptake was restricted adsorption of bacterial cell surface rather than internal uptake through the membrane. The bioleaching technology of silica magnesite by using *B. licheniformis* developed at Bose Institute, Calcutta is being used for the first time for commissioning a 5 billion tonnes capacity of pilot plant at Salem Works of Burn, Standard Co. Ltd, Tamil Nadu, in collaboration with the Department of Biotechnology, Govt of India (Haideret al., 1994).