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DEPARTMENT OF MICROBIOLOGY

Course : M.Sc Year: **I** Semester: **II**

Course Material on:

ENVIRONMENTAL & AGRICULTURAL MICROBIOLOGY

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Prepared by :

Dr. A.CHOLARAJAN, M.Sc., M.Phil., Ph.D

ASSISTANT PROFESSOR / MB

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ENVIRONMENTAL AND AGRICULTURAL MICROBIOLOGY

OBJECTIVES:

To enable the students to get exposure on various aspects of environmental and agricultural microbiology

Unit I Air microbiology and Biogeochemical cycles:

Aerobiology- Significance of air microflora - Microbial air pollution- sources, biological indicators and effects on plants and human beings. Enumeration of bacteria from air, Air sampling devices, Outline of Airborne diseases (Bacterial, Fungal and Viral), Air sanitation. Biogeochemical cycles -Nitrogen, Carbon, Phosphorous, Sulphur, Iron and their importance.

Unit II Aquatic microbiology:

Microbes in marine and fresh water environment – eutrophication – Water pollution – sources and nature of pollutants in water – sewage – treatment of liquid waste – primary, secondary and tertiary treatment – water borne diseases – Assessment of water quality – BOD and COD. Solid waste treatment – saccarification and pyrolysis.

Unit III Recycling of Liquid and Solid wastes:

Recycling of Liquid and Solid wastes-Composting-Biogas, Mushroom and SCP production from waste. Biodegradation of complex polymers (Cellulose, Hemicellulose, Lignin, Chitin and Pectin), Bioremediation (In-situ, Ex-situ, Intrinsic), Bioaugmentation and Biostimulation. Bioleaching (Copper and Uranium) - Xenobiotics degradation (Heavy metals). A brief note on panchakavya.

Unit IV Soil Microbiology:

Microbial association with plants - Phyllosphere, Rhizosphere, Mycorrhizae, nitrogen fixing organism – symbiosis, asymbiosis, associate symbiosis – phosphate solubilizers – application of biofertilizers in agriculture. Biology of nitrogen fixation – genes and regulations in Rhizobium

Unit V Plant diseases and its control:

Bacterial, viral and fungal plant pathogens. Morphological, physiological changes with reference to disease establishment in plants – plant protection – phenolics – phytoalexins and related compounds. Disadvantages of chemical pesticides. Microbial pesticides- types, mechanisms, advantages and limitations.

Unit I Air microbiology and Biogeochemical cycles

Aeromicrobiology is the study of living microbes which are suspended in the *air*. These microbes are referred to as bioaerosols

Viable airborne microorganisms are not air pollutants, but should be considered as a factor affecting air quality. Air is an unfavourable environment for microorganisms, in which they cannot grow or divide. It is merely a place which they temporarily occupy and use for movement.

There are 3 elementary limiting factors in the air

- **A lack of adequate nutrients**
- **Frequent deficit of water (desiccation)**
- **Solar radiation**

The atmosphere can be occupied for the longest time by those forms which, due to their chemical composition or structure, are resistant to desiccation and solar radiation. They can be subdivided into the following groups:

- Bacterial resting forms,
- Bacterial vegetative forms which produce carotenoidal dyes or special protective layers (capsules, special structure of cell wall),
- Spores of fungi,
- Viruses with envelopes

Resting forms of Bacteria

Endospores are the best known resting forms. These structures evolve within cells and are covered by a thick multi-layer casing. Consequently, endospores are unusually resistant to most unfavourable environment conditions and are able to survive virtually endlessly in the conditions provided by the atmospheric air. They are only produced by some bacteria, mainly by Bacillus and Clostridium genera. Because each cell produces only one endospore, these spore forms cannot be used for reproduction.

Another type of resting form is produced by very common soil bacteria, the actinomycetes. Their special vertical, filiform cells, of the so-called air mycelium, undergo fragmentation producing numerous ball-shaped formations. Due to the fact that their production is similar to the formation of fungal, they are also called conidia. Contrary to endospores, the conidia are used for reproduction. There are also other bacterial resting

forms, among others, the cysts produced by azotobacters - soil bacteria capable of molecular nitrogen assimilation.

Resistant Vegetative Cells of Bacteria

The production of carotenoidal dyes ensures cells with solar radiation protection. Carotenoids, due to the presence of numerous double bonds within a molecule (-C=C-), serve a purpose as antioxidants, because, as strong reducing agents, they are oxidizedby free radicals. Consequently, important biological macromolecules are being protected against oxidation (DNA, proteins etc.). Bacteria devoid of these dyes quickly perish due to the photodynamic effect of photooxidation. That explains why the colonies of bacteria, which settle upon open agar plates, are often colored. The ability to produce carotenoids is possessed especially by cocci and rod-shaped actinomycetes. Rod-shaped actinomycetes, e.g. *Mycobacterium tuberculosis*, besides being resistant to light, also demonstrate significant resistance to drying due to a high content of lipids within their cell wall. High survival rates in air are also a characteristic for the bacteria which possess a capsule, e.g. *Klebsiella* genus, that cause respiratory system illnesses.

Fungal Spores

Spores are special reproductive cells used for asexual reproduction. Fungi produce spores in astronomical quantities, for example the giant puffball (*Calvatia gigantea*) produces 20 billion spores, which get into the air and are dispersed over vast areas. A very common type of spores found in air is that of conidia.

Conidia are a type of spore formed by asexual reproduction. They form in the end-sections of vertical hyphae called conidiophores and are dispersed by wind. The spores of common mould fungi such as *Penicillium* and *Aspergillus* are examples of the above. Spore plants such as ferns, horsetails and lycopods also produce spores. Plant pollen is also a kind of spores.

Resistant Viruses

Besides cells, the air is also occupied by viruses. Among those that demonstrate the highest resistance are those with enveloped nucleocapsids, such as influenza viruses. Among viruses without enveloped nucleocapsids, enteroviruses demonstrate a relatively high resistance.

Of course, besides the previously mentioned resistant forms, the air is also occupied by more sensitive cells and viruses, but their survival is much shorter. It is believed, that among vegetative forms, gram-positive bacteria demonstrate greater resistance than Gram negative bacteria (especially for desiccation), mainly due to the thickness of their cell wall. Viruses are usually more resistant than bacteria.

Factors Affecting Growth of Microorganism in Air

There are several factors which influence the ability of a bioaerosol to survive in air:

- Particular resistance for a given microorganism (morphological characteristics)
- Meteorological conditions (inter alia, air humidity, solar radiation),
- Air pollution,
- The length of time in air.

Resistance of microorganisms

It is a species dependent feature, which relies on the microorganism's morphology and physiology.

Relative humidity

The content of water in air is one of the major factors determining the ability to survive. At a very low humidity and high temperature cells face dehydration, whereas high humidity may give cells protection against the solar radiation. Microorganisms react differently to humidity variations in air, but nevertheless most of them prefer high humidity. The morphology and biochemistry of cell-surrounding structures, which may change its conformation depending on the amount of water in air, are crucial. Actually, an exact mechanism of this is not known. Forms of resting spores with thick envelopes (e.g. bacterial endospores) are not particularly susceptible to humidity variations. Gram-negative bacteria and enveloped viruses (e.g. influenza virus, myxo) deal better with low air humidity which is contrary to gram-positive bacteria and non-enveloped viruses (e.g. enteroviruses) that have higher survival rates in high air humidity.

Temperature

Temperature can indirectly affect cells by changing the relative-air humidity (the higher the temperature, the lower the relative humidity) or a direct affect, causing, in some extreme situations, cell dehydration and protein denaturation (high temperatures) or crystallization of water contained within cells (temperatures below 0°C). Therefore, it can be concluded that low temperatures (but above 0°C) are optimal for the bioaerosol. According to some researchers the optimal temperatures are above 15°C.

Solar radiation

Solar radiation has a negative affect on the survival rate of the bioaerosol, both visible as well as ultraviolet (UV) and infrared radiation due to the following factors:

- Causes mutation,
- Leads to the formation of free radicals, which damage important macromolecules.
- Creates a danger of dehydration.

Visible light rays of about 400-700 nm wavelength, create the so-called photodynamic effect, which produces free radicals within cells, especially compounds such as peroxy and hydroxyl radicals. These radicals demonstrate strong oxidizing activities and may cause damage to crucial macromolecules, e.g. DNA or proteins.

UV radiation has a much larger affect on cells than visible light does, especially the rays of 230-275 nm wavelengths. The mechanism of this effect is based on changes to DNA, both directly (e.g. by creating thymine dimer and consequently causing mutation), as well as indirectly, by creating free radicals as in the case of the visible light.

In addition, infrared (IR) radiation may have a negative effect upon cells contained in air - heating up and consequently dehydration.

Biological aerosols

Microorganisms in air occur in a form of colloidal system or the so-called bioaerosol. Every colloid is a system where, inside its dispersion medium, particles of dispersed phase occur whose size is halfway between molecules and particles visible with the naked eye. In the case of biological aerosols, it's the air (or other gases) that has the function of the dispersion medium, whereas microorganisms are its dispersed phase. However, it is quite rare to have microbes independently occurring in air. Usually, they are bound with dust particles or liquid droplets (water, saliva etc.), thus the particles of the bioaerosol often exceed microorganisms in size and may occur in two phases:

- Dust phase (e.g. bacterial dust) or
- Droplet phase (e.g. formed as the result of water-vapour condensation or uring sneezing).

The dust particles are usually larger than the droplets and they settle faster. The difference in their ability to penetrate the respiratory tract is dependent on the size of the particles; particles of the droplet phase can reach the alveoli, but dust particles are usually retained in the upper respiratory tract. The number of

microorganisms associated with one dust particle is greater than in the droplet phase.

The average size of bioaerosols ranges from about 0.02 μm to 100 μm. The sizes of certain particles may change under the influence of outside factors (mainly humidity and temperature) or as a result of larger aggregates forming. By using size criterion, the biological aerosol can be subdivided into the following:

- Fine particles (less than 1μm) and
- Coarse particles (more than 1μm)

Fine particles are mainly viruses, endospores and cell fragments. They possess hygroscopic properties and make-up the so-called nucleus of condensation of water vapour. At high humidity water collects around these particles creating a droplet phase.

Then, the diameter of the particles increases. Coarse particles consist mainly of bacteria and fungi, usually associated with dust particles or with water droplets.

Biological aerosols as a human hazard source .

- What types of dangers are connected to the presence of microorganisms in air?
- Infectious diseases (viral, bacterial, fungal and protozoan),
- Allergic diseases,
- Poisoning (exotoxins, endotoxins, mycotoxins).

Bioaerosols may carry microorganisms other than those which evoke respiratory system diseases. The intestinal microorganisms contained in aerosols may, after settling down, get into the digestive system (e.g. by hands) causing various intestinal illnesses.

Infectious Airborne Diseases

The mucous membrane of the respiratory system is a specific type of a 'gateway' for most airborne pathogenic microorganisms. Susceptibility to infections is increased by dust and gaseous air-pollution, e.g. SO_2 reacts with water that is present in the respiratory system, creating H_2SO_4, which irritates the layer of mucous. Consequently, in areas of heavy air pollution, especially during smog, there is an increased rate of respiratory diseases.

Bioaerosols may, among other things, carry microbes that penetrate organs via the respiratory system. After

settling, microbes from the air may find their way onto the skin or, carried by hands, get into the digestive system (from there, carried by blood, to other systems, e.g. the nervous system). Fungi that cause skin infections, intestinal bacteria that cause digestive system diseases or nervous system attacking enteroviruses are all examples of the above.

Viral diseases

After penetrating the respiratory system with inhaled air, particles of viruses reproduce inside the cuticle cells of both the upper and lower respiratory system. After reproduction some of the viruses stay inside the respiratory system causing various ailments (runny nose, colds, bronchitis, pneumonia), whereas others leave the respiratory system to attack other organs (e.g. chickenpox viruses attack the skin). The most noteworthy viruses are:

Influenza (orthomyxoviruses) Influenza, measles, bronchitis, mumps and pneumonia among newborns (paramyxoviruses)

- German measles (similar to paramyxoviruses)
- Colds (rhinoviruses and Coronaviruses)
- Cowpox and true pox (pox type viruses)
- Chickenpox (cold sore group of viruses)
- Foot-and-mouth disease (picorna type viruses)
- Meningitis, pleurodynia (enteroviruses)
- Sore throat, pneumonia (adenoviruses)

Bacterial diseases

Similarly to viruses, some bacteria that find their way to the respiratory system may also cause ailments of other systems. Especially staphylococcus infections assume various clinical forms (bone marrow inflammation, skin necrosis, intestinal inflammation, pneumonia). Often, a susceptible base for development of various bacterial diseases is first prepared by viral diseases, e.g. S*taphylococcus pneumonia* is usually preceded by a flu or mumps. Bacterial airborne diseases include:

- Tuberculosis (*Mycobacterium tuberculosis*),
- Pneumonia (*Staphylococcus*, *Pneumococci*, *Streptococcus pneumoniae*, less frequently chromatobars of *Klebsiella pneumoniae*),
- Angina, scarlet fever, laryngitis (*Streptococcus*),
- Inflammation of upper and lower respiratory system and meningitis (*Haemophilus influenzae*),

- Whooping cough (chromatobars of *Bordetella pertussis*),
- Diphtheria (*Corynebacterium diphtheriae*),
- Legionnaires disease (chromatobars of *Legionella* genus, among others *L. pneumophila*),
- Nocardiosis (oxygen actinomycetes of *Nocardia* genus).

Fungal diseases

Many potentially pathogenic airborne fungi or the so-called saprophytes live in soil. They usually have an ability to break down keratin (keratinolysis) - difficult to decompose proteins found in horny skin formations, e.g. human or animal hair, feathers, claws. Some of the keratinolytic fungi, the so-called dermatophytes, cause mycosis of the outer skin (dermatosis), as the break down of keratin enables them to penetrate the epidermis. Other fungi, after penetrating the respiratory system, cause deep mycosis (organ), e.g. attacking lungs. The following are examples of airborne fungi diseases:

- Mycosis (*Microsporum racemosum*),
- Deep mycosis: aspergillosis (*Aspergillus fumigatus*), cryptococcus (*Cryptococcus neoformans*).

Protozoan diseases

Some protozoa, which are able to produce cysts that are resistant to dehydration and solar radiation, may also infect humans by inhalation. The most common example of the above is *Pneumocystis carinii* which causes pneumonia. Dangers connected with pathogenic bioaerosols do not concern only human diseases. Other significant diseases are those that attack cultivated plants or farm animals. The following are examples of the above:

- Blight - grain disease caused by *Puccinia graminis*, and
- Aphthous fever - very infectious disease that attacks artiodactylous animals.

Basic Sources of Bioaerosol Emission

There are two basic sources of bioaerosol:

Natural sources: These are mainly soil and water, from which microorganisms are being lifted up by the movement of air, and from organisms such as fungi, that produce gigantic amounts of spores that are dispersed by the wind. Therefore, there are always a given number of microorganisms in the air, as a natural background. It is estimated, that the air is considered to be clean, if the concentration of bacteria and fungi cells does not exceed $1000/m^3$ and $3000/m^3$ respectively. This latter statement is only true when the

concentration of microorganisms consists of saprophytic organisms, not pathogenic organisms. If the concentration of microorganisms in the air exceeds the above values, or contains microorganisms dangerous to humans, then such air is considered to be microbiologically polluted.

Human activities: From the hygienic point of view, living sources of bioaerosols related to human activity, are more important than the natural sources. The emissions from these sources are dangerous due to the following two reasons

- They may distribute pathogenic microorganisms,
- They often cause a high increase of microorganisms in the air, significantly exceeding the natural background.

The emission sources of biological aerosols can have a localized character (e.g. aeration tank) or a surface character (e.g. sewage-irrigated field).

The most important sources of bioaerosol emission are:

- Agriculture and farming-food industry,
- Sewage treatment plants,
- Waste management.

Microbiology of Inside Air

Bacteria are microscopic organisms found in indoor environments typically come from human sources (skin and respiration) or from the outdoors. Like mold, most of the bacteria found in the air in buildings are saprobes meaning they grow on dead organic matter. As far as building envelopes are concerned the primary concern is about bacteria colonies that may grow in damp areas. Most of the bacteria are shed from human skin surfaces. It is not surprising to find hundreds of thousands of bacteria per gram of dust in carpets. As long as the bacterial types are a mixture of those listed below, there is generally no cause for concern. Bacteria may also enter with outdoor air or floodwater and grow in indoor environmental reservoirs. Common indoor reservoirs are water systems, air handling unit and wet organic material. Inadequately maintained air handling system is an important source for bacterial exposure that may lead to allergic type disease. Air handling system must be check for the contaminated water where chest tightness, cough, and fever are associated with a particular indoor environment.

The most abundant bacteria present include

Micrococcus sp

Micrococcus species are human shed bacteria and are caused by the normal shed of skin. It is found in areas of higher occupant density and/or inadequate ventilation. Micrococcus species are generally regarded as being harmless bacteria. Normally, these bacteria are removed through ventilation systems or cleaning procedures such as mopping or vacuuming.

Bacillus sp

Bacillus sp mainly associated with soil and dust. Appropriate temperature and moisture with deposited dust on hard surfaces allow for ideal growing conditions. Most are not serious pathogens.

Staphylococcus sp

Staphylococcus sp is an inhabitant and shed from of the skin surfaces. Among the *Staphylococcus* species that are commonly found indoors is *Staphylococcus aureus*, which is an important pathogen in hospital environments. It shouldn't be a matter of concern unless it is the predominating colony found on air or surface samples in indoor environment.

Gram positive rod

Gram positive rod bacteria mainly associated with soil and dust. Appropriate temperature and moisture allow for ideal growing conditions on carpet, wall, furniture's etc. Most are not serious pathogens. These bacteria can be removed by good house keeping practice and adequate ventilation systems.

Gram negative rod

These organisms are rare in indoor environments, if they found in higher concentration may be related to the bio aerosol of contaminated water or other contamination of wet/moist surfaces or materials, or possibly air handling units systems in which they are proliferating. Some Gram negative bacteria (or endotoxin extracted from their walls) have been shown to provoke symptoms of fever. Occasionally, growth in air handling units has been great enough for aerosols to be generated which contained sufficient allergenic cells to have caused the acute pneumonia like symptoms. If there has been a sewage spill or flood, then Gram negative bacteria are to be expected and such environments should be thoroughly cleaned with disinfectant.

Identification of bacteria by cultural analysis is based on morphology (e.g., spherical, rod-shaped, etc.), by staining reactions (e.g. Gram positive or negative) and by the pattern of results from a series of biochemical tests.

Enumeration of bacteria from air:

Various methods commonly applied for enumeration and detection of microorganisms can be subdivided into:

- **Microscopic methods**
- **Culture methods**
- **Combination of both**

Microscopic Methods

These consist of

- Letting air through a membrane filter or placing a glass coated with a sticky substance (e.g. vaseline), in the path of air
- Staining of the trapped microorganisms and
- Microscopic testing consisting of cell counting

Staining with acridine orange and examination under a fluorescence microscope is often applied. The final result is given as a total number of microbes in 1 m^3 of air. The advantage of this method is that it allows the detection of live and dead microbes in air, as well as those, which do not abundantly flourish in culture media. Due to this, the number of microbes determined is usually higher by one order of magnitude than in culture methods. In addition, it is possible to detect and identify other biological agents e.g. plant pollen, allergenic mites, abiotic organic dust (fragments of skin, feathers, plants, etc.).

However the methods have a serious drawback: inability to determine the species of microbes (bacteria, fungi, viruses).

Culture Methods

These methods consist of transferring microbes from air onto the surface of the appropriate culture medium. After a period of incubation at optimal temperature, the formed colonies are counted and the result is given as cfu/m^3 of air (colony forming units). Because a colony can form not only from a single cell, but also from a cluster of cells, the air may contain more microbes than suggested by the CFU result. Besides, the method allows the detection of only the cells that are viable and those which are able to grow upon the medium used. Microbes transferred to the culture medium require resuscitation as they were subjected to the influence of unfavourable conditions. Therefore it is recommended to supplement the culture mediums are required to be supplemented with components such as betaine and catalase. Betaine, the methylic derivative of the glycine amino acid, is utilized by bacteria to maintain osmotic balance, and as a donor of methylic groups it is essential during the processes of biosynthesis. Catalase however breaks down harmful peroxides created in air as a result of UV radiation.

However, testing of viruses differs significantly from the methods utilized for other organisms because:

- They may develop only in living cells, therefore they require tissue cultures (e.g. the epithelium of human trachea or monkey's kidney) or, in the case of bacteriophages, bacterial cultures,
- Species identification of detected viruses is meticulous and, among other things, consists of performing electrophoresis or utilizing antiserum that contains antibodies of common viruses,
- Drawing large quantities of air is essential (over 1000 dm^3, at least one order of magnitude higher than in the case of bacteria), as the amount of viruses in air is rather small (this especially concerns the enteroviruses).

After transferring the viruses onto the surface of a single-layer culture, the viruses penetrate the cells, reproduce in them, and after their destruction attack the neighboring cells. Consequently, the areas around the initial places of the cell infections get cleared of cells – this clearing is called plaques. Therefore, the number of viruses detected is given as the number of units that form the plaques, in short pfu/m^3 (plaque forming units). It has to be pointed out though, that such a method only allows the detection of viruses capable of infecting the utilized cells.

Sampling of Air

There are four basic ways of sampling the air for use in culture methods:

- Koch's sedimentation method
- Filtration method (also used in microscopic methods)
- Centrifugation
- Impact methods

Sedimentation Method

This ‘Settling Plate Technique’ based on this approach is the simplest and is often used by air microbiologists. The principle behind this method is that the bacteria carrying particles are allowed to settle onto the medium for a given period of time and incubated at the required temperature. A count of colonies formed shows the number of settled bacteria containing particles. In this method petridishes containing an agar medium of known surface area are selected so that the agar surface is dry without any moisture. Choice of the medium depends upon the kind of microorganisms to be enumerated. For an overall count of pathogenic, commensal and saprophytic bacteria in air blood agar can be used. For detecting a particular pathogen which may be present in only small numbers, an appropriate selective medium may be used. Malt extract agar can be used for molds. The plates are labeled appropriately about the place and time of sampling, duration of exposure etc. Then the plates are uncovered in the selected position for the required period of time. A Petri dish containing agar medium is kept covered and, at the time of sampling, the cover is removed from the Petri dish so that the agar surfaces is exposed to air for a few minutes. The Petri dish is now incubated. One can see a certain number of colonies developing on agar medium. Each colony represents a particle carrying microorganisms which has fallen on the agar surface. The optimal duration of exposure should give a significant and readily countable number of well isolated colonies, for example about 30-100 colonies. Usually it depends on the dustiness of air being sampled. In occupied rooms and hospital wards the

time would generally be between 10 to 60 m. During sampling it is better to keep the plates about I metre above the ground. Immediately after exposure for the given period of time, the plates are closed with the lids. Then the plates are incubated for 24 hrs at 37℃ for aerobic bacteria and for 3 days at 22℃ for saprophytic bacteria. For molds incubation temperature varies from 10-50℃ for 1-2 weeks. After incubation the colonies on each plate are counted and recorded as the number of bacteria carrying particles settling on a given area in a given period of time.

The use of settle plates is not recommended when sampling air for fungal spores, because single spores can remain suspended in air indefinitely. Settle plates have been used mainly to sample for particulates and bacteria either in research studies or during epidemiologic investigations. Results of sedimentation sampling are typically expressed as numbers of viable particles or viable bacteria per unit area per the duration of sampling time (i.e. CFU/area/time); this method can not quantify the volume of air sampled. Because the survival of microorganisms during air sampling is inversely proportional to the velocity at which the air is taken into the sampler, one advantage of using a settle plate is its reliance on gravity to bring organisms and particles into contact with its surface, thus enhancing the potential for optimal survival of collected organisms. This process, however, takes several hours to complete and may be impractical for some situations.

Limitation

Though the method has the advantage of simplicity, it has certain limits.

- In this method only the rate of deposition of large particles from the air, not the total number of bacteria carrying particles per volume, is measured.
- Growth of bacteria in the settled particles may be affected by the medium used since not all microorganisms are growing well on all media.
- Moreover since air currents and any temporary disturbances in the sampling area can affect the count, many plates have to be used.
- Since only particles of certain dimensions tend to settle on to the agar surface and, also, the volume of air entering inside the Petri dish is not known, this technique gives only a rough estimate and can be used only to isolate air-borne microorganisms.
- However, one can gather information about the kind of air-borne microbes occurring in a particular area by repeated use of settling plate technique for a fixed period of time.

Agar plates showing colonies of microbes present in Air

Filtration Methods

The methods consist of using an aspirator to suck in a given volume of air, passing it through a sterile absorbing substance (liquid or solid) and transferring the filtered microbes onto the appropriate culture medium. After a pre-determined time of incubation the resulting colonies are counted. Most often, a membrane filter or a physiological solution (0.85% NaCl) is utilized for the filtration of air. Filtration using liquids (sometimes classified as the impact method) is one of the most often used and highly valued techniques of sampling bioaerosol. It results in high output of microbe isolation as well as significant survival of the filtered microbes. The method may be utilized in virus testing as long as the remaining microbes are neutralized (e.g. with chloroform) and the liquid is concentrated before its introduction into the cell culture.

The filtration process through membrane filters allows the utilization of both culture methods (filters containing microbes are placed directly upon the culture media or are rinsed and then inoculated) as well as the microscopic methods (filters are stained and observed under a microscope).

These are simple methods for collecting particles from air. The filter can be made of any fibrous or granular material like sand, glass fibre and alginate wool (in phosphate buffer). However, recovery of organisms for culture is not so easy. The membrane filter devices are adaptable to direct collection of microorganisms by filtration of air. These methods are also rather inexpensive and not complicated; they possess two significant advantages over the sedimentation methods:

- The volume of the air tested is known,
- It is possible to detect the very small aerosol that creates the respiratory fraction (nevertheless it is still impossible to determine its size

Tubesampler

This is one of the oldest devices for collecting and enumerating microorganisms in the air. It consists of a tube with an inlet at the top and an outlet at the bottom which is narrower than the top end. Near the bottom there is a filter of wet sand which is supported by a cotton plug below. The entire device can be sterilized. After sterilization the air to be sampled is allowed to pass through the sand and cotton. Microorganisms as well as dust particles containing microorganisms in the air are deposited in the sand filter as the air passes through it. Later the sand is washed with broth and a plate count is made from the broth by taking aliquotes of the broth.

Millipore filter

This type of filters is made of pure and biologically inert cellulose ethers. They are prepared as thin porous, circular membranes of about 150 µm thickness. The filters have different porosity. The assemblage contains a funnel shaped inlet and a tube like outlet. In between these two the filter is fitted. The outlet may be connected to a vacuum pump to suck known amount of air. After collecting required volume of air through the filter, it can directly be placed onto the surface of a solid medium. After incubation colonies formed can be

counted.

However, the disadvantage of this method is that it has a significantly low output as the process of passing the air through pores of the filter creates resistance. That's why the method is not recommended for microbe testing, but is routinely put to use in detection of endotoxins in air.

Centrifugation Methods

Air centrifuge

The first primitive type of air centrifuge was developed by Wells in 1993. The principle of air centrifuge is that the particles from air are centrifuged onto the culture medium. In his air centrifuge sampled air was passed along a tube which was rotated rapidly on its long axis. The inner surface of the tube was lined with culture medium and any bacteria containing particle deposited on it grew into a colony on incubation. A modern version of this centrifuge is the Reuter centrifugal air sampler, which is portable and battery powered. It resembles a large cylindrical torch with an open ended drum at one end. The drum encloses impeller blades which can be rotated by battery power when switched on. A plastic strip coated with culture medium can be inserted along the inner side of the drum. Air is drawn into the drum and subjected to centrifugal acceleration. This causes the suspended particles to impact on the culture medium. After sampling the strip is removed from the instrument and incubated at 37°C for 48 h. Later the colonies can be counted. Advantage of this sampler is that it is very convenient for transportation and use. However, the disadvantage is that it is less efficient than the slit sampler in detecting particle below 5 mm in diameter. More over the size of the air being sampled cannot be accurately controlled.

Impact methods

These methods consist of using an aspirator to suck in a pre-determined amount (volume) of air, which collides with the nutrient agar at high speed. It causes the microbes in the air to stick to the surface, which after a specific time of incubation, form colonies. The impact methods are the most highly valued and most often used methods of detecting microbes in air. Their biggest advantage is the possibility of detecting and determining the respiratory fraction of the bioaerosol, in other words, determining the size distribution of its particles. The methods can be utilized to test viruses (trapped microbes are swept from the surface of the culture medium and, after the elimination of other microbes with chloroform, introduced into the cell culture).

A disadvantage for the impact method is a decline in the microbes viability caused by the shock of a sudden collision with nutrient agar and also a possibility of the nutrient culture getting overgrown in cases of high air pollution. The above stated methods are usually not cheap. The most widely known device that is based on the impact technique is the Andersen's apparatus, in which the air is drawn in passes through six vertically positioned sieves. A petri dish with nutrient agar is placed underneath each sieve. The speed of the passing air increases as it passes through the consecutive sieves, consequently causing greater impact force as it collides with the sieves. As a result, the heaviest (largest) particles settle upon the first sieve, whereas the lighter (smaller) ones are drawn in by the current of the passing air. As they pass through the consecutive sieves, the increasingly smaller and faster particles collide with the nutrient agar. Consequently the particles of the biological aerosol are sorted according to their size and the colonies are then derived from particles of particular size. This way, by counting the colonies upon the consecutive plates, it is possible to determine the

ratio of particles which settle in the upper (higher positioned plates) and lower respiratory system (lower plates).

Sampling of measured volume of air

An improvised method wherein a measured volume of air is sampled has also been developed. These are sieve and slit type devices. A sieve device has a large number of small holes in a metal cover, under which is located a petridish containing an agar medium. A measured volume of air is drawn, through these small holes. Airborne particles impinge upon the agar surface. The plates are incubated and the colonies counted. In a slit device the air is drawn through a very narrow slit onto a petridish containing agar medium. The slit is approximately the length of the petridish. The petridish is rotated at a particular speed under the slit. One complete turn is made during the sampling operation

Selection of air sampler

The following factors must be considered when choosing an air sampling instrument:

- Viability and type of the organism to be sampled
- Compatibility with the selected method of analysis
- Sensitivity of particles to sampling
- Assumed concentrations and particle size
- Whether airborne clumps must be broken (i.e. total viable organism count vs. particle count)
- Volume of air to be sampled and length of time sampler is to be continuously operated
- Background contamination
- Ambient conditions
- Sampler collection efficiency
- Effort and skill required to operate sampler
- Availability and cost of sampler, plus back-up samplers in case of equipment malfunction
- Availability of auxiliary equipment and utilities (e.g. vacuum pumps, electricity, and water)

Airborne Diseases

Airborne disease can spread when people with certain infections cough, sneeze, or talk, spewing nasal and throat secretions into the air. Some viruses or bacteria take flight and hang in the air or land on other people or surfaces.

When you breathe in airborne pathogenic organisms, they take up residence inside you. You can also pick up germs when you touch a surface that harbors them, and then touch your own eyes, nose, or mouth.

Because these diseases travel in the air, they’re hard to control. Keep reading to learn more about the common types of airborne diseases and what you can do to protect yourself from catching them.

Common airborne diseases

Particles that cause airborne diseases are small enough to cling to the air. They hang on dust particles, moisture droplets, or on the breath until they are picked up. They are also acquired by contact with bodily fluids, such as mucus or phlegm.

Once the pathogens are inside the body, they multiply until someone has the disease.

Common airborne diseases include:

- **Influenza**: The seasonal "flu" virus spreads easily from person to person. There are many strains of the flu, and it continually changes to adapt to the human immune system.
- **The common cold**: The condition called "a cold" is usually caused by a rhinovirus. There are many rhinoviruses, and the strains change to make it easier to infect humans.
- **Varicella zoste**r: This virus causes chickenpox and spreads easily among young children. The rash is typically widespread on the body and made up of small red spots that turn into itchy blisters, which scab over in time. Chickenpox is spread for about 48 hours before a rash shows, which is how it infects others so successfully. It is usually spread through the air or by touching the rash.
- **Mumps**: This virus affects the glands just below the ears, causing swelling and, in some cases, loss of hearing. Vaccination is considered important to prevent the disease.
- **Measles**: This illness is caused by contact with a person who has the measles virus, or by inhaling particles from their sneezes or cough. As with mumps, vaccination is essential for preventing the spread of this disease.
- **Whooping cough (pertussis)**: This is a contagious, bacterial illness that causes the airways to swell. The hacking cough that results is persistent and generally treated with antibiotics early on to prevent damage.

Uncommon airborne diseases include:

- **Anthrax**: This is a bacterial disease that infects the body when a person inhales anthrax spores. It causes nausea and flu symptoms. Inhaled anthrax is difficult to diagnose because it resembles other diseases such as flu. Anthrax is treated with antibiotics to stop it worsening.
- **Diphtheria**: A rare bacterial disease, diphtheria damages the respiratory system and attacks the heart, kidneys, and nerves. Its rarity may be due to widespread vaccination. Diphtheria can be treated with antibiotics.

- **Meningitis**: Meningitis swells the membranes around the brain and spinal cord. It is a bacterial or viral infection, but is also caused by an injury or fungal infection. Common symptoms include a persistent headache, fever, and skin rash.

The length of an illness caused by a common airborne disease can vary from a few days to weeks, but it is usually dealt with easily. Uncommon airborne diseases may require additional treatment.

Prevention

Airborne diseases are wipespread and easily treatable, in most cases. Complete prevention is difficult, but there are some ways to reduce exposure to the pathogens that cause them.

The Maine Department of Health and Human Services suggest that carrying out good sanitary habits can greatly reduce the risk of transmitting airborne diseases.

Wearing a hospital mask in public, and covering sneezes and coughs with an elbow or tissue, are some of the good habits that are recommended.

Regular hand-washing can also help lower the spread of bodily fluids that may contain disease-causing germs.

Ventilation and air management

The United States Environmental Protection Agency (EPA) recommend increasing ventilation to help exchange air between the inside and outside of a building.

In an unventilated area, pathogens, pollutants, and moisture can build up to unsafe levels. Cleaning the air with a filter is another part of keeping an area as free of pollutants and pathogens as possible.

A few basic filtering methods include mechanical air filters, UV purification, HEPA filters, and ion generators.

Symptoms

Many airborne diseases have symptoms similar to the common cold or influenza. They include:

- cough
- chill
- muscle and body aches

- fatigue
- congestion
- sneezing
- runny or stuffy nose
- sore throat
- slight body aches or headaches
- sinus pressure

Some people also experience a low fever or general sluggishness with these symptoms.

Treatment

It is important for people to talk to a doctor as soon as they experience symptoms to avoid any complications and to begin treatment.

Symptoms of the common cold can be treated, but the illness tends to go away without treatment. The flu runs its course over a few days before someone starts to recover. In the case of chickenpox, the immune system usually deals with the virus on its own.

While airborne diseases are common, serious complications are much more rare and normal vaccinations reduce the risk, substantially.

Air Sanitation:

- ✓ An **air sanitizer** is a sanitizer that acts on airborne microbiological organisms or microorganisms.
- ✓ A sanitizer is a disinfectant that is intended to disinfect or sanitize, reducing or mitigating growth or development of microbiological organisms including bacteria, fungi or viruses on inanimate surfaces in the household, institutional, and/or commercial environment and whose labeled directions for use result in the product being discharged to publicly owned treatment works

Need for Air Sanitation:

- ❖ Microbial diseases transferred over respiratory droplets or dust is classified as airborne. Some common air borne diseases include flu, tuberculosis, measles, etc.

- ❖ Excess patient overload in emergency service areas mean that poor attention is paid to thorough cleaning, disinfection of rooms and air-conditioning ducts, which otherwise would require closing the

concerned area. Over a period of time, this leads to accumulation of lint, fibre, dust and fungal growth.

- ❖ Fumigation allows overcoming many critical aspects of wiping in both procedure and validation

Method of Air Sanitation

Traditionally, we relied on pressurized steam sterilization (autoclaving), radiation (UV and gamma rays), and other various liquid chemical disinfectants.

Fumigation is an effective form of disinfection, as gas can spread rapidly within the space to be disinfected, and all the hidden corners and blocked surfaces. The disinfectant is vapourized into fine droplets using a fogger and blown into the air. Upon settling down, these droplets reveal their effect.

Conventional Air disinfectants

Formaldehyde is a disinfectant belonging to the aldehyde group, conventionally used for air sterilization. Although effective against bacteria, fungi, and many viruses and spores, formaldehyde is known to be highly carcinogenic. Glutaraldehyde is rapidly effective against a wide range of microorganisms, but has toxic health effects, and may leave a greasy residue

Disadvantages

- Formaldehyde is slow acting, and has poor penetration.
- It is also toxic in nature, causing burning sensations, pulmonary oedema, pneumonitis, etc.
- Carcinogenic, resulting in nasopharyngeal cancer in humans.
- Does not work outside its effective temperature range.
- Glutaraldehyde has adverse health effects like nausea, eye and skin irritation, etc.
- Requires assisted ventilation

Advantages of ALSTASAN SILVOX:

- ➢ Prevents air borne diseases such as Leginellosis, Swine flu, influenza, tuberculosis, pneumonia, anthrax, pox, measles, etc.
- ➢ No scent or odour
- ➢ No neutralization required
- ➢ No toxic effect on humans
- ➢ Eco-friendly, forming water and oxygen

Biogeochemical cycles:

Chemical substance moves through biotic (biosphere) and abiotic (lithosphere, atmosphere, and hydrosphere) compartments of Earth.

Nitrogen Cycle

Definition

The nitrogen cycle refers to the cycle of nitrogen atoms through the living and non-living systems of Earth. The nitrogen cycle is vital for life on Earth. Through the cycle, atmospheric nitrogen is converted to a form which plants can incorporate into new proteins.

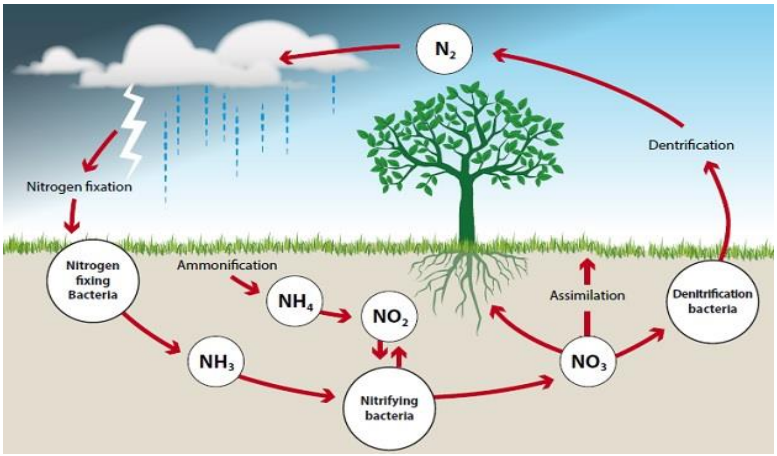
Nitrogen Cycle Explained

Nitrogen was originally formed in the hearts of stars through the process of nuclear fusion. When ancient stars exploded, they flung nitrogen-containing gases across the Universe. **When the Earth was formed, nitrogen gas was the main ingredient in its atmosphere.**

Today, the Earth's atmosphere is about 78% nitrogen, about 21% oxygen, and about 1% other gases. This is an ideal balance because too much oxygen can actually be toxic to cells. In addition, oxygen is flammable. Nitrogen, on the other hand, is inert and harmless in its gaseous form. **However, nitrogen gas is not accessible to plants and animals for use in their cells.**

Nitrogen Cycle Steps

The basic steps of the nitrogen cycle are illustrated here:



Nitrogen Fixation

In the process of nitrogen fixation, bacteria turn nitrogen gas from the atmosphere into ammonia.

These nitrogen-fixing bacteria, often called "diazotrophs," have an enzyme called "nitrogenase" which combines nitrogen atoms with hydrogen atoms. Ammonia is a nitrogen compound that can dissolve in water, and is easier for other organisms' enzymes to interact with.

Interestingly, the enzyme nitrogenase can only function when oxygen isn't present. As a result, organisms that use it have had to develop oxygen-free compartments in which to perform their nitrogen fixation!

Common examples of such nitrogen-free compartment sare the *Rhizobium* nodules found in the roots of nitrogen-fixing legume plants. The hard casing of these nodules keeps oxygen out of the pockets where *Rhizobium* bacteria do their valuable work of converting nitrogen gas into ammonia.

Nitrification

In nitrification, a host of soil bacteria participate in turning ammonia into nitrate – the form of nitrogen that can be used by plants and animals. **This requires two steps, performed by two different types of bacteria.**

First, soil bacteria such as *Nitrosomonas* or *Nitrococcus* convert ammonia into nitrogen dioxide. Then another type of soil bacterium, called *Nitrobacter*, adds a third oxygen atom to create nitrate.

These bacteria don't convert ammonia for plants and animals out of the goodness of their hearts. Rather, they are "chemotrophs" who obtain their energy from volatile chemicals. By metabolizing nitrogen along with oxygen, they obtain energy to power their own life processes.

The process can be thought of as a rough (and much less efficient) analog to the cellular respiration performed by animals, which extract energy from carbon-hydrogen bonds and use oxygen as the electron acceptor, yielding carbon dioxide at the end of the process.

Nitrates – the end product of this vital string of bacterial reactions – can be made artificially, and are the main ingredient in many soil fertilizers. You may actually hear such fertilizer referred to as "nitrate fertilizer." By pumping the soil full of nitrates, such fertilizers allow plants to grow large quickly, without being dependent on the rate at which nitrogen-fixing bacteria do their jobs!

Interestingly, high-energy environments such as lightning strikes and volcanic eruptions can convert nitrogen gas directly into nitrates – but this doesn't happen nearly enough to keep modern ecosystems healthy on its own!

Assimilation

In nitrogen assimilation, plants finally consume the nitrates made by soil bacteria and use them to make nucleotides, amino acids, and other vital chemicals for life.

Plants take up nitrates through their roots and use them to make amino acids and nucleic acids from scratch. Animals that eat the plants are then able to use these amino acids and nucleic acids in their own cells.

Ammonification

Now we have moved nitrogen from the atmosphere into the cells of plants and animals.

Because there is so much nitrogen in the atmosphere, it may seem that the process could stop there – **but the atmosphere's supply is not infinite, and keeping nitrogen inside plant and animal cells would eventually result in big changes to our soil, our atmosphere, and our ecosystems!**

Fortunately, that's not what happens. In a robust ecosystem like ours, anywhere that energy has been put into creating an organic chemical, there is another form of life that is waiting to extract that energy by breaking those chemical bonds.

A process called "ammonification" is performed by soil bacteria which decompose dead plants and animals. During the process, these decomposers break down amino acids and nucleic acids into nitrates and ammonia and release those compounds back into the soil.

There, the ammonia may be taken up again by plants and nitrifying bacteria. Alternatively, the ammonia may be converted back into atmospheric nitrogen through the process of denitrification.

Denitrification

In the final step of the nitrogen cycle, anaerobic bacteria can turn nitrates back into nitrogen gas.

This process, like the process of turning nitrogen gas into ammonia, **must happen in the absence of oxygen.** As such it often occurs deep in the soil, or in wet environments where mud and muck keep oxygen at bay.

In some ecosystems, this denitrification is a valuable process to prevent nitrogen compounds in the soil from building up to dangerous levels.

Nitrogen Cycle Important:

Nitrogen is an essential ingredient for life as we know it. Its unique chemical bonding properties allow it to create structures such as DNA and RNA nucleotides, and the amino acids from which proteins are built. **Without nitrogen, these molecules would not be able to exist.**

It's thought that the first nucleotides and amino acids formed naturally under the volatile conditions of early Earth, where energy sources like lightning strikes could cause nitrogen and other atoms to react and form complex structures

This process might have naturally produced self-replicating organic chemicals – but in order to reproduce and evolve, life needed to figure out how to make these nitrogen compounds on demand.

Today, "nitrogen fixers" are organisms that can turn nitrogen gas from the atmosphere into nitrogen compounds that other organisms can use to produce nucleic acids, amino acids, and more. These nitrogen fixers are such a vital part of the ecosystem that agriculture cannot occur without them.

Ancient peoples learned that if they did not alternate growing nitrogen-consuming crops with nitrogen-fixing crops, their farms would become fallow and unable to support growth. Today, most artificial fertilizers contain life-giving nitrogen compounds as their main ingredient to make the soil more fertile.

Carbon Cycle

Carbon cycle portrays the movement of carbon in elemental and combined states on earth. Diamond and graphite are the elemental forms of carbon and in combined state, it is found as carbonates in minerals and as carbon dioxide gas in the atmosphere.

Carbon Cycle Definition

Carbon cycle is the process where carbon compounds are interchanged among the biosphere, geosphere, pedosphere, hydrosphere, and atmosphere of the earth.

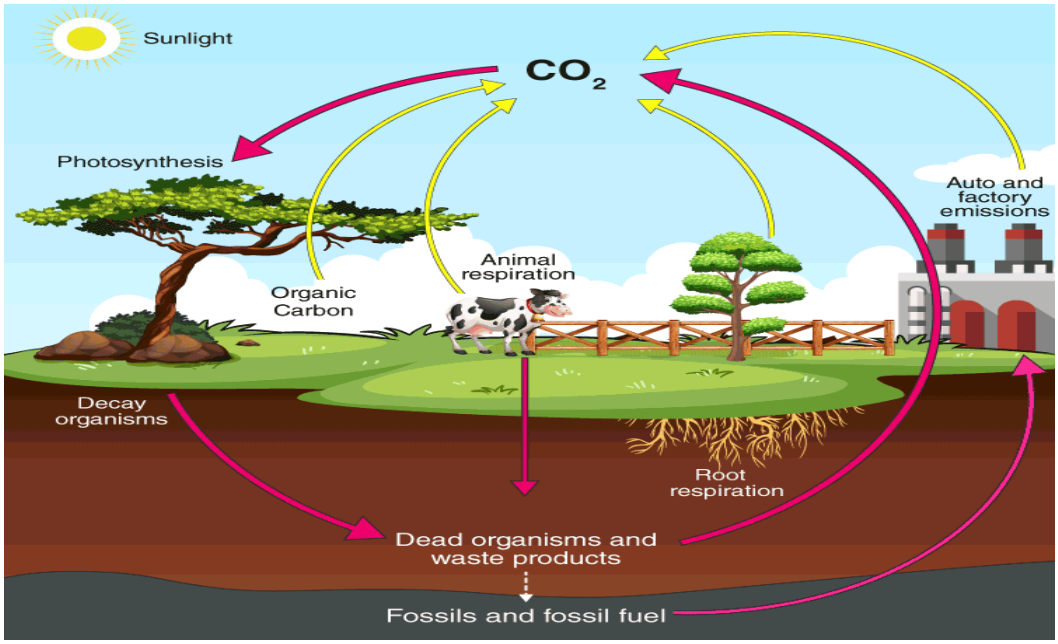
Carbon Cycle Steps

Following are the major steps involved in the process of the carbon cycle:

1. Carbon present in the atmosphere is absorbed by plants for photosynthesis.
2. These plants are then consumed by animals, and carbon gets bioaccumulated into their bodies.
3. These animals and plants eventually die, and upon decomposing, carbon is released back into the atmosphere.
4. Some of the carbon that is not released back into the atmosphere eventually become fossil fuels.
5. These fossil fuels are then used for man-made activities, which pumps more carbon back into the atmosphere.

Carbon Cycle Diagram

The carbon cycle diagram below elaborates the flow of carbon along different paths.



Carbon Cycle diagram showing the flow of carbon, its sources and paths.

Carbon Cycle on Land

Carbon in the atmosphere is present in the form of carbon dioxide. Carbon enters the atmosphere through natural processes such as respiration and industrial applications such as burning fossil fuels. The process of photosynthesis involves the absorption of CO_2 by plants to produce carbohydrates. The equation is as follows:

$$CO_2 + H_2O + \text{energy} \rightarrow CH_2O + O_2$$

Carbon compounds are passed along the food chain from the producers to consumers. The majority of the carbon exists in the body in the form of carbon dioxide through respiration. The role of decomposers is to eat the dead organism and return the carbon from their body back into the atmosphere. The equation for this process is:

$$CH_2O + O_2 \rightarrow CO_2 + H_2O$$

Importance of Carbon Cycle

Even though carbon dioxide is found in small traces in the atmosphere, it plays a vital role in balancing the energy and traps the long-wave radiations from the sun. Therefore, it acts like a blanket over the planet. If the carbon cycle is disturbed it will result in serious consequences such as climatic changes and global warming.

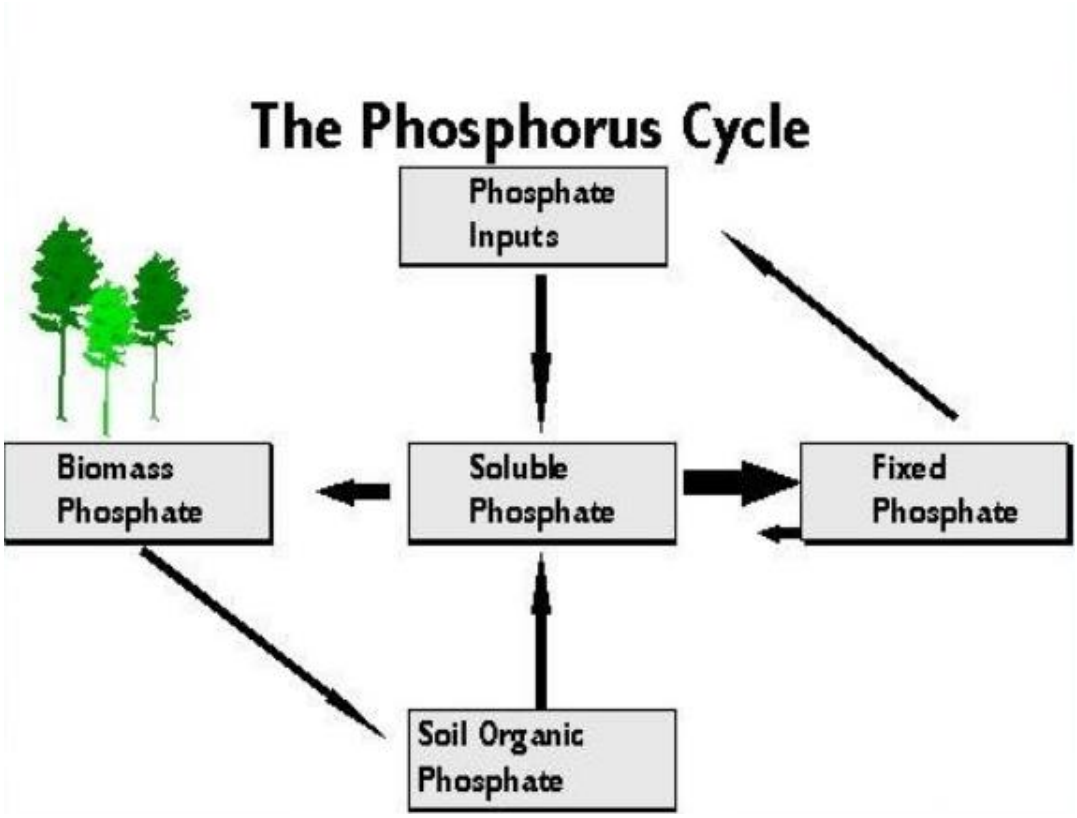
Carbon is an integral component of every life form on earth. From proteins and lipids to even our DNA. Furthermore, all known life on earth is based on carbon. Hence, the carbon cycle, along with the nitrogen cycle and oxygen cycle, plays a vital role in the existence of life on earth.

Phosphorus Cycle

The phosphorus cycle is the process by which phosphorus moves through the lithosphere, hydrosphere, and biosphere. Phosphorus is essential for plant and animal growth, as well as the health of microbes inhabiting the soil, but is gradually depleted from the soil over time. The main biological function of phosphorus is that it is required for the formation of nucleotides, which comprise DNA and RNA molecules. Specifically, the DNA double helix is linked by a phosphate ester bond. Calcium phosphate is also the primary component of mammalian bones and teeth, insect exoskeletons, phospholipid membranes of cells, and is used in a variety of other biological functions. The phosphorus cycle is an extremely slow process, as various weather conditions (e.g., rain and erosion) help to wash the phosphorus found in rocks into the soil. In the soil, the organic matter (e.g., plants and fungi) absorb the phosphorus to be used for various biological processes.

Phosphorus Cycle Steps

The phosphorus cycle is a slow process, which involves five key steps, as shown in the diagram below and described as follows:



Weathering

Since the main source of phosphorus is found in rocks, the first step of the phosphorus cycle involves the extraction of phosphorus from the rocks by weathering. Weather events, such as rain and other sources of erosion, result in phosphorus being washed into the soil.

Absorption by Plants and Animals

Once in the soil, plants, fungi, and microorganisms are able to absorb phosphorus and grow. In addition, phosphorus can also be washed into the local water systems. Plants can also directly absorb phosphorus from the water and grow. In addition to plants, animals also obtain phosphorus from drinking water and eating plants.

Return to the Environment via Decomposition

When plants and animals die, decomposition results in the return of phosphorus back to the environment via the water or soil. Plants and animals in these environments can then use this phosphorus, and step 2 of the cycle is repeated.

Importance of Phosphorous:

- ❖ Humans have had a significant impact on the phosphorus cycle due to a variety of human activities, such as the use of fertilizer, the distribution of food products, and artificial eutrophication.
- ❖ Fertilizers containing phosphorus add to the phosphorus levels in the soil and are particularly detrimental when such products are washed into local aquatic ecosystems. When phosphorus is added to waters at a rate typically achieved by natural processes, it is referred to as natural eutrophication.
- ❖ A natural supply of phosphorus over time provides nutrients to the water and serves to increase the productivity of that particular ecosystem.
- ❖ However, when foods are shipped from farms to cities, the substantial levels of Phosphorus that is drained into the water systems is called artificial or anthropogenic eutrophication.
- ❖ When levels of phosphorus are too high, the overabundance of plant nutrients serves to drive the excessive growth of algae.
- ❖ However, these algae die or form algae blooms, which are toxic to the plants and animals in the ecosystem. Thus, human activities serve to harm aquatic ecosystems, whenever excess amounts of phosphorus are leached into the water.

Sulphur Cycle

Sulphur is one of the most abundant elements on the earth. It is a yellow, brittle, tasteless, odourless non-metal. Sulphur is present in all kinds of proteins. Plants directly absorb sulphur-containing amino acids such as methionine, cystine, and cysteine.

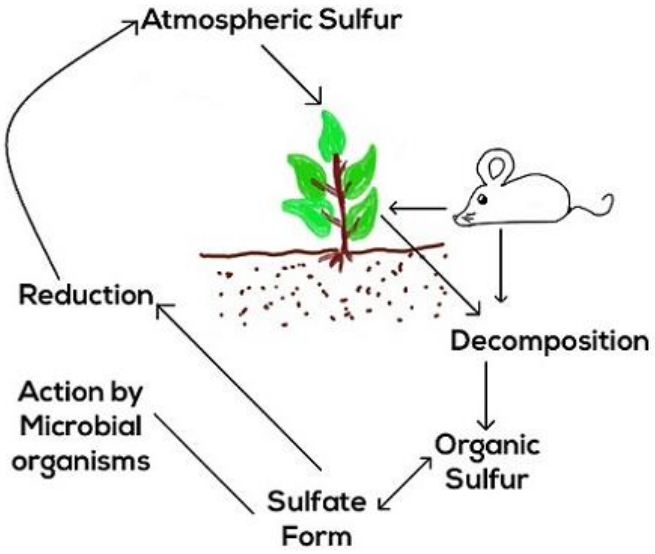
Sulphur is released into the atmosphere by the burning of fossil fuels, volcanic activities, and decomposition of organic molecules.

On land, sulphur is stored in underground rocks and minerals. It is released by precipitation, weathering of rocks and geothermal vents.

Sulphur Cycle

The process of sulphur cycle is explained below:

- The sulphur is released by the weathering of rocks.
- Sulphur comes in contact with air and is converted into sulphates.
- Sulphates are taken up by plants and microbes and are converted into organic forms.
- The organic form of sulphur is then consumed by the animals through their food and thus sulphur moves in the food chain.
- When the animals die some of the sulphur is released by decomposition while some enter the tissues of microbes.
- There are several natural sources such as volcanic eruptions, evaporation of water, and breakdown of organic matter in swamps, that release sulphur directly into the atmosphere. This sulphur falls on earth with rainfall.



Steps of Sulphur Cycle

Following are the important steps of the sulphur cycle:

Decomposition of Organic Compounds

Protein degradation releases **amino acids** that contain sulphur. Sulphates are reduced to H_2S by the action of Desulfotomaculum bacteria.

Oxidation of Hydrogen Sulphide to Elemental Sulphur

Hydrogen sulphide oxidises to produce elemental sulphur. Certain photosynthetic bacteria from the families Chlorobiaceae and Chromatiaceae initiate the oxidation process.

Oxidation of Elemental Sulphur

Elemental sulphur present in the soil cannot be utilized directly by the plants. Therefore, it is converted into sulphates by chemolithotrophic bacteria.

Reduction of Sulphates

Sulphates are reduced to hydrogen sulphide by *Desulfovibrio desulfuricans.* This occurs in two steps:

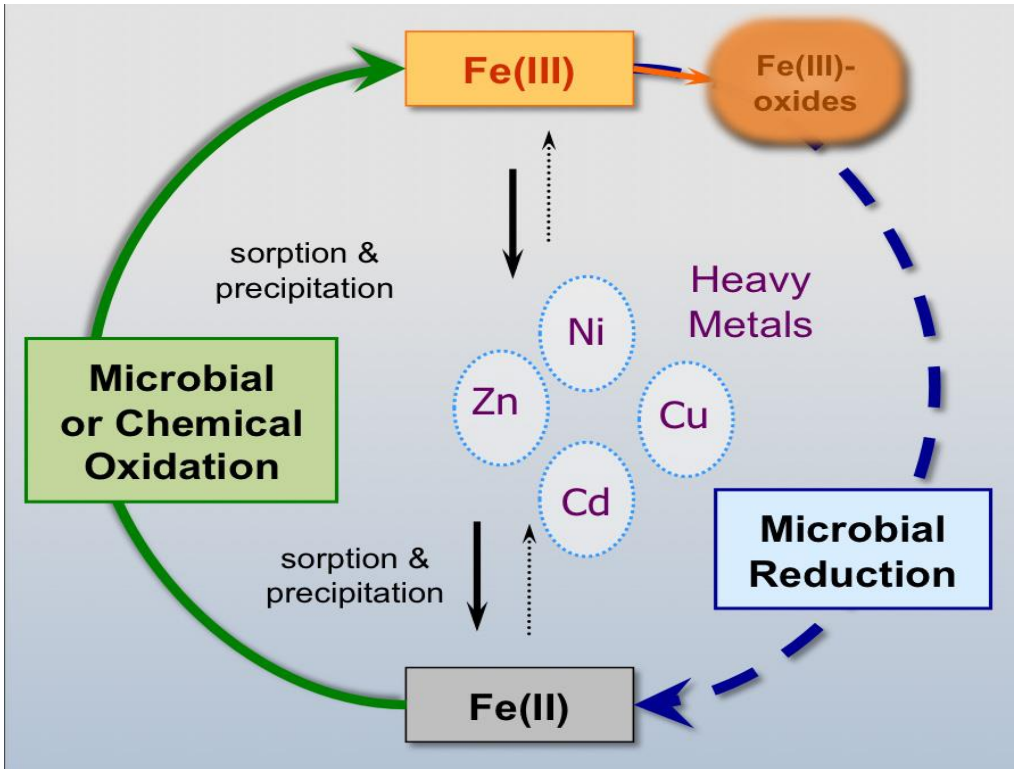
- Firstly, the sulphates are converted to sulphites utilizing ATP.
- Secondly, the reduction of sulphite to hydrogen sulphide.

Iron cycle

The **iron cycle** (Fe) is the biogeochemical cycle of iron through the atmosphere, hydrosphere, biosphere and lithosphere. While Fe is highly abundant in the Earth's crust,it is less common in oxygenated surface waters. Iron is a key micronutrient in primary productivity,and a limiting nutrient in High-Nutrient, Low-Chlorophyll (HNLC) regions of the ocean. A critical component of the iron cycle is aeolian dust, which is transported from the Earth's land via the atmosphere to the ocean.

Iron exists in a range of oxidation states from -2 to +7; however, on Earth it is predominantly in its +2 or +3 redox state. The cycling of iron between its +2 and +3 oxidation states is referred to as the iron cycle. This process can be entirely abiotic or facilitated by microorganisms. Some examples of this include the rusting of iron-bearing metals (in this case, Fe^{2+} is abiotically oxidized to Fe^{3+}) by oxygen, and the abiotic reduction of Fe^{3+} to Fe^{2+} by iron-sulfide minerals or the biological cycling of Fe^{2+}-oxidizing microbes.

Iron is an essential micro-nutrient for almost every life form, and is a primary redox-active metal on Earth. Due to the high reactivity of Fe^{2+} with oxygen and low solubility of Fe^{3+}, iron is a limiting nutrient in most regions of the world. Thus, the iron cycle is intrinsically linked to the cycling of other biologically-important elements.



Unit II: Aquatic microbiology:

Water microbiology refers to the study of the microorganisms that live in water, or which can be transported from one habitat to another by water.

Water can support the growth of many types of microorganisms. This can be advantageous. For example, the chemical activities of certain strains of yeasts provide us with beer and bread. As well, the growth of some bacteria in contaminated water can help digest the poisons from the water.

Many microorganisms are found naturally in fresh and saltwater. These include bacteria, cyanobacteria, protozoa, algae, and tiny animals such as rotifers. These can be important in the food chain that forms the basis of life in the water. For example, the microbes called cyanobacteria can convert the energy of the sun into the energy it needs to live. The plentiful numbers of these organisms in turn are used as food for other life. The algae that thrive in water is also an important food source for other forms of life.

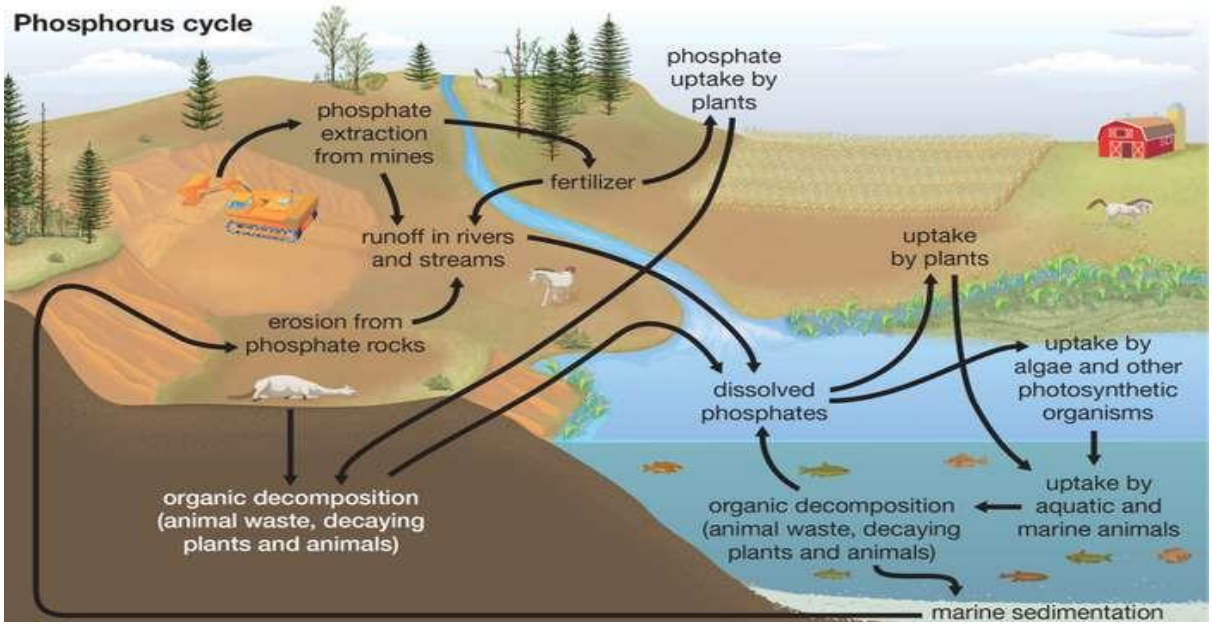
A variety of microorganisms live in fresh water. The region of a water body near the shoreline that is termed the littoral zone is well lighted, shallow, and warmer than other regions of the water. Photosynthetic algae and bacteria that use light as energy thrive in this zone. Further away from the shore is the limnitic zone, which can be colder and sunlight only in the upper 100 feet or so. Photosynthetic microbes also live here. As the water deepens, temperatures become colder and the oxygen concentration and light in the water decrease. Now, microbes that require oxygen do not thrive. Instead, purple and green sulfur bacteria, which can grow without oxygen, dominate. Finally, at the bottom of fresh waters (the benthic zone), few microbes survive. Bacteria that can survive in the absence of oxygen and sunlight, such as methane producing bacteria, thrive.

Salt water presents a different environment to microorganisms. The higher salt concentration, higher pH, and lower nutrients, relative to freshwater, are lethal to many microorganisms. But, salt loving (halophilic) bacteria abound near the surface, and some bacteria that also live in freshwater are plentiful (i.e., *Pseudomonas* and *Vibrio*).The role of archaebacteria in the ocean food chain is not yet known, but must be of vital importance.

Eutrophication

Eutrophication, the gradual increase in the concentration of phosphorus, nitrogen, and other plant nutrients in an aging aquatic ecosystem such as a lake. The productivity or fertility of such an ecosystem naturally increases as the amount of organic material that can be broken down into nutrients increases. This material enters the ecosystem primarily by runoff from land that carries debris and products of the reproduction and death of terrestrial organisms. Water blooms, or great concentrations of algae and

microscopic organisms, often develop on the surface, preventing the light penetration and oxygen absorption necessary for underwater life. Eutrophic waters are often murky and may support fewer large animals, such as fish and birds, than non-eutrophic waters.



Effects

The disturbance of aquatic equilibria may be more or less evident according to the enrichment of water by nutrients (phosphorus and nitrogen). An aquatic environment with a limited availability of phosphorus and nitrogen is described as "oligotrophic" while one with high availability of these elements is called "eutrophic"; a lake with intermediate availability is called "mesotrophic".When the eutrophication phenomenon becomes particularly intense, undesirable effects and environmental imbalances are generated. The two most acute phenomena of eutrophication are hypoxia in the deep part of the lake (or lack of oxygen) and algal blooms that produce harmful toxins, processes that can destroy aquatic life in the affected areas abundance of particulate substances (phytoplankton, zooplankton, bacteria, fungi and debris) that determine the turbidity and colouration of the water;

- abundance of inorganic chemicals such ammonia, nitrites, hydrogen sulphide etc. that in the drinking water treatment plants induce the formation of harmful substances such as nitrosamines suspected of mutagenicity;

- abundance of organic substances that give the water disagreeable odours or tastes, barely masked by chlorination in the case of drinking water. These substances, moreover, form complex chemical compounds that prevent normal purification processes and are deposited on the walls of the water purifier inlet tubes, accelerating corrosion and limiting the flow rate;

- the water acquires disagreeable odours or tastes (of earth, of rotten fish, of cloves, of watermelon, etc.) due to the presence of particular algae;
- disappearance or significant reduction of quality fish with very negative effects on fishing (instead of quality species such as trout undesirable ones such as carp become established);
- possible affirmation of toxic algae with potential damage to the population and animals drinking the affected water;
- prohibition of touristic use of the lake and bathing, due to both the foul odour on the shores caused by the presence of certain algae, as well as the turbidity and anything but clean and attractive appearance of the water; bathing is dangerous because certain algae cause skin irritation;
- reduction of oxygen concentration, especially in the deeper layers of the lake at the end of summer and in autumn.

Control

In the past, the traditional eutrophication reduction strategies, including the alteration of excess nutrients, physical mixing of the water, application of powerful herbicides and algaecides, have proven ineffective, expensive and impractical for large ecosystems (Michael F. Chislock, 2013). Today, the main control mechanism of the eutrophic process is based on prevention techniques, namely removal of the nutrients that are introduced into water bodies from the water.It would be sufficient to reduce the concentrations of one of the two main nutrients (nitrogen and phosphorus), in particular phosphorus which is considered to be the limiting factor for the growth of algae, acting on localised loads (loads associated with waste water) and widespread loads (phosphorus loads determined by diffuse sources such as land and rain). The load is the quantity (milligrams, kilograms, tons, etc.) of nutrients introduced into the environment due to human activity.

The possible activities to be undertaken to prevent the introduction of nutrients and to limit phosphorus loads can be summarized as follows:

- improvement of the purifying performance of waste water treatment plants, installing tertiary treatment systems to reduce nutrient concentrations;
- implementation of effective filter ecosystems to remove nitrogen and phosphorus present in the run-off water (such as phyto-purification plants);
- reduction of phosphorous in detergents;
- rationalisation of agricultural techniques through proper planning of fertilization and use of slow release fertilisers;
- use of alternative practices in animal husbandry to limit the production of waste water.

In cases where water quality is already so compromised as to render any preventive initiative ineffective, "curative" procedures can be implemented, such as:

- removal and treatment of hypolimnetic water (deep water in contact with the sediments) rich in nutrients since in direct contact with the release source;
- drainage of the first 10-20 cm of sediment subject to biological reactions and with high phosphorus concentrations;
- oxygenation of water for restore the ecological conditions, reducing the negative effects of the eutrophic process, such as scarcity of oxygen and formation of toxic compounds deriving from the anaerobic metabolism;
- chemical precipitation of phosphorous by the addition of iron or aluminium salts or calcium carbonate to the water, which give rise to the precipitation of the respective iron, aluminium or calcium orthophosphates, thereby reducing the negative effects related to the excessive presence of phosphorus in the sediments.

Water Pollution

Water pollution occurs when harmful substances—often chemicals or microorganisms—contaminate a stream, river, lake, ocean, aquifer, or other body of water, degrading water quality and rendering it toxic to humans or the environment.

Causes of Water Pollution

Water is uniquely vulnerable to pollution. Known as a "universal solvent," water is able to dissolve more substances than any other liquid on earth. It's the reason we have Kool-Aid and brilliant blue waterfalls. It's also why water is so easily polluted. Toxic substances from farms, towns, and factories readily dissolve into and mix with it, causing water pollution.

Categories of Water Pollution

Groundwater

When rain falls and seeps deep into the earth, filling the cracks, crevices, and porous spaces of an aquifer (basically an underground storehouse of water), it becomes groundwater—one of our least visible but most important natural resources. Nearly 40 percent of Americans rely on groundwater, pumped to the earth's surface, for drinking water. For some folks in rural areas, it's their only freshwater source. Groundwater gets polluted when contaminants—from pesticides and fertilizers to waste leached from landfills and septic systems—make their way into an aquifer, rendering it unsafe for human use. Ridding groundwater of

contaminants can be difficult to impossible, as well as costly. Once polluted, an aquifer may be unusable for decades, or even thousands of years. Groundwater can also spread contamination far from the original polluting source as it seeps into streams, lakes, and oceans.

Surface water

Covering about 70 percent of the earth, surface water is what fills our oceans, lakes, rivers, and all those other blue bits on the world map. Surface water from freshwater sources (that is, from sources other than the ocean) accounts for more than 60 percent of the water delivered to American homes. But a significant pool of that water is in peril. According to the most recent surveys on national water quality from the U.S. Environmental Protection Agency, nearly half of our rivers and streams and more than one-third of our lakes are polluted and unfit for swimming, fishing, and drinking. Nutrient pollution, which includes nitrates and phosphates, is the leading type of contamination in these freshwater sources. While plants and animals need these nutrients to grow, they have become a major pollutant due to farm waste and fertilizer runoff. Municipal and industrial waste discharges contribute their fair share of toxins as well. There's also all the random junk that industry and individuals dump directly into waterways.

Ocean water

Eighty percent of ocean pollution (also called marine pollution) originates on land—whether along the coast or far inland. Contaminants such as chemicals, nutrients, and heavy metals are carried from farms, factories, and cities by streams and rivers into our bays and estuaries; from there they travel out to sea. Meanwhile, marine debris—particularly plastic—is blown in by the wind or washed in via storm drains and sewers. Our seas are also sometimes spoiled by oil spills and leaks—big and small—and are consistently soaking up carbon pollution from the air. The ocean absorbs as much as a quarter of man-made carbon emissions.

Effects of Water Pollution

On human health

To put it bluntly: Water pollution kills. In fact, it caused 1.8 million deaths in 2015, according to a study published in *The Lancet*. Contaminated water can also make you ill. Every year, unsafe water sickens about 1 billion people. And low-income communities are disproportionately at risk because their homes are often closest to the most polluting industries.

Waterborne pathogens, in the form of disease-causing bacteria and viruses from human and animal waste, are a major cause of illness from contaminated drinking water. Diseases spread by unsafe water include cholera, giardia, and typhoid. Even in wealthy nations, accidental or illegal releases from sewage treatment facilities, as well as runoff from farms and urban areas, contribute harmful pathogens to waterways.

On the environment

In order to thrive, healthy ecosystems rely on a complex web of animals, plants, bacteria, and fungi—all of which interact, directly or indirectly, with each other. Harm to any of these organisms can create a chain effect, imperiling entire aquatic environments.

When water pollution causes an algal bloom in a lake or marine environment, the proliferation of newly introduced nutrients stimulates plant and algae growth, which in turn reduces oxygen levels in the water. This dearth of oxygen, known as eutrophication, suffocates plants and animals and can create "dead zones," where waters are essentially devoid of life. In certain cases, these harmful algal blooms can also produce neurotoxins that affect wildlife, from whales to sea turtles.

Prevent Water Pollution

- Reduce your plastic consumption and reuse or recycle plastic when you can.
- Properly dispose of chemical cleaners, oils, and non-biodegradable items to keep them from ending up down the drain.
- Maintain your car so it doesn't leak oil, antifreeze, or coolant.
- If you have a yard, consider landscaping that reduces runoff and avoid applying pesticides and herbicides.
- If you have a pup, be sure to pick up its poop.

Treatment and Management of Liquid

Waste

Liquid wastes mainly consist of waste water from residential, commercial and industrial areas in towns and cities. This waste water contains many dissolvable unwanted and rejected substances. In cities and towns, waste water is transported through sewerage system having a network of underground pipes called sewers.

Sewage is waste water containing solid and liquid excreta coming from houses, streets, industries etc. Silage is another term applied to waste liquid not containing excreata. Sewage water mainly has 99.9 percent of water and rest 0.1 percent of organic and inorganic substances.

This waste water carries many bacteria which cause diseases. Organic matter decomposes to give different colour to the water and it also gives bad odour to the liquid. The sewage water is managed to get it free from pollution and can be reused for agricultural and other uses.

The treatment to such sewage mainly focused on three things. They are:

(a) Remove the suspended matters

(b) To reduce the organic matter through decomposition by bacterial action.

(c) To produce germ free water safe for environment.

Management of liquid waste through sewage treatment:

There are three stages for treatment of sewage water. They are:

1. Primary or physical treatment.

2. Secondary or Biological treatment.

3. Tertiary or chemical treatment.

1. Primary treatment:

It is the process of mechanically removing the solid materials present in water through metal screening. Grit chambers and sedimentation. Metal screening removes large floating objects such as small piece of woods, rags, masses of garbage and death insects and animals.

The Grit chamber allows the settlement of heavier solids such as sand into the bottom layer. The waste water is then allowed to pass into a big sedimentation tank where the liquid spends about 6- 8 hours. During this time about 50 to 70 percent of the solids settle down under the influence of gravitational force.

During this process a small amount of decomposition takes place by the microorganisms present in sewage breaking down the organic matter present. The organic matter after breaking down settles down into a layer called sludge.

This sludge is removed mechanically. Primary treatment removes about 60 percent of floating solid bodies, 30 percent of oxygen demanding wastes, 20 percent of nitrogen compounds, and 10 percent of phosphorous compounds.

2. Secondary Treatment:

It is a biological oxidation of organic matter. It is achieved by filter method or by sludge process. In the filter method, the waste water is sprinkled over the surface of a bed of small stones of one to two metres deep. When the water percolates through the stone bed, a very complex biological growth of algae, fungi, protozoa and bacteria occurs. By these formation, the waste water gets oxidised. The oxidised waste water is then passed into the sedimentation tanks.

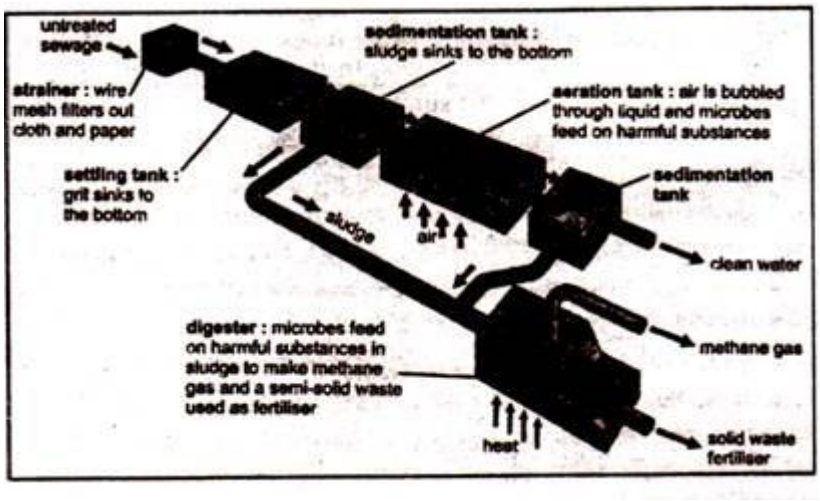
The sludge process is a modem method of management of waste water. The liquid from the sedimentation tank is mixed with sludge collected from the final tank. This sludge is called activated sludge as it is rich in aerobic bacteria (bacterial which can survive only in presence of oxygen). This activated sludge is then subjected to aeration. By aeration the organic matter of waste liquid gets oxidized into carbon dioxide, water and nutrients. Organisms causing diseases like typhoid, cholera are destroyed is the stage.

The oxidised waste liquid is then passed into a secondary sedimentation tank where activated sludge is collected. The volume and characteristics of the sludge is reduced through anaerobic (devoid of oxygen) auto digestion. In this process, complex compounds are broken down into water, carbon dioxide, methane and ammonia. This substance works as a good fertiliser.

3. Tertiary Treatment:

The residue from earlier two treatment process still leave about 10 percent of suspended solid bodies, 10 percent of the oxygen demanding wastes, 30 percent of toxic metal compounds, 50 percent of Nitrogen and 70 percent of phosphorous. This Tertiary Treatment method is an advanced form of chemical and physical process.

The most common methods in this treatment is precipitation of suspended particles, filtration with carbon to resolve dissolve organic compounds and reverse osmosis by passage through a membrane to remove dissolve organic and inorganic materials. Chlorination is also required at the end to remove disease causing bacteria and other germs.



Common waterborne diseases

Water is certainly the source of life. But with the potentiality to harbor infectious-germs that can cause the dangerous effect to the human body, one has to take the careful precautions to determine when the water is supplied through the tap is safe to drink and when it's not.The pathogens available in the contaminant water are invisible to the human eyes and are responsible for the various form of diseases like bacteria, viruses, and protozoa to count a few.

1. Typhoid Fever

About the disease:

Typhoid fever caused by the bacterium Salmonella Typhi. S. Typhi can be a life-threatening disease. It is the same type of bacteria found in the types of eggs and chicken and is also commonly known as food poisoning or salmonella poisoning. It is the most common type of bacteria found in the developing world. A person suffering from typhoid can transfer harmful bacteria in their bloodstream and the intestinal tract.

Causes:

Typhoid could be caused by consuming the food and water that have been infected with the disease. Also, it may transfer to food and water while cooking.

Symptoms:

Patients suffering from typhoid have a continuous fever as high as 103° to 104° F (39° to 40° C). You may feel weakness, headache, loss of appetite and stomach pains. Other symptoms include the rashes across also called as the red spots. For the proper diagnosis, you should get the samples of stool or blood tested by the doctor.

Precautions:

Use soapy or hot water to wash hands, avoid drinking contaminated and untreated water. Use a high-quality water purifier which can remove the available germs from the water, eat raw fruits and vegetables.

2. Cholera

About the Disease:

Cholera is an acute diarrheal illness that is widely caused by the infection of the intestine with bacterium Vibrio cholerae. It's a severe water-borne disease which is caused by the bacterium Vibrio chohlera, typically abstained by consuming dirty water. If not treated on time, it can also cause death in some rare cases.

Causes:

The disease gets transmitted through the ingestion of feces poorly polluted with the bacterium. When the untreated water goes through the sewage into the waterways, it affects the domestic water supply and also the foods washed by it. However, the person to person transmission is rarely found.

Symptoms:

There are various types of symptoms cholera patients can experience such as the general GI tract upset, like profuse diarrhea, vomiting (it may sustain for 1 hour in severe cases), nausea, and dehydration.

Precautions:

Drink and use safe filtered water, wash your hands often with the soap. Cook your food well, especially the non-veg. Places where the family baths and washes clothes should be neat and clean.

3. Hepatitis A

About the disease:

Hepatitis A is a liver-related disease and is caused by the virus hepatitis A virus (HAV) which impact anyone. It is often found in the stool of the patient with Hepatitis A and can drastically outspread from person to person. The virus gets easily transmitted at the places having poor sanitary conditions or where the hygiene factors are not taken into consideration. If a person affected with Hepatitis A, he/she can affect rest people living in the same home.

Causes:

The major cause of source of this disease gets polluted with the feces from the infected humans, the virus gets quickly spread in the water, and can enter through various mediums such as the broken sewage systems and sewage overflows.

Symptoms:

The most common issues are jaundice or the yellowness of the skin, eyes, and dark yellowish urine, fatigue loss of appetite, fever, stomach pain and nausea.

Precautions:

First thing first, if there are people around you affected by the hepatitis A, keep a distance to them. And follow good hygiene practices especially while washing your hands. Drink and use clean water in cooking.

4. Hepatitis E

About the disease:

Hepatitis E virus is a real cause of the disease which can be largely spared by the uses of drinking contaminated water. It is widely found in the countries where human waste is allowed to throw into the rivers and ponds, without being purified. The regular consumption of polluted water leads to develop epidemics.

Causes:

Similar to other above diseases, the real cause of Hepatitis E is also the contaminated drinking water, hygiene issues and the regular intake of dirty food.

Symptoms:

The typical symptoms of Hepatitis E include the dark urine, pale stools, jaundice that leads to the yellow discoloration of the skin and the sclera of the eyes, anorexia (the loss of appetite) hepatomegaly (tender liver), and an acute illness that damages liver cells, and sometimes might also cause the death.

Precaution: Don't consume the contaminated water or street food cooked in unhealthy oil, avoid eating under-cooked meat, and wash your hands with the water and soap after using a bathroom or toilets.

5. Traveler's Diarrhea

About the Disease:

Sometimes also known as Aztec Two-Step, Montezuma's Revenge, Turista Traveler's Diarrhea is the most common waterborne disease that widely affects the travelers. Every year around twenty to fifty percent of international travelers (approx 10 million persons) who travel to the different countries get affected to this disease after returning their home.

Traveler's Diarrhea generally occurs within the first week but might also occur any time during travel and even after returning from the home. There is a high risk of determinant destinations in Asian Countries where the attack rates are for the men and women both while the primary source of infection is indigestion of the severely pestiferous water and food.

Causes:

80% of the Traveler's Diarrhea is caused by bacterial enteropathogens. Apart from the coli and other types of bacterial pathogens, there are a variety of other parasitic and viral enteric pathogens that are also the real cause of causative agents.

Symptoms

Generally, most of the Traveler's Diarrhea begin abruptly whereas the travelers experience four to five water bowel movement each day. Other common symptoms are abdominal cramping, fever, bloating, vomiting, diarrhea, and malaise. But the good side is that it's not a life-threatening disease and one can recover from it within 1 week or maximum 1 month.

Precautions:

One can manage the traveler's diarrhea by avoiding contaminated food and drinks. If the problem still continues, make sure to take the effective medical treatment which is usually the combination of antimotility and antibiotic.

6. Giardia and Cryptosporidium

About the disease:

Giardia and Cryptosporidium are the microscopic organisms or cysts that could be found in the water body. The availability of Giardia in the water causes an intestinal illness which is medically called as the giardiasis or sometimes is also referred to as "beaver fever". It's the most common sort of waterborne disease found in

both drinking as well as recreational water. On the other hand, Cryptosporidium leads to a similar type of illness called cryptosporidiosis.

Causes:

The drinking water gets contaminated while the parasites are mixed or get flushed into the water. If the right treatment is not taken, the blend of harmful parasites can lead to severe illness. A lot of communities claim that the giardiasis is closely linked to drinking normal municipal water contaminated with Giardia.

Symptoms:

Regular weight loss, abdominal cramps, malaise, and Diarrhea are the most common symptoms of Giardia. Besides, chills, headache, fever and committing may also occur. One can experience these symptoms six to sixteen days after the initial contact and can continue for around one month. The symptoms of cryptosporidiosis are also similar such as nausea, headaches, abdominal cramps etc. These symptoms may occur within two to 25 days and can last up to 15 days, while in some critical cases, it may persist for up to a month.

Precautions:

One can get in touch with Giardiasis by using contaminated recreational water which mostly includes the swimming pools. So try to avoid swimming while suffering from diarrhea, or take a shower before and after swimming. Also avoid gulping the recreational water, wash your hands before using the toilet or changing diapers.

7. Dysentery

About the Disease:

Dysentery refers to any case of infectious bloody diarrhea and is also known as the bloody flux, flux, travelers dysentery and Montezuma Revenge. This mostly affects the people living in developing areas like India where they have improper sanitation. It indicates to the response of the body to an unwanted visitor in the digestive system.

Causes:

The most common causes of Dysentery is the Entamoeba histolytica and a number of other bacteria including the shigella bacteria and E. histolytica which often thrives in the food and water polluted by the human feces. As far as the Amoebic dysentery is considered, it's usually transmitted by the polluted water.

Symptoms:

People afflicted with the disease often suffer from bloody diarrhea along with the intense fever, stomach pain,

and rapid weight loss. Besides, it may also cause diminutive stools mixed with the mucus and blood, among all cramps are the most common symptoms. Sometimes, infected individuals may also experience a painful strain.

Precautions:

The very first thing you can do to avoid Dysentery is to use germs free water. Secondly, always wash your hands before using the toilet. And avoid shaking hands with the people suffering from the Dysentery.

8. Salmonella

About the Disease:

Salmonellosis is an infection caused by the bacteria termed as Salmonella which is better-known to cause the health illness. There are various types of Salmonella bacteria spread through human or animal feces. The drinking water can also be contaminated while the wild domestic animals who leave their droppings in or near water surfaces such as rivers, lakes, ponds, streams etc.

Causes:

Salmonella virus can be found in water contaminated with the feces of infected animals or humans. The harmful waste can be mixed in the water through various mediums including polluted storm water runoff, agricultural runoff etc. Other types of common infections may occur through under-cooked poultry, eggs and other sorts of contaminated water and food.

Symptoms:

The most common symptoms of salmonellosis include fever, vomiting, diarrhea, and abdominal cramps. In the case of infants, dehydration can also occur. Sometimes individuals can also be infected without showing symptoms, and can automatically recover within 6-7 days.

Precaution:

Family members who are suffering from salmonellosis should avoid cooking food at home and avoid swimming. Avoid buying dirty or contaminated food. Also unpasteurized should also be avoided.

9. Campylobacter

About the disease:

In humans, Campylobacters are the bacteria that are a leading cause of diarrhea infused illness. They are regarded as the most generic bacterial cause of gastroenteritis globally. The disease is widely caused by the presence of campylobacters viruses. In most developing countries, the presence of Campylobacter infections

is fatigue increasing. They are broadly prevalent in food animals such as cattle, pigs, poultry, shellfish, and ostriches.

Causes:

Campylobacter can transmit into your system if you use under-cooked food derived from the poultry. The bacteria are widely present in animals such as poultry and cattle. Apart from that, unpasteurized milk can also contain Campylobacter bacteria. It spreads more quickly in the isolated cases whereas, in the developing countries, it can be found in the water and sewage.

Symptoms:

The effect of the campylobacter virus usually lasts up to a week. The most common symptoms of the disease is diarrhea, also the stool might also have the presence of blood in it that might sicken your stomach and make you vomit. Other signs include bloating, fever, and belly cramps.

Precaution:

In order to avoid the effect of the respective virus, you should cook the poultry to least 165 F. Also avoid eating pink meat, it has to be white and never add the under-cooked chicken in your meal. For further safety, drink clean purified water, wash your hands properly with soap and water after using a toilet or bathrooms.

10. Legionellosis

About the Disease:

Often referred as the form of pneumonia, Legionnaires is a lung inflammation which is usually caused by the infection. But the good news is that, this disease doesn't spread from person to person contact. Most people get affected by the diseases by inhaling the water droplets from the contaminated water. The most common victims of this disease are the smokers, adults and those who have weakened immune systems.

Causes:

It's widely caused by a bacterium medically known as Legionella which mostly survives in soil and contaminated water. Legionella bacterial can twofold in all sorts of water systems like hot tubs, air conditioners and the mist sprayers available in grocery store produce departments. Also, inhabitants may also get effected these diseases from the home plumbing systems by the outbreaks usually occurred in large buildings.

Symptoms:

Legionellosis develops in 2 to 10 days after its exposure to Legionella bacteria and can frequently release the symptoms like- chills, muscle pain, headache, and the fever (104 F (40 C) or higher).

Precautions:

Infection spread by Legionellosis is can be prevented. All you have to do is just the meticulous cleaning and disinfection of water, pools and spas.

11. Botulism

About the disease:

Botulism is a fatal illness which is usually caused by the botulinum toxins. If not treated on time, it can also cause paralysis that often starts with the face and can spread to limbs. If, in any case it reaches to the breathing muscles, it can result into wicked respiratory failure. There are different types of botulism such as- Wound botulism, Infant botulism, Latrogenic botulism and Adult intestinal colonization.

Causes:

It's basically a botulinum toxin, a poison produced by the bacterium Clostridium botulinum and usually exists in the untreated water and soil. Even the minimal consumption of toxins can cause terrible poisoning.

Symptoms:

Some signs and symptoms may include- respiratory difficulties, slow or improper reflexes, floppiness and poor muscle tone, no gag reflex, constipation, poor feeding, a bad temper, excessive drooling when feeding, sagging eyelids, flat facial expression, weak crying weakly, unfocused eyes, weak sucking.

Precaution:

In order to reduce the risk of Botulism, one needs to follow the good practice of food hygiene. Avoid canning food at home, wash vegetables and fruits with filtered water, boil home processed food for at least 10 minutes, ensure all foods are well cooked, and drink clean water.

12. Vibrio Illness

About the disease:

Produced by the Vibrio bacteria, also known as the Vibrosis, Vibrio habitats in the coastal water. There are around 12 species of vibrio that can cause gastrointestinal illness (gastroenteritis) in humans. They are usually find in the months between May – October when the weather is warmer.

Causes:

The real cause of the vibrio illness it the habit of eating undercooked food, mostly cooked in the untreated water. While the open would is exposed to the salty water, vibrio can also cause severe skin infection.

Symptoms:

When ingested, the vibrio virus can also cause watery diarrhea that may result in painful abdominal cramping, vomiting, nausea, fever and chills. Such symptoms might occur within 24 hours of ingestion.

Precautions:

All you can do to stay away to such virus is just use filtered water and eat well cooked vegetables. Once you see the sign of Vibrio, do consult a good doctor for further prevention.

To wrap it up in a nutshell, it is pivotal to have a clean, hygienic water supply system in the house, which ensures that you and your family get clean water 24*7. Water borne diseases may be quick to creep into our homes, but can be avoided with the help of some precautionary measures such as boiling water, disinfecting the house, especially the kitchen and bathroom area, and properly disposing off sewage waste so that it does not accumulate and become a breeding ground for viruses and bacteria. Also, Installing a water purifier that will take care of your daily water needs, while preventing you from water borne diseases. Get one today, and live a clean, disease free life.

COD & BOD?

Simple enough, because most NPDES stormwater & process water permits require that folks test for these two parameters. Usually we see these parameters limited by an effluent limitation.

By why test for ***oxygen***?

I mean, we all need it to breath. It's in our air, our water, so what's the big deal?

If the oxygen levels in our air changed, it would be a big issue. If they decreased, it would be a problem. Now imagine if the same thing happened in the water. You might not think it, but it would also be a big issue.

There are certain ways that we can impact the oxygen levels in the water, and that's why we're testing for oxygen.

A simple way to think of it is this. When you place organic chemicals or biological matter into water, it will eventually break down. Just like composting, or how a fallen tree rots in the woods.

Oxygen is necessary for breaking down material. The more material to break down, the more oxygen needed.

Additionally, aquatic organisms need oxygen to breathe.

A normal, naturally balanced ecosystem will have a certain amount of oxygen. There will be a happy equilibrium of oxygen so everything gets what it needs. The fish, the algae, the decaying matter, it'll all have just enough oxygen so nothing gets out of sync.

Now imagine there was too much organic matter breaking down. Or aquatic organisms were reproducing at an alarming rate.

You guessed it, the oxygen would be out of balance. Something that would normally need oxygen wouldn't be getting it.

Now we aren't talking about controlling population here, I'll let you talk to a biologist to cover that matter, we're talking about the breaking down, or decay of matter, which our stormwater runoff can contribute to.

When too much organic matter, either from a chemical or biological source, is added to a system, the natural balance gets out of sync, and things like fish kills occur, or extreme algae blooms. Point is, what we do, can have a serious impact on a naturally balanced ecosystem.

So how do we prevent this? We monitor for COD & BOD.

chemical oxygen demand (COD)

You'll notice that both these terms contain the word **oxygen**.

That means that when either of them break down in a waterbody, they place a demand on the amount of available… oxygen! They consume it as they decompose or breakdown.

COD comes from, you guessed it, chemical sources. It is a measurement of the amount of chemical organic matter being added to a waterbody.

Organic pollutants at a site could be things like chemicals, petroleum, solvents, cleaning agents, etc. Not what you immediately think of when you see the word organic, but that's what it means.

When these pollutants get spilled, mixed, etc., and end up in stormwater, they'll eventually break down, and add an additional strain on the oxygen demand in the water. That's not a good thing.

So COD is looking for the amount of these pollutants in your stormwater runoff. Simple enough, right?

biological oxygen demand (BOD)

It's pretty similar to COD.

The difference? It's biological sources vs. organic sources.

There's a fair amount of natural, organic matter which makes its way to waterbodies via stormwater runoff. Same as chemical sources, biological sources place a burden on the ecosystem by needing oxygen to breakdown.

This would be things like sewage, plant and animal matter, etc.

Here's what Wikipedia says on the matter

Biochemical oxygen demand (BOD) is the amount of dissolved oxygen needed by aerobic biological organisms in a body of water to break down organic material present in a given water sample at certain temperature over a specific time period. The term also refers to a chemical procedure for determining this amount. This is not a precise quantitative test, although it is widely used as an indication of the organic quality of water. The BOD value is most commonly expressed in milligrams of oxygen consumed per liter of sample during 5 days of incubation at 20 °C and is often used as a robust surrogate of the degree of organic pollution of water.

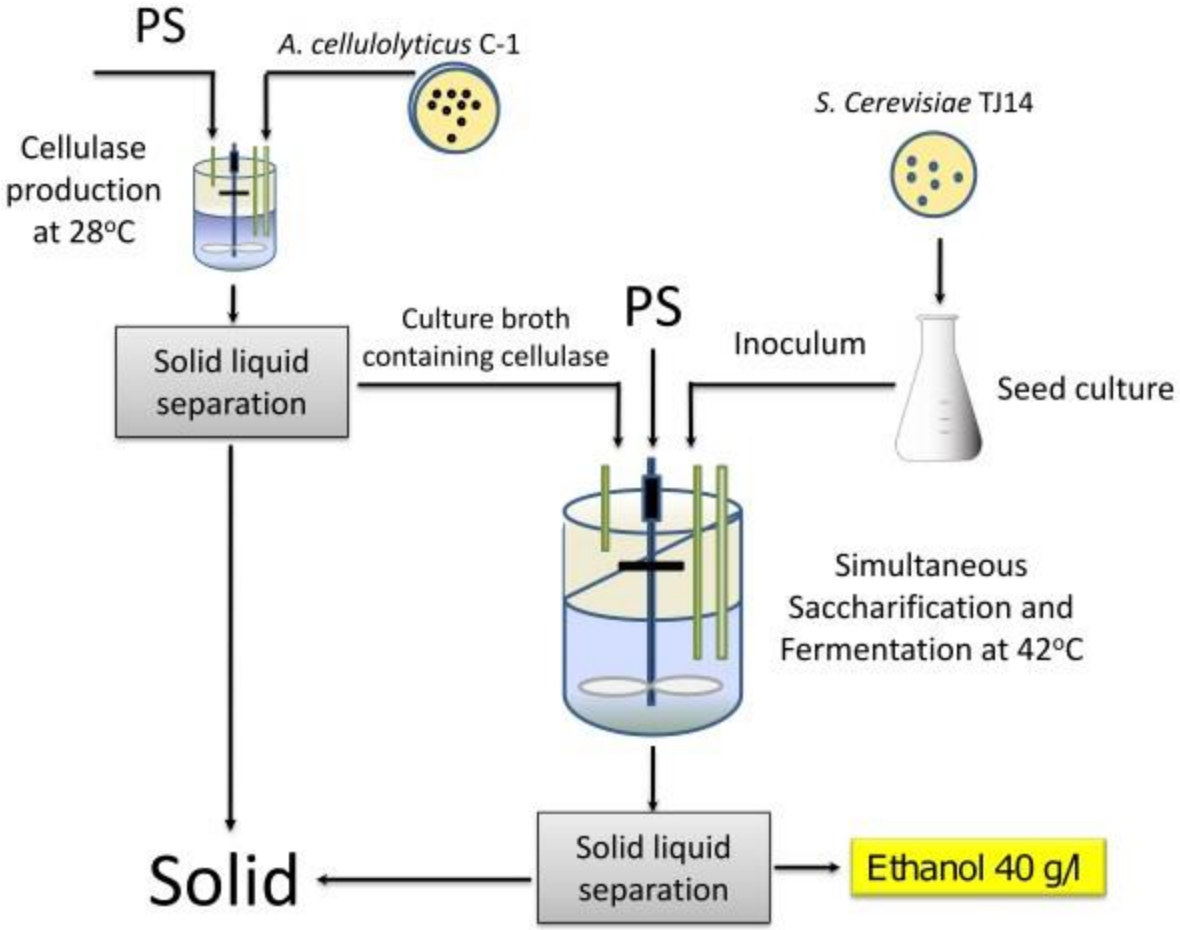
So, just like COD, this is a methodology of determining a degree of pollution in your runoff.

In environmental chemistry, there does seem to be a correlation between COD and BOD, so sometimes one is used as an indicator of both of them.

Solid-waste management

Solid-waste management, the collecting, treating, and disposing of solid material that is discarded because it has served its purpose or is no longer useful. Improper disposal of municipal solid waste can create unsanitary conditions, and these conditions in turn can lead to pollution of the environment and to outbreaks of vector-borne disease—that is, diseases spread by rodents and insects. The tasks of solid-waste management present complex technical challenges. They also pose a wide variety of administrative, economic, and social problems that must be managed and solved.

Saccharification is the complete degradation of starch to simpler sugars such as glucose, maltose, maltotriose, and to some extent, dextrins.



Pyrolysis

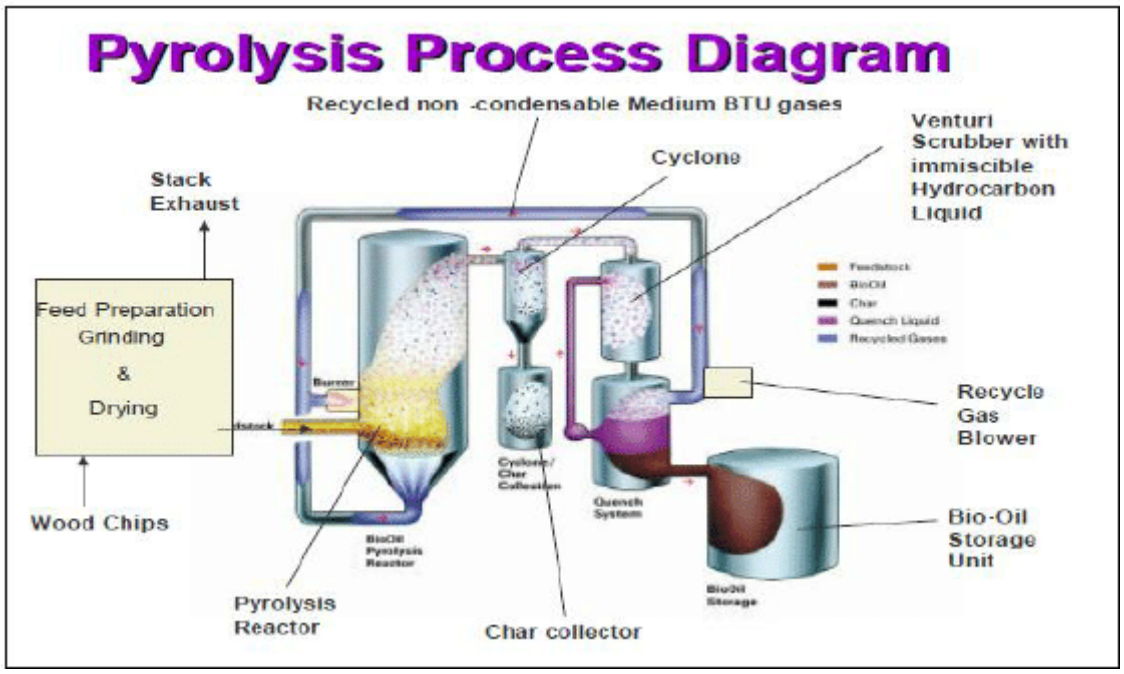
Pyrolysis is a thermochemical treatment, which can be applied to any organic (carbon-based) product. It can be done on pure products as well as mixtures. In this treatment, material is exposed to high temperature, and **in the absence of oxygen** goes through chemical and physical separation into different molecules. The decomposition takes place thanks to the limited thermal stability of chemical bonds of materials, which allows them to be disintegrated by using the heat.

Thermal decomposition leads to the formation of new molecules. This allows to receive products with a different, often more superior character than original residue. Thanks to this feature, pyrolysis becomes

increasingly important process for today industry – as it allows to bring far greater value to common materials and waste.

Pyrolysis is frequently associated with thermal treatment. But in contrary to combustion and gasifications processes, which involve entire or partial oxidation of material, pyrolysis bases on heating in the absence of air. This makes it mostly endothermic process that ensure high energy content in the products received.

Pyrolysis products always produce solid (charcoal, biochar), liquid and non-condensable gases (H2, CH4, CnHm, CO, CO2 and N). As the liquid phase is extracted from pyrolysis gas only during it's cooling down, in some applications, these two streams can be used together when providing hot syngas directly to the burner or oxidation chamber



UNIT III

1. **SOLID WASTE** - Solid wastes include solid portions of the discarded material such as glass bottles, crockeries, plastic containers, metals and radioactive wastes. The solid wastes may be biodegradable or non-biodegradable. The biodegradable solid wastes are agricultural wastes, food wastes, paper, food processing by products, manure, yard wastes etc. The non-biodegradable wastes include plastic, metals, synthetic materials, radioactive waste etc.

The solid waste management involves disposal of solid waste to land (or ocean) or recovering and reproducing useful substances from the waste through recycling.

The entire methodology of solid waste management is based on:

- Collection of Waste
- Disposal
- Resource recovery

Collection of Waste: The solid wastes are usually collected by a covered truck.

Disposal of Waste: After the collection of wastes, the wastes are disposed of by any one of the methods as follows:

- Dumping
- Sanitary landfills
- Incineration
- pyrolysis
- Composting
- Bio gas technology etc

Resource recovery (Recycling):

By the process of recycling, a number of useful products can be obtained from the solid wastes. Some important products obtainable from solid wastes are described below:

- Waste papers and cardboards from sugar cane bagasse can be used for the preparation of unbreakable dolls, packing cardboards etc.

- Metals can be recycled from the industrial scrap.
- Waste glasses can be used for the preparation of new glass bottle.
- Electricity can be generated through incineration.

2. **LIQUID WASTE** - Liquid wastes are the liquid part of the waste material. Liquid waste includes effluents of industries, fertilizer and pesticide solutions from agricultural fields, leachate from landfills, urban runoff of untreated waste water and garbage, mining wastes etc. The liquid waste may contain nontoxic inorganic substances or toxic organic substances.

Some important liquid waste management methods are as follows

1. **Sewage treatment** - This process involves the following methodology:

- Dilution
- Mechanical treatment
- Biological treatment
- Chemical treatment

2. **Removal of ammonia-** The treatment of industrial effluents in Effluent Treatment involves chemical or primary treatment (by methods of neutralization, sedimentation, coagulation, precipitation etc.) followed by biological or secondary treatment (by activated sludge and trickling filter method) and tertiary treatment (by methods of ion exchange, reverse osmosis, chemical oxidation).
3. Effluent water can be used to grow algae and aquatic plants to produce biomass for biogas plants.
4. The sewage with organic nutrients is stored in specially constructed shallow ponds called as oxidising or stabilizing pond. In the pond, green algae and bacteria grow in presence of sun light, consuming organic nutrients. This water contains enough nitrogen, phosphorous and potassium and is highly helpful for the growth of plants.

3. GASEOUS WASTE - Gaseous waste are waste products in gas form resulting from various human activities like manufacturing, processing, material consumption, biological processes etc. The gaseous wastes include carbon dioxide (CO2), methane (CH4), chlorofluorocarbon (CFC), oxides of nitrogen (NOx), carbon monoxide (CO), oxides of sulphur (SOx) etc. These gaseous wastes can cause serious environmental hazards. Therefore, it is highly essential to take appropriate steps for the proper management and control of gaseous wastes in the environment.

Some of the control measures are:

1. The gaseous pollutant like SO2, H2S, NH3 etc can be removed by absorption in (using appropriate liquid) wet scrubbers.
2. The industries should use precipitators, scrubbers and filters to check production of particulate matter.
3. There should be large scale of plantation which will reduce CO2 level and increase O2 level of atmosphere.
4. Air cleaning devices like gravity settlers, cyclone separators, wet collectors, electrostatic precipitators etc. should be used for the cleaning of air before their discharge into atmosphere.
5. Public awareness should be created regarding hazards of air pollutant accumulation in environment.

COMPOSTING – Definitions:

- Compost is decomposed organic material. Compost is made with material such as leaves, shredded twigs, and kitchen scraps from plants.
- **Compost** is **organic matter** that has been **decomposed** in a process called composting. This process **recycles** various organic materials otherwise regarded as waste products and produces a **soil conditioner**

Examples: decay of fallen leaves in forests, decay of wood in a stand and animal carcasses decaying in a preserve. These natural processes in nature return organic material to the ecosystem. To gardeners, compost is considered **"black gold"** because of its many benefits in the garden. **Compost is a great material for garden soil.**

Raw materials for compost preparation:

- Animal manure
- Used stable straw
- Spoiled fruits and vegetables
- Field refuse
- Vineyard and orchard pruning
- Rotted hay
- Other agricultural waste products
- Some of the more unusual **raw materials** used to make **compost** include seaweed, chicken feathers, peanut shells, and hair clippings

Determine the composting process for you:

Next, you have to decide which composting process is for you. The most common composting processes are:

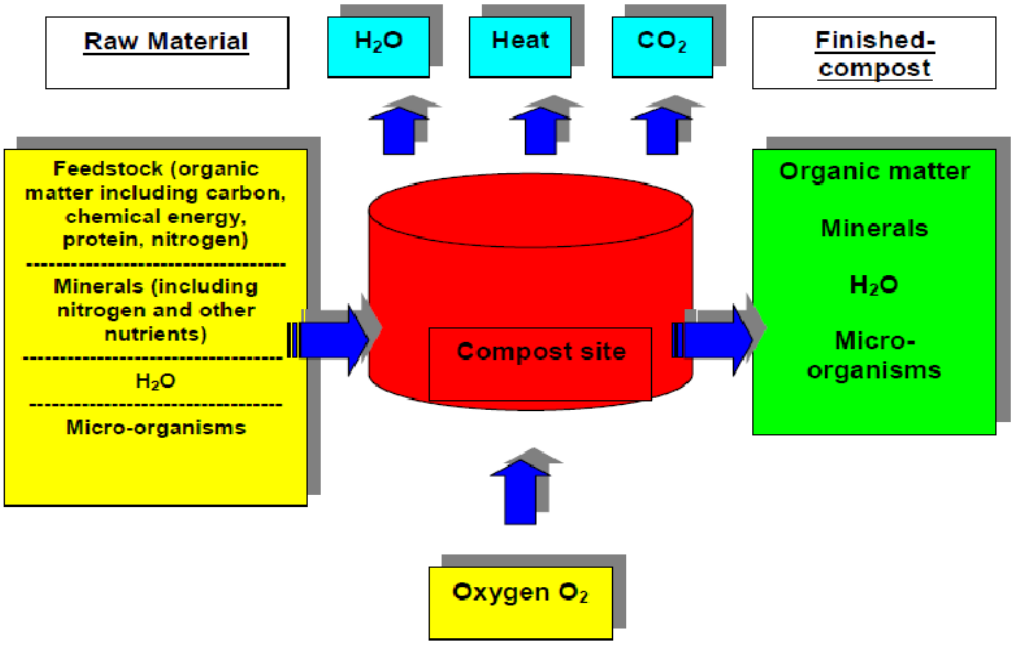
- **Compost Pile or Heap** - This passive method requires the least amount of effort. Simply pile up compostable materials, and let nature do the job. Be prepared to wait.... several months to a year. Some consider it unsightly.
- **Burying Compost** - Dig a hole or a trench. Put compostable material in it. Then, bury it. ... Out of sight, out of mind.
- **Compost Bins and Barrels** - It's an aesthetically neat and tidy way to store compost while it is decomposing. Unless you turn it from time to time, it is largely passive and will take a while to decompose.
- **Compost Tumblers** - The fastest way to make compost, is to use a compost tumbler. It makes turning and aerating the materials a cinch. Under ideal conditions, it makes finished compost in as little as 30 days!

Composting process:

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.

1. Gather compostable materials. Add organic matter from your garden and yard.
2. Chip or shred larger branches and plant stalks. The more surface area bacteria have to work, the quicker the decomposition.
3. You don't have to add all the materials at once. You can add them as you get them, until the barrel, bin or pile is full.
4. Include vegetable and plant waste from your kitchen, including egg shells, coffee grounds, tea bags, spoiled vegetables and fruits, etc.
5. Alternate layers of green and brown materials. This helps to get the decomposition process started, and to keep it working. When decomposition is going on, we often call it "cooking".
6. Keep the pile moist, but not wet.
7. If you are able, turn the pile over from time to time, to maximize air circulation to the bacteria and microbes that "eat" and convert your pile into rich compost.

8. Use the compost after it has almost completely turned into soil. It is rich in nutrients and minerals.
9. Apply compost liberally around your plants as mulch, or mix it into the soil.



Uses / Applications:

- Compost is rich in nutrients
- It is used, for example, in gardens, landscaping, horticulture, urban agriculture and organic farming
- The compost itself is beneficial for the land in many ways, including as a soil conditioner, a fertilizer, addition of vital humus or humic acids, and as a natural pesticide for soil
- In ecosystems, compost is useful for erosion control, land and stream reclamation, wetland construction, and as landfill cover
- The benefits of using compost are numerous
- It builds good soil structure
- Enables soil to retain nutrients, **water**, and air
- Protects against drought
- Helps maintain a neutral pH, and protects plants from many diseases commonly found in the garden
- It also feeds earthworms and other microbial life in the soil.

BIOGAS

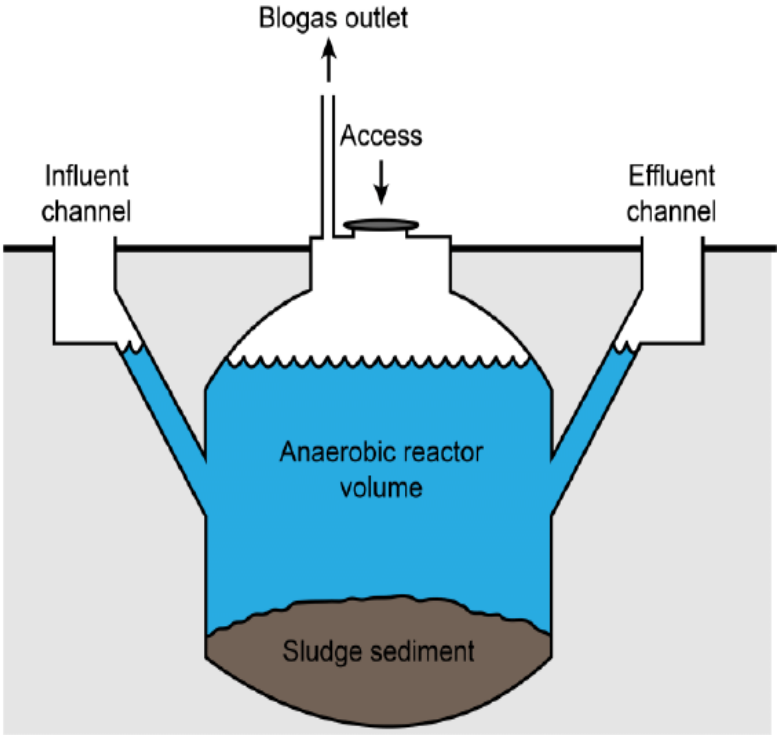
Biogas refers to a mixture of different gases **produced** by the breakdown of organic matter in the absence of oxygen. **Biogas** can be **produced** from raw materials such as agricultural waste, manure, municipal waste, **plant** material, sewage, green waste or food waste. **Biogas** is a renewable energy source.

Raw materials for biogas production

- Cattle dung has been recognized as the chief raw material for bio-gas plants
- Other materials like
    - Night-soil
    - Poultry litter
    - Agricultural wastes can also be used.

Components of biogas plants

- **Mixing tank** - The feed material (dung) is collected in the mixing tank. Sufficient water is added and the material is thoroughly mixed till a homogeneous slurry is formed.
- **Inlet pipe** - The substrate is discharged into the digester through the inlet pipe/tank.
- **Digester** - The slurry is fermented inside the digester and biogas is produced through bacterial action.
- **Gas holder or gas storage dome** - The biogas gets collected in the gas holder, which holds the gas until the time of consumption.
- **Outlet pipe -** The digested slurry is discharged into the outlet tank either through the outlet pipe or the opening provided in the digester.
- **Gas pipeline -** The gas pipeline carries the gas to the point of utilization, such as a stove or lamp.



Steps involved:

This **process** is called methane fermentation and result of decomposition of organic substances by two main groups of microorganisms – acidic and methane bacteria.

1. Hydrolysis
2. Acidification
3. Fermentation
4. Methane formation / Methanogenesis

1. Hydrolysis:

- Biomass is normally comprised of large organic polymers proteins, fats and carbohydrates

- These are broken down into smaller molecules such as amino acids, fatty acids, and simple sugars - - It is the essential first step in anaerobic fermentation; fermentative bacteria hydrolyze the complex organic matter into soluble molecules

- Some of the products of hydrolysis, including hydrogen and acetate may be used by methanogens later in the anaerobic digestion process

- Majority of the molecules, which are still relatively large, must be further broken down in the process of acidogenesis so that they may be used to create methane.

2. Acidogenesis:

- Acidogenesis is the next step of anaerobic digestion where acidogenic microorganisms further break down the biomass and organic products after hydrolysis

- These fermentative bacteria produce an acidic environment in the digestive tank while creating ammonia, H2, CO2, H2S, shorter volatile fatty acids and organic acids, as well as trace amounts of other byproducts

- The principal acids produced are acetic acid, propionic acid, butyric acid etc.

3. Fermentation /Acetogenesis:

- In general, acetogenesis is the creation of acetate, a derivative of acetic acid, from carbon and energy sources by acetogens

- These microorganisms catabolize many of the products created in acidogenesis into acetic acid, CO_2 and H_2

- Acetogens break down the biomass to a point to which methanogens can utilize much of the remaining material to create methane.

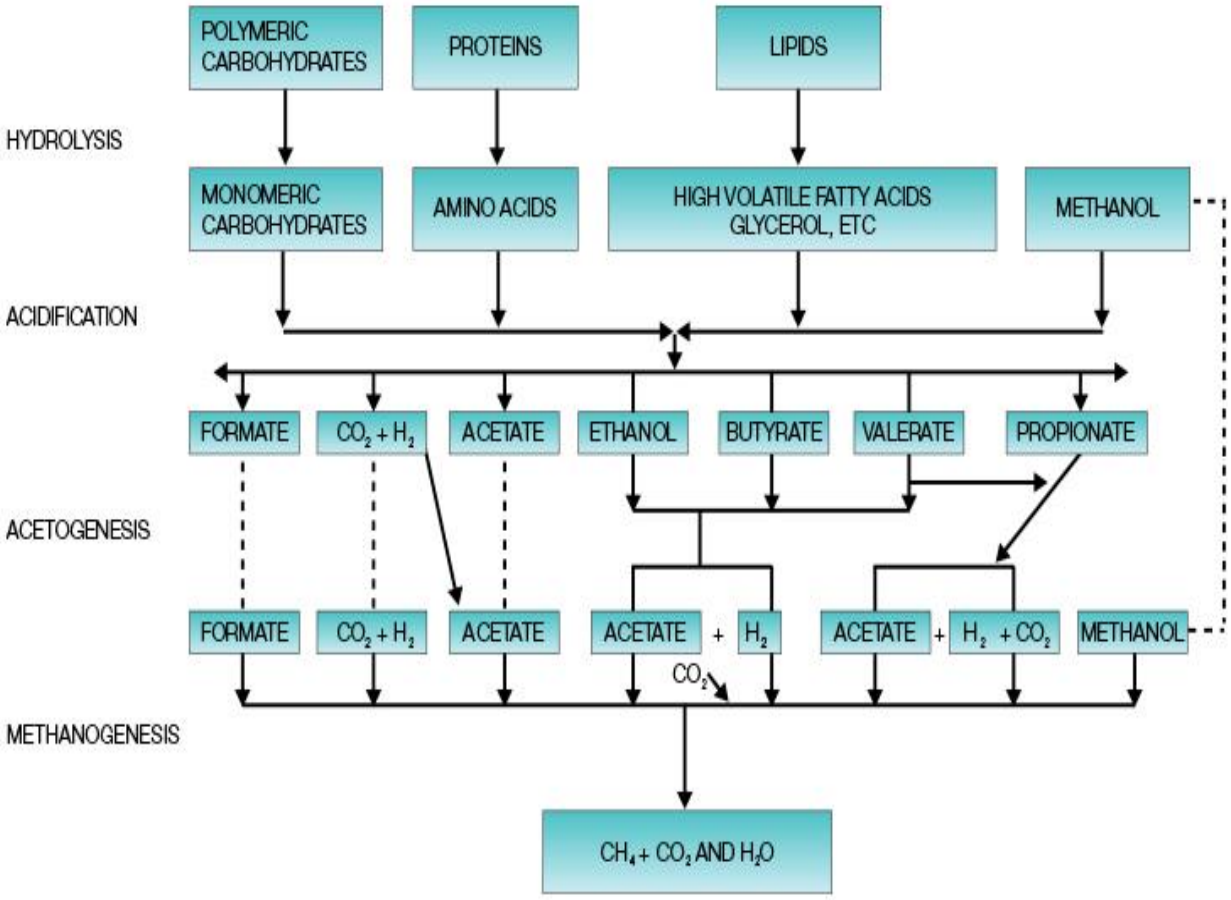
4. Methanogenesis:

- Methanogenesis constitutes the final stage of anaerobic digestion in which methanogens create methane from the final products of acetogenesis as well as from some of the intermediate products from hydrolysis and acidogenesis

- There are two general pathways involving the use of **acetic acid** and **carbon dioxide**, the two main products of the first three steps of anaerobic digestion, to create methane

- Methanogenesis: $CO_2 + 4\ H_2 \rightarrow CH_4 + 2H_2O$ $CH_3COOH \rightarrow CH_4 + CO_2$ While CO_2 can be converted into methane and water through the reaction, the main mechanism to create methane in

methanogenesis is the path involving acetic acid. This stage leads to generation of methane and CO2, the two main products of anaerobic digestion.



Uses or Advantages of biogas production

- It is a eco-friendly fuel.
- The required raw materials for biogas production are available abundantly in villages.
- It not only produces biogas, but also gives us nutrient rich slurry that can be used for crop production.
- It prevents the health hazards of smoke in poorly ventilated rural households that use dung cake and fire-wood for cooking.
- It helps to keep the environment clean, as there would be no open heap of dung or other waste materials that attract flies, insects and infections
- Availability of biogas would reduce the use of firewood and hence trees could be saved.

MUSHROOM CULTIVATION FROM AGRI WASTES:

Definition of Mushrooms:

The Greeks and Romans described mushrooms as **"food for the god".** During that period, people consumed the mushrooms after collecting them from their natural habitat.

Mushrooms are fruiting bodies of various species of fungi. They are eaten raw or used to add flavor to dishes. Many species are gathered wild, but care must be taken as some are poisonous.

The most commonly cultivated species is *Agaricus bisporus* and other types of commercial importance include shiitake, straw mushrooms, oyster mushrooms and winter mushrooms.

Classification of Mushrooms

Accordingly mushrooms can be grouped into four categories (Structural):

(1)**Edible mushrooms:** are those which are fleshy and edible (e.g., *Agaricus bisporus*)

(2)**Medicinal mushrooms:** which are considered to have medicinal applications (e.g., *Ganoderma lucidum)*

(3)**Poisonous mushrooms:** are those proven to be or suspected of being poisonous (e.g., *Amanita phalloides*)

(4**) "Other mushrooms"**: a miscellaneous category, which includes a large number of mushrooms whose properties remain less well defined.

Cultivation of Paddy Straw Mushroom (Volvariella Volvacea):

The paddy straw mushroom is also called tropical, straw or Chinese mushroom. The genus Volvariella belongs to the family Pluteaceae under the order Agaricales of Basidiomycotina.

The genus Pleurotus contains more than 50 species, of which ***P. flabellatus***, ***P. ostreatus***, ***P. sajor-caju, P. sapidus, P. fossulatus, P. cornu- copieae, P. sapathulatus*** and ***P. florida*** have been cultivated in India.

Substrates: In addition to **paddy straw**, other substrates like **water hyacinth, cotton waste**, **banana leaves**, **sawdust**, **sugarcane thrash** (bagasse) etc., are used as substrate due to the presence cellulose, hemicellulose and lignin.

In India, the cultivation of this mushroom was first initiated in Coimbatore, Tamil Nadu, and now it is popular in different tropical parts due to the requirement of temperature ranges between 30-45°C.

The process of cultivation of straw mushroom is as follows:

1. Requirements,

2. Preparation of spawn,

3. Cultivation procedure,

4. Harvesting of fruit bodies, and

5. Preservation of fruit bodies.

1. Requirements:

i. Spawn of V. volvacea (600-800 gms grain spawn/bed),

ii. Bricks,

iii. Bamboo frame (1 m x 1 m),

iv. Small water tank,

v. Paddy straw (preferably from aman variety), apx. 36 kg,

vi. Loose straw 5-6 kg,

vii. Powder of Gram or Arhar seeds 200-250 gm,

viii. Thermometer (0-100°C scale), and ix. White polythene sheet.

2. Preparation of Spawn:

The spawn can be prepared following the same procedure as adopted in ***Agaricus brunnescens***. But in addition to grains of wheat or sorghum, the rice straw can also be used as substrate.

3. Cultivation Procedure:

Fresh paddy straw, not more than one year old and preferably from the Aman variety, should be collected from farmer or from any store. 24 straw bundles of about 1.5 kg each along with some loose straw are immersed completely in a water-filled tank by putting some weight on the bundles for about 12-15 hours.

Then take out the straw bundles from the tank and keep them in stack on cement floor to drain off excess water.

4. Preparation of Bed and Spawning:

One square bed of 1 m x 1 m x 1 m or 1 m x 0.75 m x 1 m is prepared with pre-soaked straw, keeping the butt ends (basal region) at one side, placed close to each other and arranged length-wise on a bamboo frame, supported on 4 pillars made of bricks. Same number of soaked straw bundles are placed on the previous one by keeping the butt ends in opposite direction.

Inoculate the bed with spawn. The bids of spawn are placed about 8-10 cm inside the margin, maintaining a space of about 5 cm from each other. About 160-200 grams spawn is required for each layer. Powders of Cram or Arhar seeds of about 50 gms or more are spread along the line of spawning.

Second and third layers are arranged and inoculated in a similar process, but 2nd layer is placed at right angle to the 1st layer and the third layer is like the 1st layer. The spawn and seed powder on the 2nd layer will be given like the 1st layer, but on the 3rd layer those will be distributed uniformly throughout the bed.

Finally, cover the top layer with loose straw. Loosely bind the bed with rope made of wheat straw at the three regions, one in the middle and one on each side. Press the bed with the help of wooden board to release the internal air and thus the spawn get compressed with the wet straw bundles. Cover the bed with polythene sheet.

Watering should be done once or twice with the help of micro-sprayer. The temperature of the bed should remain 30-35°C after spawning and it should not go below 30°C during the growing season. The relative humidity should be between 80-90%.

Polythene sheet should be removed after 7-10 days of spawning for the appearance of button of the mushroom. After that the buttons quickly develop into fruit bodies.

The straw once used in the mushroom cultivation can be used again. The bed should be prepared under shade away from direct sunlight and rain and also in well-aerated condition, but wind should not blow very fast.

5. Harvesting of Mushroom:

The fruit bodies are harvested by gentle twisting when the volva is about to rupture or is just ruptured. The production continues for 25- 30 days, but in two phases. The total production per bed is approximately 3 kg. The production of second phase is comparatively less.

6. Preservation:

The fruit bodies are consumed fresh or can be preserved by drying or in refrigerator for 27- 48 hours. Drying can be done either in the sun or in oven at 50-60°C temperature.

Uses / Applications:

i. Mushrooms are the richest source of vegetable protein.

ii. The protein content varies from 1.1-4.98% in common cultivable mushroom (much higher than pulses, vegetables and fruits).

iii. All the essential amino acids including lysine (550 mg/gm) are present in much higher amount than even egg.

iv. Mushrooms contain sufficient quantities of mineral elements such as Na, K, Ca, P and Fe.

v. Mushrooms contain folic acid.

vi. Mushrooms contain vitamins like B, C, D and K.

vii. They contain little amount of fat (0.35- 0.65% dry wt.) and starch (0.02% dry wt.).

SCP PRODUCTION FROM AGRI WASTES:

Definition - single-cell protein. :

Protein that consists of processed microorganisms (such as yeasts or bacteria) grown in culture and that is used as a source of food especially for livestock.

Producing Microbes:

Microbes employed include:

- *Yeast*
  - *Saccharomyces cerevisiae*
  - *Pichia pastoris*
  - *Candida utilis*
  - *Torulopsis*
  - *Geotrichum candidum*
- Fungi (Mycoprotein)
  - *Aspergillus oryzae*
  - *Fusarium venenatum*
  - *Sclerotium rolfsii*
  - *Polyporus*
  - *Trichoderma*
  - *Scytalidium acidophilum*[18]
- Bacteria
  - *Rhodobacter capsulatus*
- Algae
  - spirulina (dietary supplement)
  - *Chlorella*

Substrates:

The nature of the raw materials supplying substrates is very crucial for SCP production. The cost of raw material significantly influences the final cost of SCP. The most commonly used raw materials may be grouped in the following categories.

1. High-energy sources e.g. alkanes, methane, methanol, ethanol, gas oil.

2. Waste products e.g. molasses, whey, sewage, animal manures, straw, bagasse.

3. Agricultural and forestry sources e.g. cellulose, lignin.

4. Carbon dioxide, the simplest carbon source.

Production of SCP from Wastes:

There are several materials that serve no useful purpose and they are collectively referred to as wastes e.g. molasses, whey, animal manures, sewage, straw, date wastes. These waste products, formed in various industries and other biological processes, largely contribute to environmental pollution. There are several advantages of utilizing wastes for the production of SCP.

These include the conversion of low-cost organic wastes to useful products, and reduction in environmental pollution. However, there has been very limited success for the large scale production of SCP from wastes. This is mainly because of transportation cost and technical difficulties. The technology adopted and the organism employed for SCP production depends on the waste being used as the substrate. Thus, Saccharomyces cerevisiae is used for molasses, Kluyveromyces fragile for cheese whey.

Symba process:

Symba process is a novel technology developed in Sweden to produce SCP by utilizing starchy wastes by employing two yeasts, ***Endomycopsis fibuligira*** and ***Candida utilis***. The Symba process is carried out in three phases.

Phase I:

The waste material containing starch is sterilized by passing through a heat exchanger.

Phase II:

The sterilized material is passed through two bioreactors. The first reactor contains E. fibuligira which hydrolyses starch. When this hydrolysate is passed to the second bioreactor, the organism, C. utilis grows to form biomass.

Phase III:

The microbial biomass can be separated by centrifugation. The samples of SCP can be dried, packaged and stored.

Applications of Symba product:

The yeast biomass produced in Symba process is of good nutritive value. It is widely used as an animal feed for pigs, calves and chicken. The animals grow quite well and no adverse effects have been reported.

APPLICATIONS

1. **As protein supplemented food**
    - Also source of vitamins, amino acids, minerals, crude fibers, etc.
    - Supplemented food for undernourished children.
2. **As health food**
    - Controls obesity• Provides instant energy
    - Example- Spirulina- part of diet of US Olympic team.
3. **In therapeutic and natural medicines**
    - Reduce body weight, cholesterol, stress
    - Lowers blood sugar level in diabetic(due to presence of B - linolenic acid)
    - Prevents accumulation of cholesterol in body
    - Healthy eyes and skin (beta carotene)• Beta carotene (anti cancer substance- UN National Cancer Research Institute)
    - Increase lactation.
4. **In cosmetics**
    - Important role in maintaining healthy hair (vitamin A and B)
    - Many herbal beauty products
    - Biolipstics and herbal face cream(Phycocyanin)
    - Capable of replacing coal tar dye based cosmetics.

5. **Poultry and cattle feed**

- Excellent, convenient source of protein and other nutrients.

- Used to feed cattle, fishes etc.

BIODEGRADATION OF COMPLEX POLYMERS

- The term "polymer" derives from the ancient Greek word polus, meaning "many, much" and meros, meaning "parts", and refers to a molecule whose structure is composed of multiple repeating units.
- The term was coined in 1833 by Jons Jacob Berzelius
- A polymer is a large molecule (macromolecules) composed of many repeated subunits, known as monomers. monomers can be linked together in various ways to give linear, branched and cross linked polymers etc

Cellulose:

- Cellulose is the most abundant organic matter in nature.
- It is a polysaccharide composed of glucose molecules linked together in a linear chain of 1-4-glycosidic linkage.
- Several microorganisms are capable of degrading Cellulose:

1. **Fungi:** *Aspergillus, Fusarium, Trichoderma, Curvularia*
2. **Bacteria:** *Bacillus, Achromobacter, Pseudomonas, Vibrio,Cellulomonas, Streptomyces, Nocardia*

- Cellulase splits the long chain cellulose to yield glucose or may split into cellbiose.
- Cellobioseis a two molecules of glucose linked together. Cellobiose in turn can be split by cellobiase to yield two molecules of glucose.

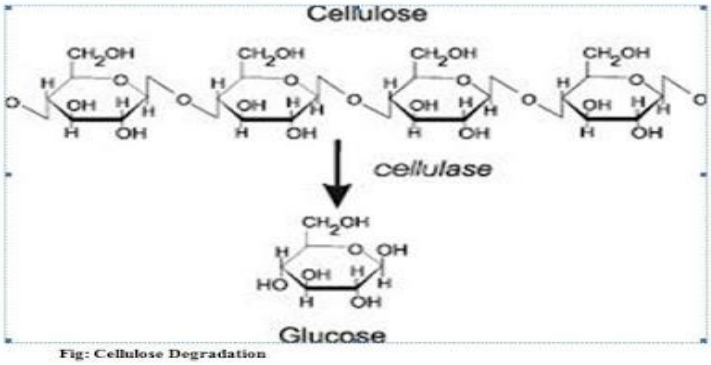


Fig: Cellulose Degradation

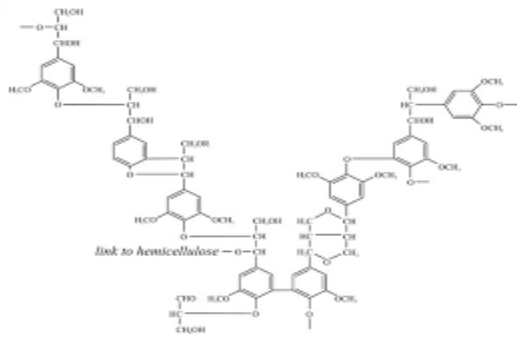
Hemicellulose:

- Hemicullose are water soluble polysaccharides and consists of hexoses, pentoses and uronic acids.
- Glucose, galactose, mannose, xylose, arabinose, glucoronic acid, and galactouronic acids are commonly found in hemicellose plants.
- The molecules contains aromatic ring as the building blocks.
- Hemicellulase degrade the hemicellulose and release the constituent components

Bacillus, Paenibacillus, Streptomyces or the actinobacteria group, while the Gramnegative strains were assigned to the genera *Pseudomonas, Acinetobacter, Ochrobactrum*, and to genera belonging to the family Enterobacteriaceae are generally involved in hemicellolose digestion.

Lignin:

- Lignin is one of the most resistant organic substances for the microorganisms to degrade.
- Many Basidiomycetes have been found to possess special capacity in degradation of lignin
- Only rarely bacteria have been found to reduce lignin.• The fungi commonly found are : *Fomes, Ganoderma, Agaricus, Armilaria, Polyporous, etc.*
- These fungi utilizes high lignin containing materials.
- The enzymatic breakdown of lignin may result in simpler aromatic compounds syringaldehyde, vanillin, *p*-hydroxy-benzaldehyde and ferulic acid.
- The final cleavage of the aromatic ring may take place with the involvement of other fungi and bacteria to yield low molecular weight organic acids, carbondioxide, methane etc.
- Fungi degrade lignin by secreting enzymes collectively termed “ligninases”. Ligninases can be classified as either phenol oxidases (laccase) or heme peroxidases [lignin peroxidase (LiP), manganese peroxidase (MnP) and versatile peroxidase (VP)]



Starch:

- Starch serves as a storage products of plants and is present in several specialized parts such as tubers, bulbs, rhizome, and seed.
- It contains two components:

1. Amylose
2. Amylopectin

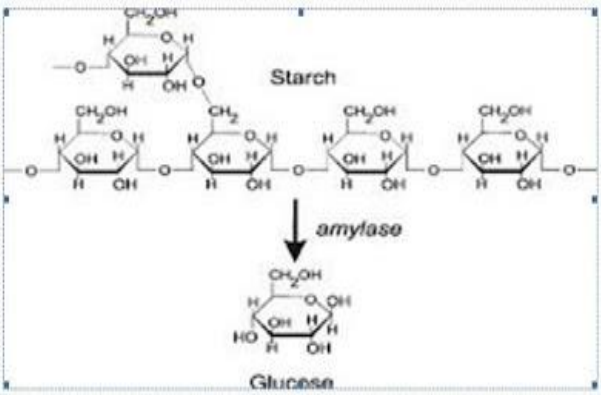


Fig: Microbial degradation of Starch

In amylose, the glucose molecules are linked together by an α-1,4- glycosidic bondage whereas in amylopectin, besides the same linkage of glucose molecules, side chains are attached through α-1,6- glycosidic bondage.

- Aerobically, the microbes fully utilize starch to produce carbondioxide and low molecular weight organic acids.
- Anaerobically, fermentation takes place to yield methane, acetic acids, lactic acids and butyric acids.
- Bacteria, actinomycetes and fungi have the physiological capacity to utilize starch as a carbon source for growth and multiplication.

Pectin:

- Pectic substances are polysaccharides found in the constituents of middle lamella and in the primary and secondary cell walls of plants.
- They are made up of galactourinic acid units bound in a long chain.
- There are three types of pectic substances, viz. pectin, protopectin, and pectic acid.
- These compounds, though, closely related requires a specific enzymes to get hydrolysed.
- Microorganisms involved in pectin degradation:

  Bacillus, Clostridium, Pseudomonas, Erwinia,

  Several Fungi

  Streptomyces
- The enzymes involved in pectin digestion are collectively known as pectinases.

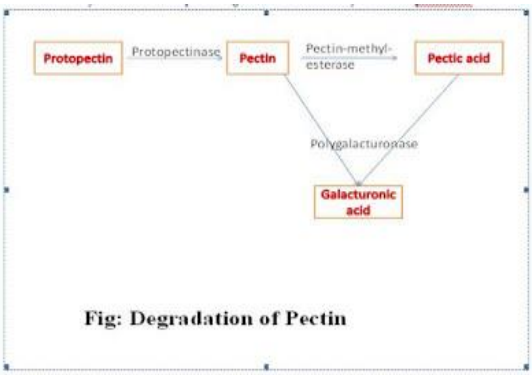
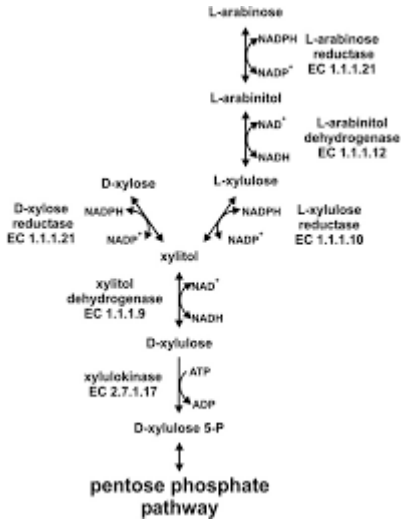


Fig: Degradation of Pectin

Chitin:

- Chitin is a polysaccharide whose basic unit is an amino sugar.
- It is structural components giving mechanical strength to several plants and animals.
- It is also present in fungal cell wall and insects.
- This is one of the hardiest organic molecules for microbial action in soil.
- The organisms involved are:

  Streptomyces, Nocardia, Micromonospora, Aspergillus, Penicillium, Mucor, Trichoderma, etc
- The breakdown products are: glucosamine, acetic acid, ammonia, carbondioxide,
- Chitinase initiates the process to reduce chitin to diacetylchitobiase, and chitobiase reduces the latter into acetylglucosamine.



Proteins:

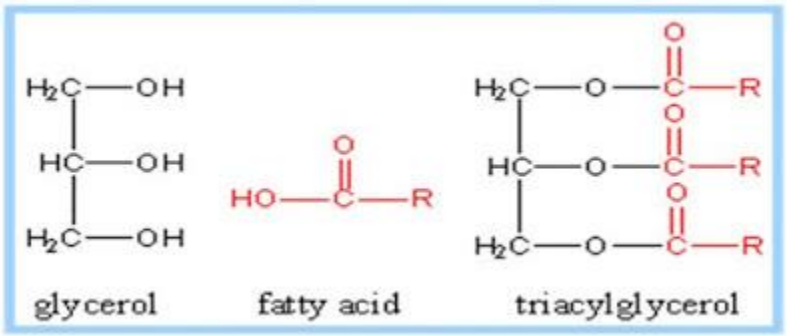
- The proteins are made up of amino acids.
- These are the structural components of plants as well as many other living things.
- The residual proteins that are found in soil are hydrolyzed by several organisms.
- The microorganisms splits protein into polypeptides and finally into simpler amino acids.
- These amino acids are either utilized by the microorganisms for body build-up, or acted upon by some bacteria and fungi producing ammonia, carbondioxide, various organic acids, and alcohol.
- Under anaerobic conditions, various amino acids and mercaptans are formed, producing offensive odour.
- The organisms mainly involved are:

E. coli, Bacteroides, Propionibacterium, Streptococcus, Clostridium, Bacillus, Staphylococcus.

Fats & Oils (Lipids):

- Lipids are hydrolyzed to glycerol and fatty acids
- The organisms involved are: *Clostridium. Acinetobacter, Bacillus* etc

The enzyme involved in lipid degradation is lipases.



BIOREMEDIATION

Bioremediation is the process of using organisms to neutralize or remove contamination from waste. It is very important to understand that this form of waste remediation uses no toxic chemicals, although it may use an organism that can be harmful under certain circumstances. A gross, but simple explanation of bioremediation is the use of maggots in wound care control.

Wounds that have contamination can have maggots introduced to them. The maggots then eat the contamination, allowing the wound to heal correctly. That is a form of medical bioremediation but there are many other types that are used to control different waste contamination.

Definition:

"Bioremediation is a waste management technique that involves the use of organisms to remove or neutralize pollutants from a contaminated site."

Important of Bioremediation

Bioremediation is important for two reasons.

1. It uses no chemicals – One of the issues with using man-made chemicals in the treatment and removal of contamination is that the chemicals eventually make it into the water supply. There were many chemicals used at the beginning of the waste management era that we now know were very harmful to plant, animal and human life once they reached the water supply.

2. It can allow waste to be recycled – Another major reason that bioremediation is preferred is that once the waste is treated and the contamination neutralized or removed, the waste itself can then be recycled. When chemical remediation types are used, the waste is still contaminated just with a less toxic substance and in general, cannot then enter into the recycle process. Bioremediation allows for more waste to be recycled while chemical methods still create waste that cannot be used and has to be stored somewhere.

Organisms involved in bioremediation

Bioremediation makes use of living organisms to break down the pollutant into harmless, natural compounds. Bioremediators, the organisms used for bioremediation, are most often **bacteria**, **archaea** and **fungi** due to their rapid **growth** rate, variable metabolic needs and ability to be genetically manipulated.

2 classes of Bioremediation used

There are two classes of bioremediation used. Don't confuse the class type with the actual types of bioremediation available, the classes describe the general application of the organisms. The two classes are:

- **In-situ** – In situ refers to when contaminated waste is treated right at its point of origin. For example, there may be soil that is contaminated. Rather than remove the soil from its point of origin, it is treated right where it is. The benefit to in situ treatment is that it prevents the spread of contamination during the displacement and transport of the contaminated material.

- **Ex-situ** – Ex situ refers to treatment that occurs after the contaminated waste has been removed to a treatment area. To use soil as the example again, the soil may be removed and transported to an area where the bioremediation may be applied. The main advantage to this is it helps to contain and control the bioremediation products, as well as making the area that was contaminated available for use.

Types of Bioremediation

There are far more than 9 types of bioremediation, but the following are the most common ways in which it is used.

1. Phytoremediation – use of plants to remove contaminants. The plants are able to draw the contaminants into their structures and hold on to them, effectively removing them from soil or water.

2. Bioventing – blowing air through soil to increase oxygen rates in the waste. This is an effective way to neutralize certain oxygen sensitive metals or chemicals.

3. Bioleaching – removing metals from soil using living organisms. Certain types of organisms are draw to heavy metals and other contaminants and absorb them. One new approach was discovered when fish bones were found to attract and hold heavy metals such as lead and cadmium.

4. Landfarming – turning contaminated soil for aeration and sifting to remove contaminants, or deliberately depleting a soil of nitrogen to remove nitrogen based organisms.

5. Bioreactor – the use of specially designed containers to hold the waste while bioremediation occurs

6. Composting – containing waste so a natural decay and remediation process occurs.

7. Bioaugmentation – adding microbes and organisms to strengthen the same in waste to allow them to take over and decontaminate the area

8. Rhizofiltration – the use of plants to remove metals in water.

9. Biostimulation – the use of microbes designed to remove contamination applied in a medium to the waste.

Factors Responsible For Microbial Bioremediation to be Effective

The major advantage of the bioremediation methods is that it allows for contamination to be treated, neutralized or removed and then produces a waste product itself that is more easily disposed of. In some cases, there is no need for disposal at all. In the case of the plants used in phytoremediation and rhizofiltration, the plant is able to do something called bioaccumulation. This means is holds onto the contaminant. As the plant is still growing, there is no need to remove and destroy it. In many ways it is similar to having a rechargeable battery. In the case of contaminated waste, it is the plant that keeps growing to allow for more storage of waste. This is a uniquely cost effective solution for contaminated waste.

Microbial Population: Suitable kinds of organisms that can biodegrade all of the contaminants

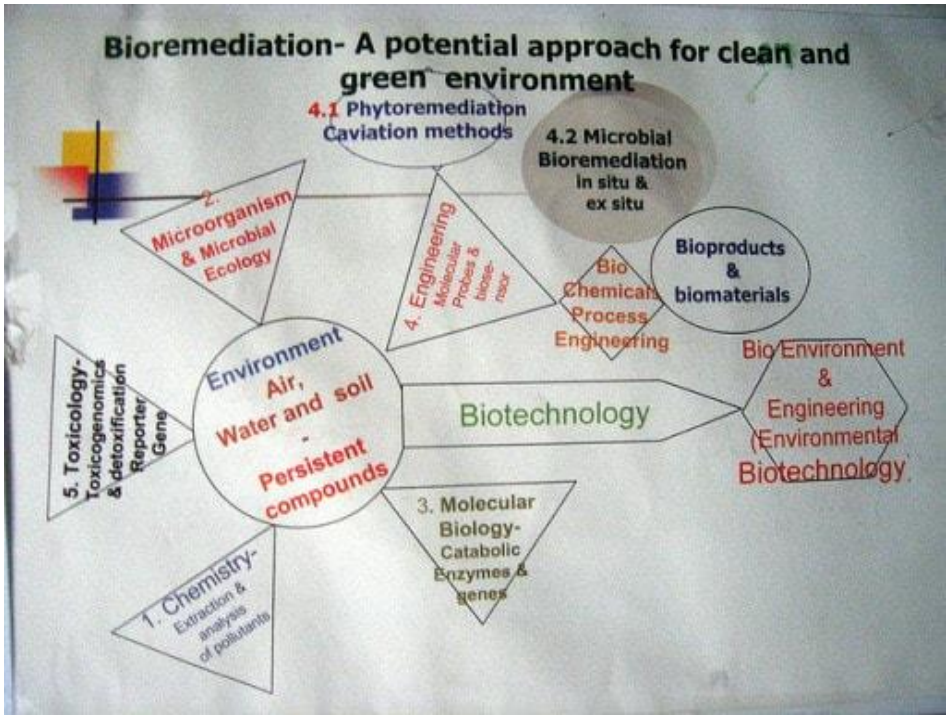
Oxygen: Enough to support aerobic biodegradation (about 2% oxygen in the gas phase or 0.4 mg/liter in the soil water)

Water: Soil moisture should be from 50–70% of the water holding capacity of the soil

Nutrients: Nitrogen, phosphorus, sulfur, and other nutrients to support good microbial growth

Temperature: Appropriate temperatures for microbial growth (0–40˚C)

pH: Best range is from 6.5 to 7.5



BIOAUGMENTATION:

Bioaugmentation is the practice of adding cultured microorganisms into the subsurface for the purpose of biodegrading specific soil and groundwater contaminants.

Bioaugmentation and biostimulation:

Biostimulation involves the modification of the environment to stimulate existing bacteria capable of bioremediation. ... **Biostimulation** can be enhanced by **bioaugmentation**. This process, overall, is referred to as bioremediation and is an EPA-approved method for reversing the presence of oil or gas spills.

Bioaugmentation for Chlorinated Contaminants

In many cases, cultured microorganisms used for bioaugmentation are “specialists” in degrading specific target contaminants. For example, some microbes may be able to degrade the chlorinated compounds cis-1,2 dichloroethylene (cDCE) and vinyl chloride (VC) more quickly than the naturally-occurring microbial community at a particular site. As a result, the remediation community has shifted toward a more prescriptive approach with the use of bioaugmentation to accelerate the reductive dechlorination process, achieve remediation targets, and realize cost savings.

Specific strains of anaerobic microorganisms have been isolated, cultured and are commercially available for the biodegradation of the chlorinated contaminants cDCE and VC. Bio-Dechlor INOCULUM® Plus is a widely used bioaugmentation culture designed specifically for this purpose. It is typically co-applied with electron donor solutions such as 3-D Microemulsion® and HRC® to facilitate full and rapid reductive dechlorination.

Bioaugmentation for Petroleum Hydrocarbons

Relative to bioaugmentation for the degradation of petroleum hydrocarbons or any aerobically degradable contaminants in soil and groundwater, it is rare if ever that aerobic degrader augmentation is required to facilitate enhanced aerobic biodegradation.

Biostimulation

Biostimulation involves the modification of the environment to stimulate existing bacteria capable of bioremediation. This can be done by addition of various forms of rate limiting nutrients and electron acceptors, such as phosphorus, nitrogen, oxygen, or carbon (e.g. in the form of molasses).

Alternatively, remediation of halogenated contaminants in anaerobic environments may be stimulated by adding electron donors (organic substrates), thus allowing indigenous microorganisms to use the halogenated contaminants as electron acceptors.EPA Anaerobic Bioremediation Technologies

Additives are usually added to the subsurface through injection wells, although injection well technology for biostimulation purposes is still emerging. Removal of the contaminated material is also an option, albeit an expensive one.

Biostimulation can be enhanced by bioaugmentation. This process, overall, is referred to as bioremediation and is an EPA-approved method for reversing the presence of oil or gas spills. While biostimulation is usually associated with remediation of hydrocarbon or high production volume chemical spills, it is also potentially useful for treatment of less frequently encountered contaminant spills, such as pesticides, particularly herbicides.

Bioleaching:

Bioleaching is the extraction of metals from their ores through the use of living organisms. This is much cleaner than the traditional heap leaching using cyanide.

The most commonly used microorganisms in bioleaching are;

- ***Thiobacillus thiooxidants***
- ***Thiobacillus ferrooxidants***
- Other microorganisms which may also be used are; ***Bacillus Licheniformis, B. luteus, B megaterium, B polymyxa, B leptospirillum ferrooxidants, Pseudomonas flurescens, Sulfolobus acidocaldarius***, etc;

Chemistry of Bioleaching:

- *Thiobacillus thiooxidant* and *T. ferrooxidants* have always been found to be present on the leaching dump
- The specie of thiobacillus is most extensively¬ studied gram negative bacteria which derives energy from oxidation of fe2
- The reactions mechanisms are two types, i.e.,

  • Direct bacterial leaching

  • Indirect bacterial leaching

- **Direct bacterial leaching**

In this process, a physical contact exist between bacteria and ores and oxidation of minerals takes place though enzymatically catalysed steps

Example; pyrite is oxidised to ferric sulphate

$$2FeSo4+2H2So4 \rightarrow 2FeS2+7O2+2H2O$$

- **Indirect bacterial leaching**

In this process the microbes are not in direct contact with minerals, but leaching agents are produced by these microbes which oxidize the ores.

There are three commercial process used in bioleaching;

a. **Slope leaching**
b. **Heap leaching**
c. **In situ leaching**

a. **Slope Leaching:**

- Here the ores are first ground to get fine pieces and then dumped into large leaching dump
- Water containing inoculum of *thiobacillus* is continuously sprinkled over the ore
- Water is collected from the bottom and used to extract metals and generate bacteria in an oxidation pond

b. **Heap Leaching:**

- Here the ore is dumped into large heaps called leach heaps
- Water containing inoculum of *thiobacillus* is continuously sprinkled over the ore
- Water is collected from the bottom and used to extract metal and generate bacteria in an oxidation pond

c. **In situ Leaching:**

- In this process the ore remains in its original position in earth.
- Surface blasting of earth is done to increase the permeability of water.
- Water containing thiobacillus is pumped through drilled passages to the ores
- Acidic water seeps through the rock and collects at bottom
- Again, water is pumped from bottom
- Mineral is extracted and water is reused after generation of bacteria

Example Copper Leaching:

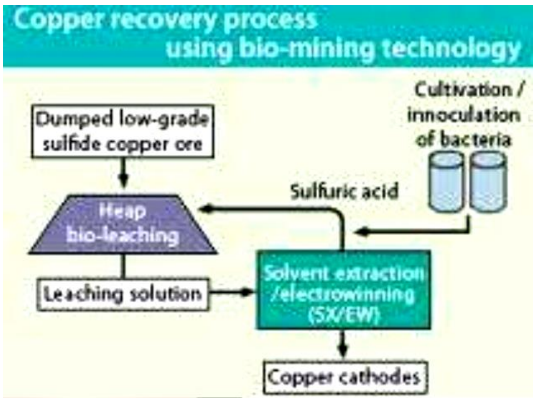
- Ores of copper from which copper is recovered are,

- Chalcocite(Cu2S)
- Chalcopyrite(CuFeS2)
- Covellite(CuS)

- Copper leaching is operated as simple heap leaching and in situ leaching process
- Dilute sulphuric acid is percolated down through the pile
- Liquid coming out of bottom of pile reach in mineral
- Liquid is collected and transported to precipitation plant
- Metal is precipitated an purified

Reactions:

- Chalcocite is oxidized to soluble form of copper
  - CuS+Cu2+ +H2O →Cu2S+O2+
- Thereafter chemical reactions occur, i.e. Cu+8Fe+SO4+8H →CuS+8Fe +4H2O Copper is removed,
  - Cu+Fe2+ → Fe0+Cu Fe2+ is transferred to oxidation pond Fe3+ +1/2(H2O) →Fe+1/4(O2)+H+
- Fe3+ ions produced is an oxidation of ore
- It is pumped back to pile
- Sulphuric acid is added to maintain pH



Uranium Leaching:

- Uranium is extracted when insoluble tetravalent uranium is oxidized with a hot H2So4/FeSo4 solution to make hexavalent uranium sulphate
- pH required for the reaction is 1.5-3.5
- Temperature: around 35 degree C following reaction takes place,

  UO2SO4+2FeSO4 →UO2+Fe2(SO4)3
- Uranium leaching is an indirect process
- When T.ferrooxidants are involved in uranium extraction, they do not directly attack on ore but on the iron oxidants.
- The pyrite reaction is used for the initial production of Fe
- Reaction;

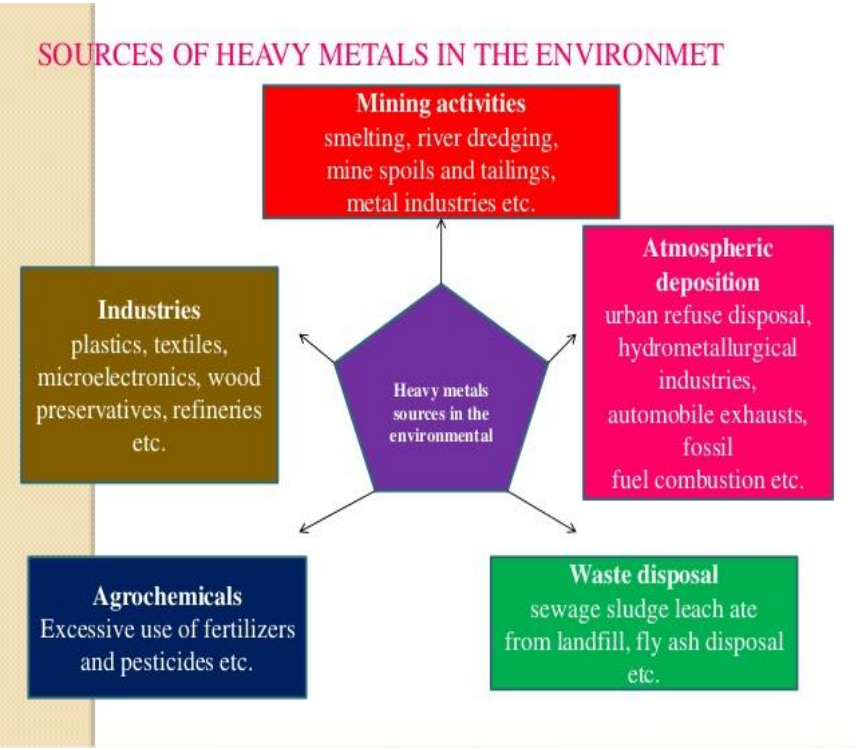
  - Fe2 [SO4]3+ H2SO4 →2FeS+H2O+7 ½[O2]

XENOBIOTICS:

The term **Xenobiotic** comes from the Greek for xeno (foreign) and biotics (of or pertaining to life). **Xenobiotics** are compounds that are foreign to an organism or are not part of **its** normal nutrition. **Examples** of **Xenobiotics** are compounds that include drugs, food additives, and environmental pollutants.

Sources of Xenobiotics

1. **Petrochemical industry**: -oil/gas industry, refineries. - produces basic chemicals e.g. vinyl chloride and benzene
2. **Plastic industry** : - closely related to the petrochemical industry - uses a number of complex organic compounds -such as anti-oxidants, plasticizers, cross-linking agents
3. **Pesticide industry :** - most commonly found. -structures are benzene and benzene derivatives,
4. **Paint industry** : - major ingredient are solvents, - xylene, toluene, methyl ethyl ketone, methyl
5. **Others :** - Electronic industry, Textile industry, Pulp and Paper industry, Cosmetics and Pharmaceutical industry, Wood preservation



BIOREMEDIATION OF XENOBIOTICS

Methods:

1. Biodegradation
2. Biotransformation
3. Co – metabolism
4. Bio sorption

- Heavy metals cannot be destroyed by microbes
- But can be transformed from one oxidation or organic state to another
- This may result in either of the following result
    - (i) more water soluble
    - (ii) inherently less toxic
    - (iii) less water soluble
    - (iv) volatilized

BIOLEACHING:

- Some micro organisms can produce metal leaching¬ organic acids. E.g. : Thiobacillus sp. are capable of producing sulphuric acid which can form metal chelates.
- Many microorganisms produce siderophores, iron¬ complexing molecules, some of which have high affinity for heavy metals and form metal chelates. e.g. : Pseudomonas aeruginosa for Cu and Zn

BIOSORPTION :

- Metabolism – independent sorption of heavy metals to¬ biomass.
- Can use low cost waste biomass sources such as spent¬ brewery yeast.
- Some bacteria (e.g. : Alcaligenes eutrophus) can also¬ adsorb metals like Cd and Zn.

BIOIMMOBILIZATION :

- Immobilize metals by precipitation.
- Microbes precipitate heavy metals by changing their¬ valence.
- The process is called Enzyme - Catalyzed¬ Transformation.
- E.g. : U (VI) is highly soluble. But U (IV) formed through¬ enzymatic reduction is highly insoluble.
- Also include formation of insoluble metal phosphates¬ and sulphates (biomineralization).

PANCHAGAVYA

1. Panchagavya

2. Preparations

3. Beneficial effect on commercial crops

4. Recommended dosage

5. Panchagavya for animal health

1. Panchagavya

Panchagavya, an organic product has the potential to play the role of promoting growth and providing immunity in plant system. Panchagavya consists of nine products viz. cow dung, cow urine, milk, curd, jaggery, ghee, banana, Tender coconut and water. When suitably mixed and used, these have miraculous effects.

- Cow dung - 7 kg
- Cow ghee - 1 kg

Mix the above two ingredients thoroughly both in morning and evening hours and keep it for 3 days

- Cow Urine - 10 liters
- Water - 10 liters

After 3 days mix cow urine and water and keep it for 15 days with regular mixing both in morning and evening hours. After 15 days mix the following and panchagavya will be ready after 30 days.

- Cow milk - 3 liters
- Cow curd - 2 liters
- Tender coconut water - 3 liters
- Jaggery - 3 kg
- Well ripened poovan banana – 12 nos.

2. Preparation

All the above items can be added to a wide mouthed mud pot, concrete tank or plastic can as per the above order. The container should be kept open under shade. The content is to be stirred twice a day both in morning and evening. The Panchagavya stock solution will be ready after 30 days. (Care should be taken not to mix buffalo products. The products of local breeds of cow is said to have potency than exotic breeds). It should be kept in the shade and covered with a wire mesh or plastic mosquito net to prevent houseflies from laying eggs and the formation of maggots in the solution. If sugarcane juice is not available add 500 g of jaggery dissolved in 3 liter of water.

Ingredients of Panchagavya

Cow dung

Cow urine

Cow ghee

Milk

Water

Cow curd

Jaggery

Tender Coconut

Well ripened poovan banana

Physico chemical and biological properties of Panchagavya

Chemical composition		
pH	:	5.45
EC dSm2	:	10.22
Total N (ppm)	:	229
Total P (ppm)	:	209
Total K (ppm)	:	232
Sodium	:	90
Calcium	:	25
IAA (ppm)	:	8.5
GA (ppm)	:	3.5

Microbial Load		
Fungi	:	38800/ml
Bacteria	:	1880000/ml
Lactobacillus	:	2260000/ml
Total anaerobes	:	10000/ml
Acid formers	:	360/ml
Methanogen	:	250/ml

Physico-chemical properties of Panchagavya revealed that they possess almost all the major nutrients, micro nutrients and growth harmones (IAA & GA) required for crop growth.

Predominance of fermentative microorganisms like yeast and lactobacillus might be due to the combined effect of low pH, milk products and addition of jaggery/sugarcane juice as substrate for their growth.

The low pH of the medium was due to the production of organic acids by the fermentative microbes as evidenced by the population dynamics and organic detection in GC analysis.

Lactobacillus produces various beneficial metabolites such as organic acids, hydrogen peroxide and antibiotics, which are effective against other pathogenic microorganisms besides its growth. GC-MS analysis resulted in following compounds of fatty acids, alkanes, alconol and alcohols.

Fatty acids	**Alkanes**	**Alconol and Alcohols**
Oleic acids	Decane	Heptanol
Palmitic acid	Octane	Tetracosanol
Myristic	Heptane	Hexadecanol
Deconore	Hexadecane	Octadeconol
Deconomic	Oridecane	Methanol, Propanol, Butanol and Ethanol
Octanoic		
Hexanoic		
Octadeconoic		
Tetradeconoic		
Acetic, propionic, butyric, caproic and valeric acids		

Beneficial effects of Panchagavya on commercial crops

Mango

- Induces dense flowering with

Acid lime

- Continuous flowering

more female flowers

- Irregular or alternate bearing habit is not experienced and continues to fruit regularly
- Enhances keeping quality by 12 days in room temperature
- Flavour and aroma are extraordinary

Guava

- Higher TSS
- Shelf life is extended by 5 days

Turmeric

- Enhances the yield by 22%
- Extra long fingers
- Ensure low drainage loss
- Narrows the ratio of mother and finger rhizomes
- Helps survival of dragon fly, spider etc which in turn reduce pest and disease load
- Sold for premium price as mother/seed rhizome

is ensured round the year

- Fruits are plumpy with strong aroma
- Shelf life is extended by 10 days

Banana

In addition to adding with irrigation water and spraying, 3% solution (100 ml) was tied up at the naval end of the bunch after the male bud is removed. The bunch size becomes uniform. One month earlier harvest was witnessed. The size of the top and bottom hands was uniformly big.

Jasmine

- Exceptional aroma and fragrance
- No incidence of bud worm
- Continuous flowering throughout the year

Vegetables

- Yield enhancement by 18% and in few cases like Cucumber, the yield is doubled
- Wholesome vegetables with shiny and appealing skin
- Extended shelf life
- Very tasty with strong flavour

Generally panchagavya is recommended for all the crops as foliar spray at 30 % level (3 litre panchagavya in 100 litres of water).

4. Recommended dosage

Spray system

3% solution was found to be most effective compared to the higher and lower concentrations investigated. Three litres of Panchagavya to every 100 litres of water is ideal for all crops. The power sprayers of 10 litres capacity may need 300 ml/tank. When sprayed with power sprayer, sediments are to be filtered and when sprayed with hand operated sprayers, the nozzle with higher pore size has to be used.

Flow system

The solution of Panchagavya can be mixed with irrigation water at 50 litres per hectare either through drip irrigation or flow irrigation

Seed/seedling treatment

3% solution of Panchagavya can be used to soak the seeds or dip the seedlings before planting. Soaking for 20 minutes is sufficient. Rhizomes of Turmeric, Ginger and sets of Sugarcane can be soaked for 30 minutes before planting.

Seed storage

3% of Panchagavya solution can be used to dip the seeds before drying and storing them.

Periodicity

Pre flowering phase : Once in 15 days, two sprays depending upon duration of crops

Flowering and pod setting stage: Once in 10 days, two sprays

Fruit/Pod maturation stage : Once during pod maturation

Time of application of Panchagavya for different crops is given as follows

Crops	**Time schedule**
Rice	10,15,30 and 50th days after transpalnting
Sunflower	30,45 and 60 days after sowing
Black gram	Rainfed: 1st flowering and 15 deays after flowering Irrigated: 15, 25 and 40 days after sowing
Green gram	15, 25, 30, 40 and 50 days after sowing
Castor	30 and 45 days after sowing
Groundnut	25 and 30th days after sowing
Bhendi	30, 45, 60 and 75 days after sowing
Moringa	Before flowering and during pod formation
Tomato	Nursery and 40 days after transplanting: seed treatment with 1 % for 12 hrs
Onion	0, 45 and 60 days after transplanting
Rose	At the time of pruning and budding
Jasmine	Bud initiation and setting
Vanilla	Dipping setts before planting

5. Panchagavya for animal health

Panchagavya is a living elixir of many micro organisms, bacteria, fungi, proteins, carbohydrates, fats, amino acids, vitamins, enzymes, known and unknown growth promoting factors micronutrients trace elements antioxidant and immunity enhancing factors.

When taken orally by animals and human beings, the living micro organisms in the Panchagavya stimulate the immune system and produce lot of antibodies against the ingested microorganisms. It acts like vaccine. This response of the body increases the immunity of animals and humans and thus helps to prevent illness and cures disease. It slows down the aging process and restores youthfulness. The other factors present in Panchagavya improve apetite, digestion and assimilation and elimination of toxins in the body. Constipation is totally cured. Thus the animals and humans become hale and healthy with shining hair and skin. The weight gains are impressive.

Pigs

Panchagavya was mixed with drinking water or feed at the rate of 10 ml – 50 ml/pig depending upon the age and weight. The pigs became healthy and disease free. They gained weight at a faster rate. The feed to weight conversion ratio increased tremendously. This helped the piggery owners to reduce the feed cost and to get very good returns due to increased weight.

Goats and Sheep

The goats and sheep became healthy and gained more weight in a short period after having administered 10 ml to 20 ml Panchagavya per animal per day depending upon the age.

Cows

By mixing Panchagavya with animal feed and water at the rate of 100 ml per cow per day, cows become healthier with increased milk yield, fat content and SNF. The rate of conception increased. The retained placenta, mastitis and foot and mouth disease became things of the past. Now the skin of the cow is shiny with more hair and looks more beautiful. Instead of spraying urea on paddy straw (hay) before staking, a few farmers sprayed the 3 percent solution of Panchagavya, layer after layer during the staking and allowed it to ferment. The cows preferred such hay compared to unsprayed hay stock.

Poultry

When mixed with the feed or drinking water at the rate of 1 ml per bird per day, the birds became disease-free and healthy. They laid bigger eggs for longer periods. In broiler chickens the weight gain was impressive and the feed-to-weight conversion ratio improved.

Fish

Panchagavya was applied daily with fresh cow dung in fish ponds. It increased the growth of algae, weeds and small worms in the pond, thus increasing the food availability to fish. The only precaution is that fresh water must be added to the ponds at frequent intervals. Otherwise, the growth of algae, weeds and other organisms will compete with the fish for available soluble oxygen in water. Alternatively, mechanical agitators can also be used to increase the oxygen content in the water. In ten months time each fish grew to a weight of 2 to 3 kgs. With reduced death rate of small fingerlings and increased weight of marketable fish, the fisheries became more profitable.

UNIT IV- SOIL MICROBIOLOGY

Soil microbiology is the study of microorganisms in soil, their functions, and how they affect soil properties.

Importance of Soil Microbiology:

Living organisms both plant and animal types constitute an important component of soil. Though these organisms form only a fraction (less than one percent) of the total soil mass, but they play important role in supporting plant communities on the earth surface.

1. Soil as a living system
2. Soil microbes and plant growth
3. Soil microorganisms and soil structure
4. Organic matter decomposition
5. Humus formation
6. Biogeochemical cycling of elements
7. Soil microorganisms as bio-control agents
8. Soil microbes and seed germination
9. Biological N2 fixation
10. Degradation of pesticides in soil.

1**. Soil as a living system:** Soil inhabit diverse group of living organisms, both micro flora (fungi, bacteria, algae and actinomycetes) and micro-fauna (protozoa, nematodes, earthworms, moles, ants). The density of living organisms in soil is very high i.e. as much as billions / gm of soil, usually density of organisms is less in cultivated soil than uncultivated / virgin land and population decreases with soil acidity. Top soil, the surface layer contains greater number of microorganisms because it is well supplied with Oxygen and nutrients. Lower layer / subsoil is depleted with Oxygen and nutrients hence it contains fewer organisms. Soil ecosystem comprises of organisms which are both, autotrophs (Algae, BOA) and heterotrophs (fungi, bacteria). Autotrophs use inorganic carbon from CO2 and are "primary producers" of organic matter, whereas heterotrophs use organic carbon and are decomposers/consumers.

2. Soil microbes and plant growth: Microorganisms being minute and microscopic, they are universally present in soil, water and air. Besides supporting the growth of various biological systems, soil and soil microbes serve as a best medium for plant growth. Soil fauna & flora convert complex organic nutrients into simpler inorganic forms which are readily absorbed by the plant for growth. Further, they produce

variety of substances like IAA, gibberellins, antibiotics etc. which directly or indirectly promote the plant growth

3. Soil microbes and soil structure: Soil structure is dependent on stable aggregates of soil particles-Soil organisms play important role in soil aggregation. Constituents of soil are viz. organic matter, polysaccharides, lignins and gums, synthesized by soil microbes plays important role in cementing / binding of soil particles. Further, cells and mycelial strands of fungi and actinomycetes, Vormicasts from earthworm is also found to play important role in soil aggregation. Different soil microorganisms, having soil aggregation / soil binding properties are graded in the order as fungi > actinomycetes > gum producing bacteria > yeasts.
Examples are: Fungi like *Rhizopus, Mucor, Chaetomium, Fusarium, Cladasporium, Rhizoctonia, Aspergillus, Trichoderma* and Bacteria like *Azofobacler, Rhizobium Bacillus* and *Xanlhomonas.*

4. Soil microbes and organic matter decomposition: The organic matter serves not only as a source of food for microorganisms but also supplies energy for the vital processes of metabolism that are characteristics of living beings. Microorganisms such as fungi, actinomycetes, bacteria, protozoa etc. and macro organisms such as earthworms, termites, insects etc. plays important role in the process of decomposition of organic matter and release of plant nutrients in soil. Thus, organic matter added to the soil is converted by oxidative decomposition to simpler nutrients / substances for plant growth and the residue is transformed into humus. Organic matter / substances include cellulose, lignins and proteins (in cell wall of plants), glycogen (animal tissues), proteins and fats (plants, animals). Cellulose is degraded by bacteria, especially those of genus *Cytophaga* and other genera *(Bacillus, Pseudomonas, Cellulomonas, and Vibrio Achromobacter)* and fungal genera *(Aspergillus, Penicilliun, Trichoderma, Chactomium, Curvularia).* Lignins and proteins are partially digested by fungi, protozoa and nematodes. Proteins are degraded to individual amino acids mainly by fungi, *actinomycetes* and *Clostridium.* Under unaerobic conditions of waterlogged soils, methane are main carbon containing product which is produced by the bacterial genera (strict anaerobes) *Methanococcus, Methanobacterium* and *Methanosardna.*

5. Soil microbes and humus formation: Humus is the organic residue in the soil resulting from decomposition of plant and animal residues in soil, or it is the highly complex organic residual matter in soil which is not readily degraded by microorganism, or it is the soft brown/dark coloured amorphous substance composed of residual organic matter along with dead microorganisms.

6. Soil microbes and cycling of elements: Life on earth is dependent on cycling of elements from their organic / elemental state to inorganic compounds, then to organic compounds and back to their elemental states. The biogeochemical process through which organic compounds are broken down to inorganic compounds or their constituent elements is known “Mineralization”, or microbial conversion of complex organic compounds into simple inorganic compounds & their constituent elements is known as mineralization.

Soil microbes plays important role in the biochemical cycling of elements in the biosphere where the essential elements (C, P, S, N & Iron etc.) undergo chemical transformations. Through the process of mineralization organic carbon, nitrogen, phosphorus, Sulphur, Iron etc. are made available for reuse by plants.

7. Soil microbes and biological N2 fixation: Conversion of atmospheric nitrogen in to ammonia and nitrate by microorganisms is known as biological nitrogen fixation.

Fixation of atmospheric nitrogen is essential because of the reasons:

1. Fixed nitrogen is lost through the process of nitrogen cycle through denitrification.
2. Demand for fixed nitrogen by the biosphere always exceeds its availability.
3. The amount of nitrogen fixed chemically and lightning process is very less (i.e. 0.5%) as compared to biologically fixed nitrogen
4. Nitrogenous fertilizers contribute only 25% of the total world requirement while biological nitrogen fixation contributes about 60% of the earth's fixed nitrogen
5. Manufacture of nitrogenous fertilizers by "Haber" process is costly and time consuming.

The numbers of soil microorganisms carry out the process of biological nitrogen fixation at normal atmospheric pressure (1 atmosphere) and temp (around 20 °C).

Two groups of microorganisms are involved in the process of BNF.
A. Non-symbiotic (free living) and B. Symbiotic (Associative)

Non-symbiotic (free living): Depending upon the presence or absence of oxygen, non symbiotic N2 fixation prokaryotic organisms may be aerobic heterotrophs (*Azotobacter, Pseudomonas, Achromobacter)* or aerobic autotrophs *(Nostoc, Anabena, Calothrix, BGA)* and anaerobic heterotrophs *(Clostridium,*

Kelbsiella. Desulfovibrio) or anaerobic Autotrophs *(Chlorobium, Chromnatium, Rhodospirillum, Meihanobacterium etc)*

Symbiotic (Associative): The organisms involved are *Rhizobium, Bratfyrhizobium* in legumes (aerobic): *Azospirillum* (grasses), Actinonycetes frantic(with *Casuarinas,* Alder).

8. Soil microbes as biocontrol agents: Several ecofriendly bioformulations of microbial origin are used in agriculture for the effective management of plant diseases, insect pests, weeds etc. eg: *Trichoderma* sp and *Gleocladium* sp are used for biological control of seed and soil borne diseases. Fungal genera *Entomophthora, Beauveria, Metarrhizium* and protozoa *Maltesia grandis. Malameba locustiae* etc are used in the management of insect pests. Nuclear polyhydrosis virus (NPV) is used for the control of *Heliothis /* American boll worm. Bacteria like *Bacillus thuringiensis, Pseudomonas* are used in cotton against Angular leaf spot and boll worms.

8. Degradation of pesticides in soil by microorganisms: Soil receives different toxic chemicals in various forms and causes adverse effects on beneficial soil micro flora / micro fauna, plants, animals and human beings. Various microbes present in soil act as the scavengers of these harmful chemicals in soil. The pesticides/chemicals reaching the soil are acted upon by several physical, chemical and biological forces exerted by microbes in the soil and they are degraded into non-toxic substances and thereby minimize the damage caused by the pesticides to the ecosystem. For example, bacterial genera like *Pseudomonas, Clostridium, Bacillus, Thiobacillus, Achromobacter etc. and* fungal genera like *Trichoderma, Penicillium, Aspergillus, Rhizopus, and Fusarium* are playing important role in the degradation of the toxic chemicals / pesticides in soil.

9. Biodegradation of hydrocarbons: Natural hydrocarbons in soil like waxes, paraffin's, oils etc are degraded by fungi, bacteria and actinomycetes. E.g. ethane *(C2* H6) a paraffin hydrocarbon is metabolized and degraded by *Mycobacteria, Nocardia, Streptomyces Pseudomonas, Flavobacterium* and several fungi.

Microbial association with plants:

Plant-associated microorganisms fulfill important functions for plant growth and health. Direct plant growth promotion by microbes is based on improved nutrient acquisition and hormonal stimulation. Diverse mechanisms are involved in the suppression of plant pathogens, which is often indirectly connected with plant growth. Whereas members of the bacterial genera *Azospirillum* and *Rhizobium*are well-studied examples for plant growth promotion, *Bacillus*, *Pseudomonas*, *Serratia*,*Stenotrophomonas*,

and *Streptomyces* and the fungal genera *Ampelomyces*, *Coniothyrium*, and*Trichoderma* are model organisms to demonstrate influence on plant health.

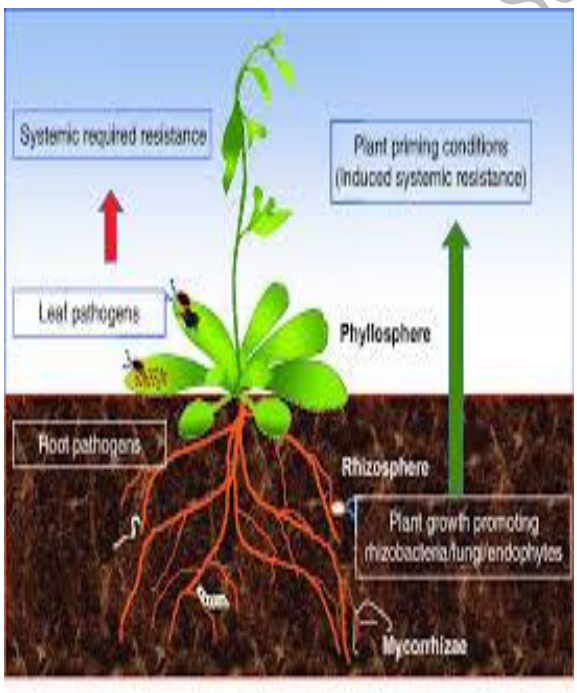
Phyllosphere:

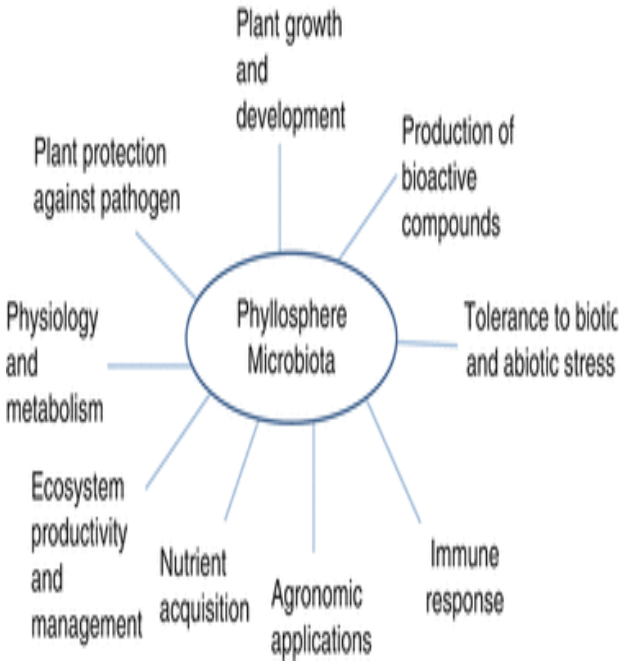
The **phyllosphere** is a term used in microbiology to refer to the total above-ground portions of plants as habitat for microorganisms.

The phyllosphere can be further subdivided into the

- **caulosphere** (stems)
- **phylloplane** (leaves)
- **anthosphere** (flowers), and
- **carposphere** (fruits).

The below-ground microbial habitats (i.e. the thin-volume of soil surrounding root or subterranean stem surfaces) are referred to as the rhizosphere and laimosphere. Most plants host diverse communities of microorganisms including bacteria, fungi, archaea, and protists . Some are beneficial to the plant, others function as plant pathogens and may damage the host plant or even kill it. However, the majority of microbial colonists on any given plant have no detectable effect on plant growth or function.

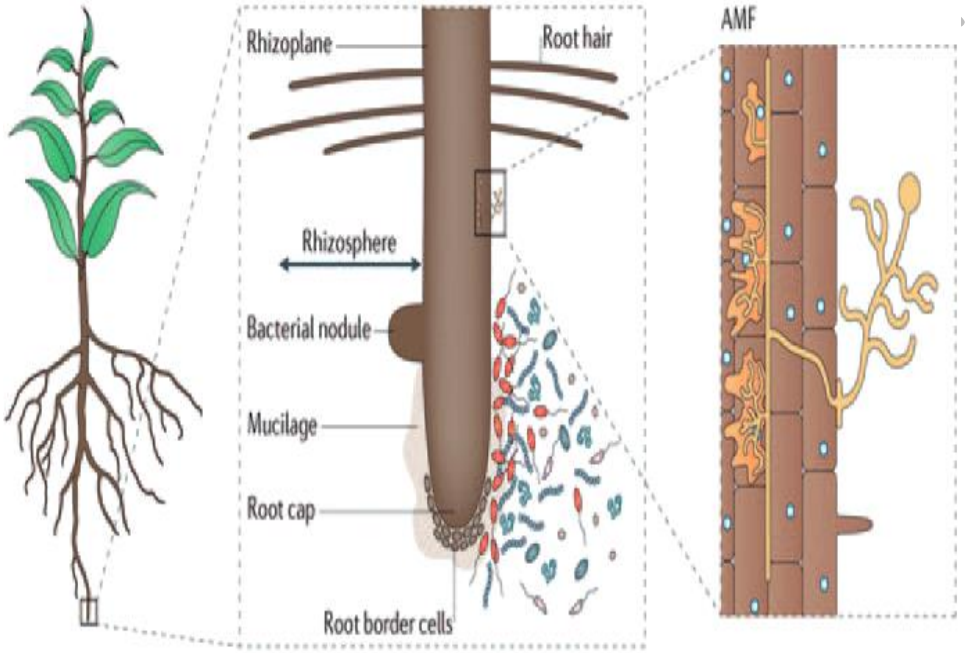




Rhizosphere:

- Microbes present in the root region

The **rhizosphere** is the narrow region of soil that is directly influenced by root secretions and associated soil microorganisms. The **rhizosphere** contains many bacteria and other microorganisms that feed on sloughed-off plant cells, termed rhizodeposition, and the proteins and sugars released by roots.



Microorganisms in the Rhizosphere:

- The rhizosphere region is a highly favorable habitat for the proliferation, activity and metabolism of numerous microorganisms.
- The rhizosphere microflora can be enumerated intensively by microscopic, cultural and biochemical techniques.
- Microscopic techniques reveal the types of organisms present and their physical association with the outer root tissue surface / root hairs.
- The cultural technique most commonly followed is "serial dilution and plate count method" which reveal the quantitative and qualitative population of microflora.

- At the same time, a cultural method shows the selective enhancement of certain categories of bacteria.
- The biochemical techniques used are designed to measure a specific change brought about by the plant or by the microflora.

Rhizosphere Effect:

- The rhizosphere effect on most commonly found microorganisms viz. bacteria, actinomycetes, fungi, algae and protozoa is being discussed.

  - **Bacteria:**

    The greater rhizosphere effect is observed with bacteria (R: S values ranging from 10-20 or more) than with actinomycetes and fungi. Gram-negative, rod shaped, non-sporulating bacteria which respond to root exudates are predominant in the rhizosphere *(Pseudomonas, Agrobacterium).* While Gram-positive, rods, Cocci and aerobic spore forming *(Bacillus, Clostridium)* are comparatively rare in the rhizosphere.
  - The most common genera of bacteria are: *Pseudomonas, Arthrobacter, Agrobacterium, Alcaligenes, Azotobacter, Mycobacterium, Flavobacter, Cellulomonas, Micrococcus* and others have been reported to be either abundant or sparse in the rhizosphere.
  - From the agronomic point of view, the abundance of **nitrogen fixing** and **phosphate solubilizing** bacteria in the rhizosphere assumes a great importance.
  - The aerobic bacteria are relatively **less in the rhizosphere** because of the **reduced oxygen levels due to root respiration.**

B. Fungi:

  -

    Rhizosphere effect is selective and significant on specific fungal genera *(Fusarium, Verticillium, Aspergillus* and *Penicillium)* which are stimulated.
  - The R:S ratio of fungal population is believed to be narrow in most of the plants, usually not exceeding to 10.
  - The zoospore / forming lower fungi such as *Phytophthora, Pythium, Aphanomyces* are strongly attracted to the roots in response to particular chemical compounds excreted by the roots and cause diseases under favorable conditions.
  - Several fungi *eg Gibberella* and *fujikurio* produces *phytohormones* and influence the plant growth.

C. Actinomycetes, Protozoa and Algae:

- ❖ Actinomycetes may also increase in number when antibacterial agents are sprayed on the crop.
- ❖ Among the actinomycete, the phosphate solublizers (eg. *Nocardia, Streptomyces)* have a dominant role to play.
- ❖ As rule actinomycetes, protozoa and algae are not significantly influenced by their proximity to the plant roots and their R: S ratios rarely exceed 2 to 3: 1 and around roots of plants, R: S ratio for these microorganisms may go to high.
- ❖ Because of large bacterial community, an increase in the number or activity of protozoa is expected in the rhizosphere. Flagellates and amoebae are dominant and ciliates are rare in the region.

Mycorrhizae:

- ➢ They are called mycorrhizae from the Greek "mukés", meaning fungus, and "rhiza," meaning roots.
- ➢ A **mycorrhiza** is a symbiotic association between a green plant and a fungus. The plant makes organic molecules such as sugars by photosynthesis and supplies them to the fungus, and the fungus supplies to the plant water and mineral nutrients, such as phosphorus, taken from the soil.

Benefits of Mycorrhizae

Mycorrhizal fungi allow plants to draw more nutrients and water from the soil. They also increase plant tolerance to different environmental stresses. Moreover, these fungi play a major role in soil aggregation process and stimulate microbial activity. According to the plant species and to the growing practices and conditions, mycorrhizae provide different benefits to the plants and to the environment:

- Produce more vigorous and healthy plants
- Increase plant establishment and survival at seeding or transplanting
- Increase yields and crop quality
- Improve drought tolerance, allowing watering reduction
- Enhance flowering and fruiting

- Optimize fertilizers use, especially phosphorus
- Increase tolerance to soil salinity
- Reduce disease occurrence
- Contribute to maintain soil quality and nutrient cycling
- Contribute to control soil erosion

Classification of Mycorrhiza

A. on the basis of tropic level *A. B. Frank* classify mycorriza into_

1. Ectotropic Mycorrhiza
2. Endotropic Mycorrhiza

B. On the basis of morphological and anatomical feature mycorrhiza divided into three types:

1. Ectomycorrhiza
2. Endomycorrhiza
3. Ectendomycorrhiza

C. But mycorrhiza bordly classify into seven categories.They are-

1. **Vesicular-arbuscular mycorrhiza (VAM)--**It was originally applied to symbiotic associations formed by all fungi in the Glomales, but because a major suborder lacks the ability to form vesicles in roots, AM is now the preferred acronym. The order Glomales is further divided into families and genera according to the method of spore formation. The spores of AM fungi are very distinctive. They range m for someμm for Glomus tenue to more than 1,000 μin diameter from 10 Scutellospora spp. The spores can vary in color from hyaline (clear) to black and in surface texture from smooth to highly ornamented. Glomus forms spores on the ends of hyphae, Acaulospora forms spores laterally from the neck of a swollen hyphal terminus, and Entrophospora forms spores within the neck of the hyphal terminus. The Gigasporineae are divided into two genera based upon the presence of inner membranous walls and a germination shield (wall structure from which the germ tube can arise) for Scutellospora or the absence of these structures for Gigaspora.

   The AM type of symbiosis is very common as the fungi involved can colonize a vast taxonomic range of both herbaceous and woody plants, indicating a general lack of host specificity among this type. However, it is important to distinguish between specificity, innate ability to colonize, infectiveness, amount of colonization, and effectiveness, plant response to colonization.

AM fungi differ widely in the level of colonization they produce in a root system and in their impact on nutrient uptake and plant growth.

2. **Ectomycorrhizae**--The diagnostic feature of ectomycorrhizae (EM) is the presence of hyphae between root cortical cells producing a netlike structure called the Hartig net, after Robert Hartig who is considered the father of forest biology. Many EM also have a sheath, or mantle, of fungal tissue that may completely cover the absorbing root (usually the fine feeder roots). The mantle can vary widely in thickness, color, and texture depending on the particular plant-fungus combination. The mantle increases the surface area of absorbing roots and often affects fine-root morphology, resulting in root bifurcation and clustering. Contiguous with the mantle are hyphal strands that extend into the soil. Often the hyphal strands will aggregate to form rhizomorphs that may be visible to the unaided eye. The internal portion of rhizomorphs can differentiate into tubelike structures specialized for long-distance transport of nutrients and water. Ectomycorrhizae are found on woody plants ranging from shrubs to forest trees. Many of the host plants belong to the families Pinaceae, Fagaceae, Betulaceae and Myrtaceae. Over 4,000 fungal species, belonging primarily to the Basidiomycotina, and fewer to the Ascomycotina, are known to form ectomycorrhizae.

3.Ericoid -- cells of the inner cortex become packed with fungal hyphae. A loose welt of hyphae grows over the root surface, but a true mantle is not formed. The ericoid mycorrhizae are found on plants such as Calluna (heather), Rhododendron (azaleas and rhododendrons) and Vaccinium (blueberries) that have very fine root systems and typically grow in acid, peaty soils. The fungi involved are ascomycetes of the genus Hymenoscyphus.

4.Arbutoid -- characteristics of both EM and endomycorrhizae are found. Intracellular penetration can occur, a mantle forms, and a Hartig net is present. These associations are found on Arbutus (e.g., Pacific madrone), Arctostaphylos (e.g., bearberry), and several species of the Pyrolaceae. The fungi involved in the association are basidiomycetes and may be the same fungi that colonize EM tree hosts in the same region.

5.Monotropoid -- the fungi colonize achlorophyllous (lacking chlorophyll) plants in Monotropaceae (e.g., Indian pipe), producing the Hartig net and mantle. The same fungi also form EM associations with trees and thereby form a link through which carbon and other nutrients can flow from the autotrophic host plant to the heterotrophic, parasitic plant.

6.Orchidaceous Mycorrhizae -- Mycorrhizal fungi have a unique role in the life cycle of plants in the Orchidaceae. Orchids typically have very small seeds with little nutrient reserve. The plant becomes colonized shortly after germination, and the mycorrhizal fungus supplies carbon and vitamins to the developing embryo. For achlorophyllous species, the plant depends on the fungal partner to supply carbon throughout its life. The fungus grows into the plant cell, invaginating the cell membrane and forming hyphal coils within the cell. These coils are active for only a few days, after which they lose turgor and degenerate and the nutrient contents are absorbed by the developing orchid. The fungi participating in the symbiosis are basidiomycetes similar to those involved in decaying wood (e.g., Coriolus, Fomes, Marasmius) and pathogenesis (e.g., Armillaria and Rhizoctonia). In mature orchids, mycorrhizae also have roles in nutrient uptake and translocation.

7.Ectendomycorrhiza--Several fungi can colonize the roots of a single plant, but the type of mycorrhiza formed is usually uniform for a host. In some cases, however, a host can support more than one type of mycorrhizal association. Alnus (alders), Salix (willows), Populus (poplars), and Eucalyuptus can have both AM and EM associations on the same plant. Some ericoid plants have occasional EM and AM colonization.
An intermediate mycorrhizal type can be found on coniferous and deciduous hosts in nurseries and burned forest sites. The ectendomycorrhiza type forms a typical EM structure, except the mantle is thin or lacking and hyphae in the Hartig net may penetrate root cortical cells. The ectendomycorrhiza is replaced by EM as the seedling matures. The fungi involved in the association were initially designated "E-strain" but were later shown to be ascomycetes and placed in the genus Wilcoxina.

Nitrogen fixing organism:

Many heterotrophic bacteria live in the soil and fix significant levels of nitrogen without the direct interaction with other organisms. Examples of this type of nitrogen-fixing bacteria include species of *Azotobacter*, *Bacillus*, *Clostridium*, and *Klebsiella*.

NITROGEN FIXERS:

Bacteria that change nitrogen gas from the atmosphere into solid nitrogen usable by plants are called nitrogen-fixing bacteria. These bacteria are found both in the soil and in symbiotic relationships with plants.

- ☆ **Nitrogen-fixing bacteria** are microorganisms present in the soil or in plant roots that change nitrogen gases from the atmosphere into solid nitrogen compounds that plants can use in the soil
- ☆ Examples: *Rhizobium sp. Frankia, Azolla*

Rhizobium - ISOLATION IDENTIFICATION CHARACTERIZATION AND APPLICATION

Rhizobium:

- ❖ They are Gram-negative
- ❖ motile
- ❖ non-sporulating rods

Yeast Mannitol Agar w/ Congo Red

Composition

Ingredients	**Gms / Litre**
Yeast extract	1.000
Mannitol	10.000
Dipotassium phosphate	0.500
Magnesium sulphate	0.200
Sodium chloride	0.100
Congo red	0.025
Agar	20.000
Final pH (at 25°C)	6.8±0.2

ISOLATION OF *Rhizobium:*

Yeast Extract Mannitol Agar (YEMA) plates were prepared and sterilized by autoclaving

⬇

Legume plants (Groundnut) were carefully uprooted and washed under running water to remove the adhesive soil particles

⬇

Healthy unbroken pink nodules were selected

↓

Surface Sterilization of root nodules by 0.1 % mercuric chloride and 3-5% hydrogen peroxides

↓

The nodules were immersed in sterilizing agents for 4-5 minutes and then washed with sterile distilled water.

↓

Then they are washed in 70% ethyl-alcohol followed by washing with sterile distilled water again

↓

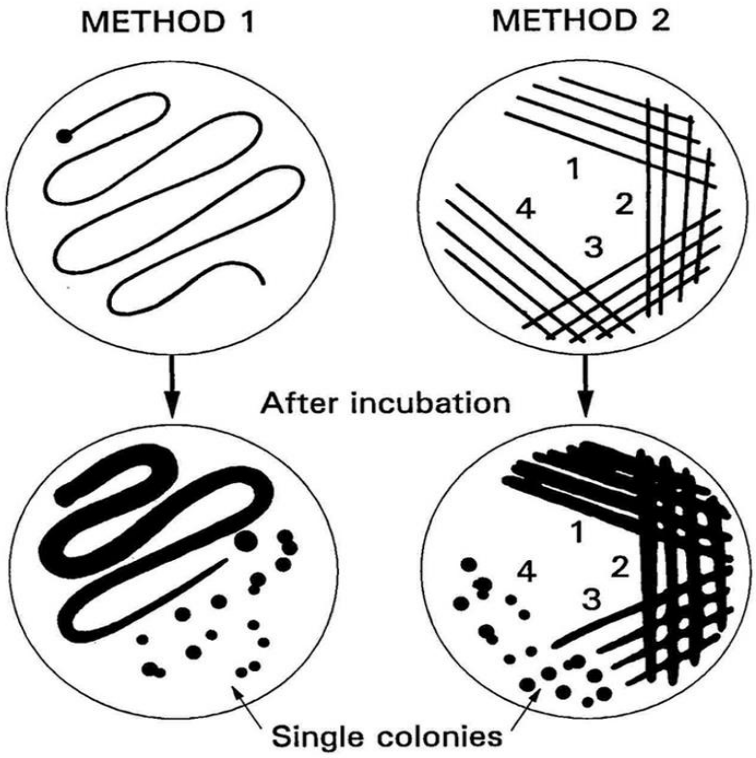
Crushed nodular extract (1 ml) suspension was diluted with 9 ml of sterile distilled water (10^{-1}) making the dilution from 10^{-2} to 10^{-8}

↓

Suspension (0.1 ml) of nodular extract was inoculated into sterile YEMA plates

↓

Inoculated Petri plates were incubated for 4-7 days in an incubator at 37°C.



IDENTIFICATION AND CHARACTERIZATION OF *Rhizobium*

CELLULAR	
Staining	Gram-negative

Morphology	Rods 0.5-0.9 x 1.2-3.0 um. Commonly pleomorphic under adverse growth conditions
Motility	Motile by one polar or subpolar flagellum or two to six peritrichous flagella
Specialized structures	Fimbriae have been described on a few strains Usually contain granules of poly-B-hydroxyl butyrate which are refractile by phase-contrast microscopy. Non spore forming
Division	

COLONIAL	
Solid surface	Colonies are circular, convex, semi translucent, raised and mucilaginous, usually 2-4 mm in diameter within 3-5 days on yeast-mannitol-mineral salts agar.
Liquid	Pronounced turbidity develops after 2 or 3 days in agitated broth

CLASSIFICATION OF *Rhizobium:*

Rhizobium is not part of a species because it is not a species. *Rhizobium* is the name of a genus of bacteria that fix nitrogen. Inside the genus is a multitude a species. Its name comes from Greek (Riza = Root and Bios = Life). Rhizobim's bacteria that are part of its genus are all aerobic bacteria.

Domain: Eubacteria

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Alpha Proteobacteria

Order: Rhizobiales:

Family: Rhizobicacea

Genus: Rhizobium

Examples of *Rhizobium sp.*

R. alamii, R. alkalisoli, R. cellulosilyticum, R. gallicum, R. indigoferae, R. leguminosarum, R. leucaenae,R. loessense, R. lupini, R. mesoamericanum, R. nepotum, R. oryzae, R. petrolearium, R. phaseoli, R. pisi

MASS PRODUCTION OF *Rhizobium* BIOFERTILIZER

Methods of Cultivation:

Following are the steps of mass cultivation of *Rhizobium:*

Sterilize the growth medium and inoculate with broth of mother culture prepared in advance

Incubate for 3-4 days at 30 - 32°C

↓

Test the cultures for its purity and transfer to a large fermenter, wait for 4-9 days for bacterial growth (for good bacterial growth make the device for its aeration)

↓

Allow to grow the bacteria either in a large fermenter containing broth or in small flasks as per demand

↓

Check the quality of broth

↓

Blend the broth with sterile carrier *e.g.* peat, lignite, farmyard manure and charcoal powder

↓

Pack the culture in polyethylene bags and keep at 25°C

↓

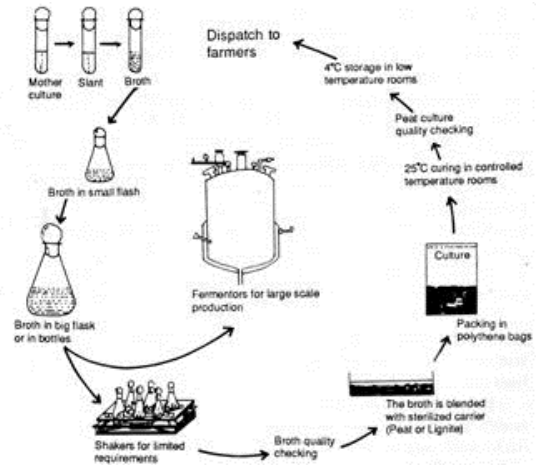
Check the quality of carrier culture

↓

Store at 4°C in a controlled-temperature room

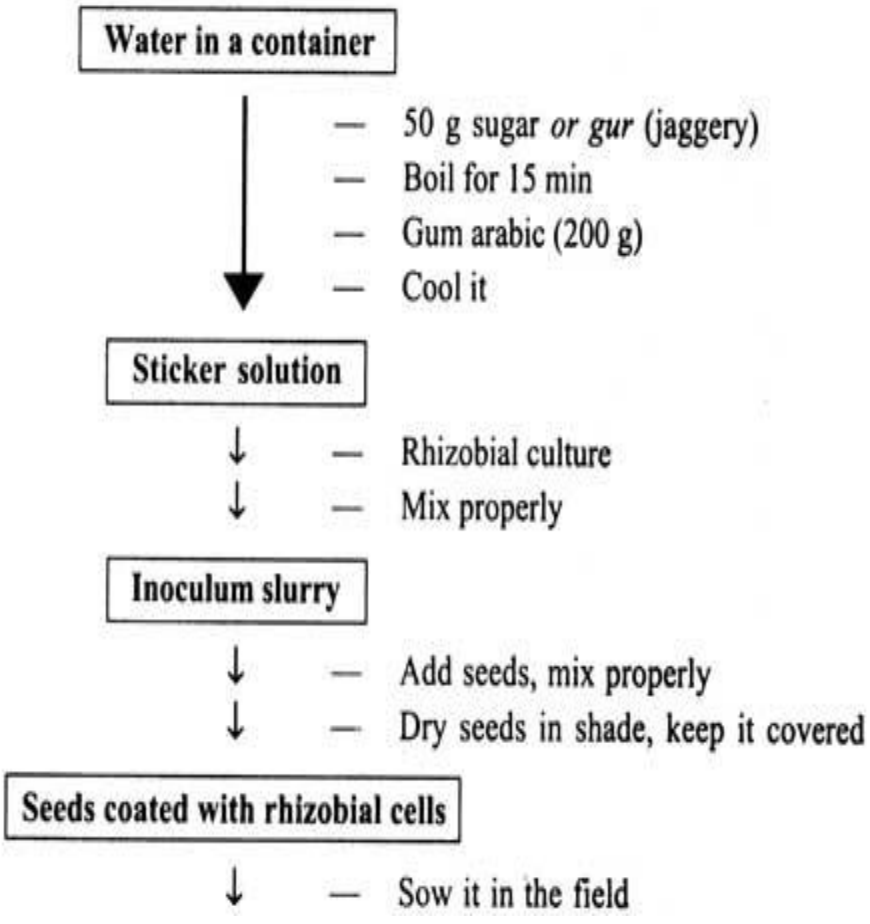
↓

Supply to farmers



METHODS OF SEED INOCULATION WITH RHIZOBIAL CULTURE:

- ✪ Dissolve 10 per cent sugar or *gur* (jaggery) in water by boiling it for some time
- ✪ Leave the content to cool down. Gum arabic solution (10%) may also be added to the solution
- ✪ This serves as sticker for *Rhizobium* cells to seeds
- ✪ Mix this carrier based culture of *Rhizobium* to form the inoculum slurry
- ✪ For one hectare, 400 g charcoal based culture would be sufficient for mixing the seeds
- ✪ Transfer the inoculum slurry on seeds and mix properly
- ✪ The number of rhizobial cells/ seed should be between 10^5 to 10^6
- ✪ Spread the seeds in shade for drying on cement floor or plastic sheets



APPLICATION OF BIOFERTILIZERS:

1. Seed treatment or seed inoculation
2. Seedling root dip
3. Main field application

Seed treatment

- ✪ One packet of the inoculant is mixed with 200 ml of rice kanji to make a slurry
- ✪ The seeds required for an acre are mixed in the slurry so as to have a uniform coating of the inoculant over the seeds and then shade dried for 30 minutes
- ✪ The shade dried seeds should be sown within 24 hours
- ✪ One packet of the inoculant (200 g) is sufficient to treat 10 kg of seeds.

Seedling root dip

- ☆ This method is used for transplanted crops
- ☆ Two packets of the inoculant is mixed in 40 litres of water
- ☆ The root portion of the seedlings required for an acre is dipped in the mixture for 5 to 10 minutes and then transplanted

Main field application

- ❖ Four packets of the inoculant is mixed with 20 kgs of dried and powdered farm yard manure and then broadcasted in one acre of main field just before transplanting.

APPLICATION OF *Rhizobium* BIOFERTILIZER:

- ➢ It is a low cost and easy technique
- ➢ It can be used by small and marginal farmers
- ➢ It is free from pollution hazards and increase soil fertility
- ➢ Secrete growth promoting substances like IAA, IBA, NAA, aminoacids, proteins, vitamins, etc. They add sufficient amount of organic matter in soil
- ➢ The bioferilizers increase physico-chemical properties of soils such as soil structure, texture, water holding capacity, cation exchange capacity and pH by providing several nutrients and sufficient organic matter
- ➢ It fix nitrogen from air to plant

MECHANISM OF BIOLOGICAL NITROGEN FIXATION

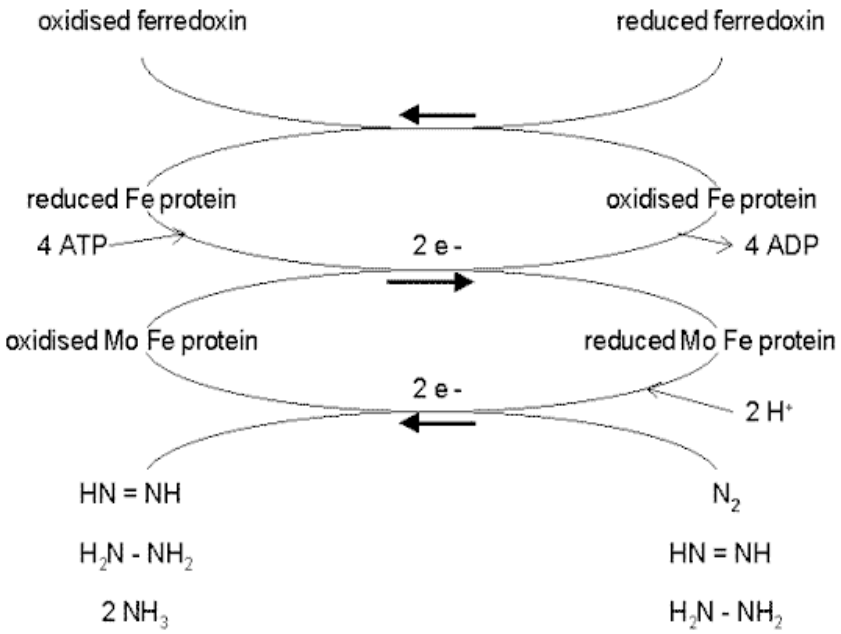
Biological nitrogen fixation can be represented by the following equation, in which two moles of ammonia are produced from one mole of nitrogen gas, at the expense of 16 moles of ATP and a supply of electrons and protons (hydrogen ions):

$N_2 + 8H+ + 8e^- + 16\ ATP = 2NH_3 + H_2 + 16ADP + 16\ Pi$

This reaction is performed exclusively by prokaryotes (the bacteria and related organisms), using an enzyme complex termed **nitrogenase**. This enzyme consists of two proteins - an iron protein and a molybdenum-iron protein, as shown below.

The reactions occur while N_2 is bound to the nitrogenase enzyme complex. The Fe protein is first reduced by electrons donated by ferredoxin. Then the reduced Fe protein binds ATP and reduces the molybdenum-iron protein, which donates electrons to N_2, producing HN=NH. In two further cycles of this process (each requiring electrons donated by ferredoxin) HN=NH is reduced to $H_2N\text{-}NH_2$, and this in turn is reduced to $2NH_3$.

Depending on the type of microorganism, the reduced ferredoxin which supplies electrons for this process is generated by photosynthesis, respiration or fermentation.



Formation of a root nodule

Multiple interactions are involved in the formation of root nodules:

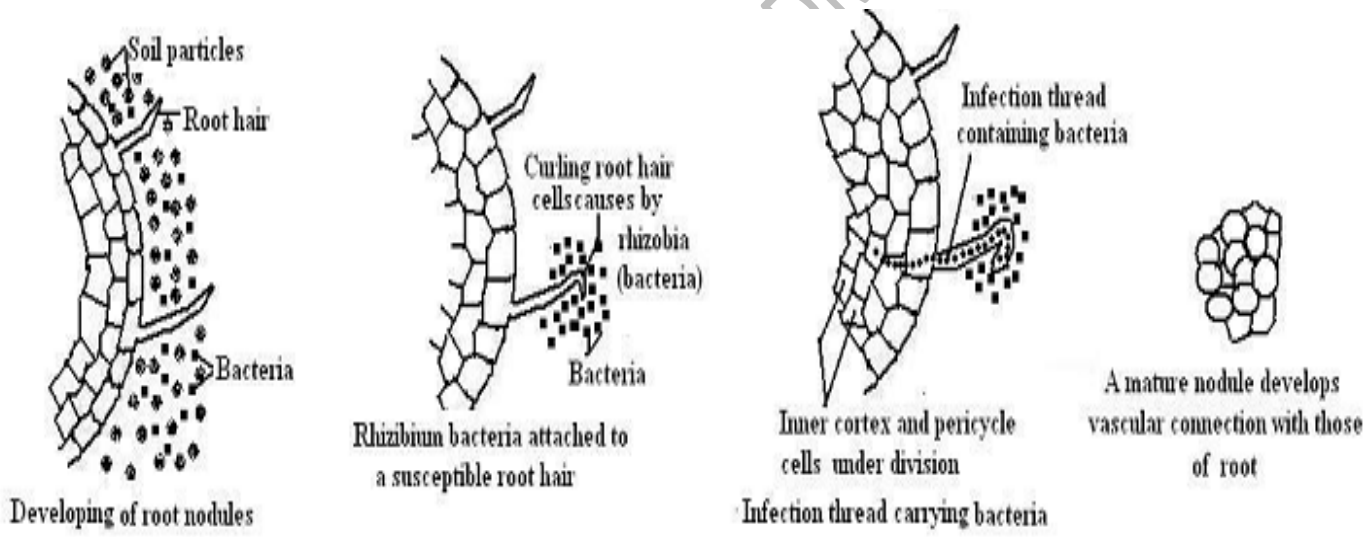
1) The Rhizobium bacteria divide and form colonies. These get attached to the root hairs and epidermal cells.

2) The root hairs get curled and are invaded by the bacteria.

3) This invasion is followed by the formation of an infection thread that carries the bacteria into the cortex of the root. The bacteria get modified into rod-shaped bacteroides.

4) As a result, the cells in the cortex and pericycle undergo division, leading to the formation of root nodules.

5) The nodules finally get connected with the vascular tissues of the roots for nutrient exchange.



***Nif* gene**

- The ***nif* genes** are genes encoding enzymes involved in the fixation of atmospheric nitrogen into a form of nitrogen available to living organisms.
- The primary enzyme encoded by the *nif* genes is the nitrogenase complex which is in charge of converting atmospheric nitrogen (N_2) to other nitrogen forms such as ammonia which the organism can use for various purposes.

- ⇨ Besides the nitrogenase enzyme, the *nif* genes also encode a number of regulatory proteins involved in nitrogen fixation.
- ⇨ The *nif* genes are found in both free-living nitrogen-fixing bacteria and in symbiotic bacteria associated with various plants.
- ⇨ The expression of the *nif* genes is induced as a response to low concentrations of fixed nitrogen and oxygen concentrations (the low oxygen concentrations are actively maintained in the root environment of host plants). The first Rhizobium genes for nitrogen fixation (nif) and for nodulation (nod) were cloned

Regulation

- ✪ In most bacteria, regulation of *nif* genes transcription is done by the nitrogen sensitive NifA protein.
- ✪ When there isn't enough fixed nitrogen available for the organism's use, NtrC triggers NifA expression, and NifA activates the rest of the *nif* genes.
- ✪ If there is a sufficient amount of reduced nitrogen or oxygen is present, another protein is activated: NifL.
- ✪ NifL inhibits NifA activity resulting in the inhibition of nitrogenase formation.
- ✪ NifL is regulated by the products of *glnD* and *glnK*.
- ✪ The *nif* genes can be found on bacterial chromosomes, but in symbiotic bacteria they are often found on plasmids or symbiosis islands with other genes related to nitrogen fixation (such as the *nod* genes).

Non-Symbiotic N_2-Fixing Bacteria:

The non-symbiotic nitrogen fixing bacteria do not require a host plant. In **1891, Winogradsky** observed that when soil was exposed to the atmosphere, the nitrogen content of the soil was recorded to be increased.

- ❖ Anaerobic bacterium ***Clostridium pasteurianum*** was found responsible for such **an increase of the nitrogen content** in soil
- ❖ In 1901, Beijerinck proved that there were also free-living aerobic bacteria, ***Azotobacter chroococcum*** that **could fix atmospheric nitrogen**
- ❖ Another bacterial group, **Granulobacter (purple colour)** obtains **nitrogen directly from the atmosphere**

- ❖ Aerobic soils of tropical climatic regions, the acid tolerant N_2-fixer ***Azotobacter beijerinckia*** is most abundant ***Azospirillum spp***. also **fixes N_2-non-symbiotically** and **help to many crops for their growth** and **yield**

Azotobacter

- ➢ *Azotobacter* is a genus of usually **motile**
- ➢ **Oval** or **spherical bacteria** that form thick-walled cysts and may produce large quantities of **capsular slime**
- ➢ They are **aerobic**
- ➢ **Free-living soil microbes** which play an important role in the **nitrogen cycle**
- ➢ **Binding atmospheric nitrogen**, which is **inaccessible to plants**, and **releasing** it in the **form of ammonium ions into the soil** (nitrogen fixation)
- ➢ **Diazotrophs** (Diazotroph: "Di": two + "A": without + "Zoo": life + "Troph": pertaining to food or nourishment. "Azote": Nitrogen (French). Named by French chemist and biologist Antoine Lavoisier, who saw it as the part of air which cannot sustain life)
- ➢ Used by humans for the production of **biofertilizers, food additives**, and some **biopolymers**
- ➢ The first representative of the genus, ***Azotobacter chroococcum***
- ➢ **Discovered** and described in **1901** by the **Dutch microbiologist** and **botanist Martinus Beijerinck**
- ➢ *Azotobacter* species are **Gram-negative bacteria**
- ➢ Found in **neutral** and **alkaline soils**

Scientific classification	
Domain:	Bacteria
Phylum:	Proteobacteria
Class:	Gammaproteobacteria
Order:	Pseudomonadales
Family:	Pseudomonadaceae/Azotobacteraceae
Genus:	***Azotobacter*** Beijerinck, 1901

Species
Azotobacter agilis
Azotobacter armeniacus
Azotobacter sp. AR

Azotobacter beijerinckii
Azotobacter chroococcum
Azotobacter sp. DCU26
Azotobacter sp. FA8
Azotobacter nigricans
Azotobacter paspali
Azotobacter salinestris
Azotobacter tropicalis
Azotobacter vinelandii

ISOLATION OF *Azotobacter:*

Ashby's medium:

Mannitol	20.0 g
K_2HPO_4	0.2 g
$MgSO_4.7H_2O$	0.2 g
NaCl	0.2 g
K_2SO_4	0.1 g
$CaCO_3$	5.0 g
Agar	15.0 g
Distilled water	1000.0 ml

Dissolve mannitol, $MgS0_4$. $7H_20$, NaCl, K_2S0_4 and $CaC0_3$ in 200 ml distilled water. Dissolve K_2S0_4 separately in 100 ml distilled water (to prevent precipitation) in another flask. Mix both solutions and make up the volume to 1000 ml. Sterilize at 15 lbs (121°C) for 15 minutes and use.

Or

Waksman medium No.77 (N-free Mannitol Agar Medium for *Azotobacter*)

Mannitol	:	10.0 g
Ca CO3	:	5.0 g
K2HPO4	:	0.5 g
Mg SO4.7H2O	:	0.2 g
NaCl	:	0.2 g
Ferric chloride	:	Trace
MnSO4.4H2O	:	Trace
N-free washed Agar	:	15.0 g
pH	:	7.0
Distilled Water	:	1000 ml

1. Pour Ashby's medium into sterile Petri plates and allow them to solidify.

2. Sieve the soil through 2 mm sieve, weigh two 10 g samples, keep one sample in an oven over night at 150°C. Weigh this sample to find out the percentage of moisture in soil.

3. Add the other 10 g soil sample into the 90 ml water blank, shake for 20-25 minutes on the magnetic shaker.

4. Make serial dilutions of this sample though sterile water blanks as mentioned under bacteria.

5. Add 1 ml of each dilution on to the agar plates, rotate the plates for even spreading of inoculum and incubate at 28°C for 3-4 days.

COLONY APPEARANCE: *Azotobacter* colonies appear as flat, soft, mucoid and milky colonies.

***MASS PRODUCTION OF* azotobacter:**

i. **Mother culture:**

A pure growth of any organism on a small scale is called as a mother culture

↓

Mother culture is always prepared in a conical flask of 500 or 1000 ml

↓

Capacity and then this mother culture is used for further production

↓

For this purpose, one liter conical flasks are taken to which 500 ml of broth of nitrogen free medium is added and these flasks are then plugged with non-absorbent cotton, sterilized in an auto slave for 15-20 minutes at 75 lbs pressure for 15 minutes

↓

Flasks are then inoculated with mother culture with the help of inoculating needle aseptically

↓

The flasks are transferred to shaker and shaking is done for 72-90 hours so as to get optimum growth of bacteria in broth. Bacteria are multiplied by binary method i.e. cell division

↓

After about 90 days, the number of per milliliters comes to about 100 crores

⬇

Total growth of bacteria in this broth means starter culture or mother culture, which should carefully be done, since further purity of biofertilizer or quality of biofertilizer depends upon how mother culture is prepared

ii. Production on a large scale:

Azotobacter is multiplied on a large scale by two ways viz. **Fermenter** and **Shaker**

⬇

The fermenter is **most automatic** and **accurate method** of multiplication of any micro-organism

⬇

In this First method, the medium is taken in a fermenter and then sterilized. **After this pH of the medium is adjusted and 1% mother culture is added**

⬇

In order to get an **optimum growth of the *Azotobacter* required temperature and oxygen supply is adjusted** so that concentrated broth is made

⬇

This concentrated **broth of the culture** is then **mixed with a carrier** previously sterilized and **bio-fertilizers are prepared**

⬇

Depending upon the demand and **supply suitable fermenter** is selected

In the 2nd method i.e. shake method, a suitable medium is prepared transferred to conical flask of suitable capacity

⬇

These flasks are then sterilized in an autoclave at 15 lbs pressure for 15 minutes

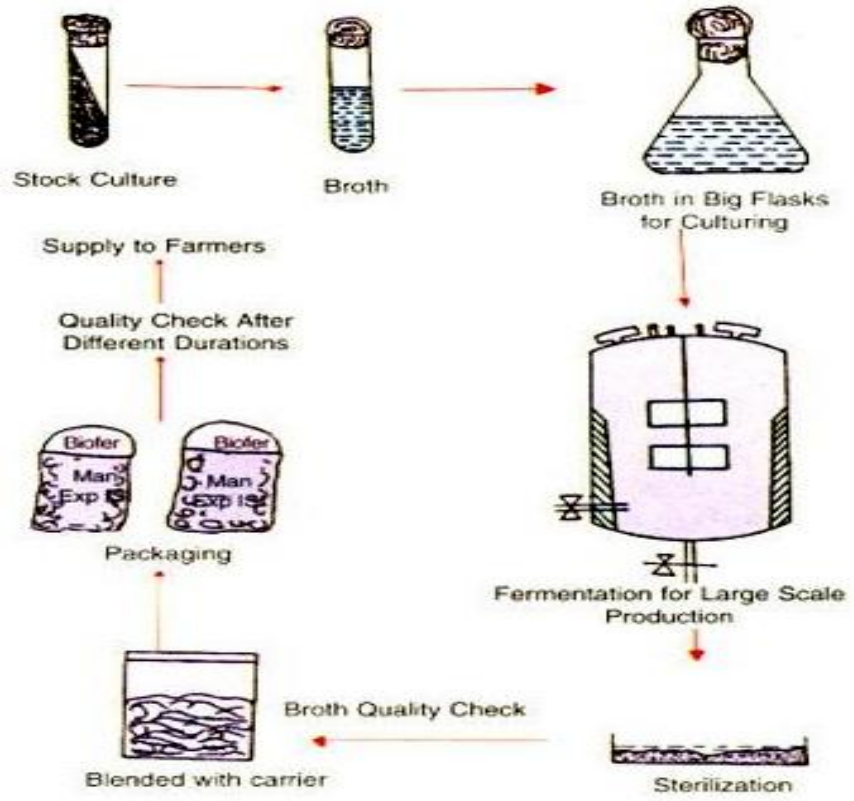
⬇

Each flask is inoclulated with 10 ml mother culture and they are transferred to shaker for multiplication where they are kept for 72-90 hours

⬇

This broth is mixed with a suitable carrier previously sterilized

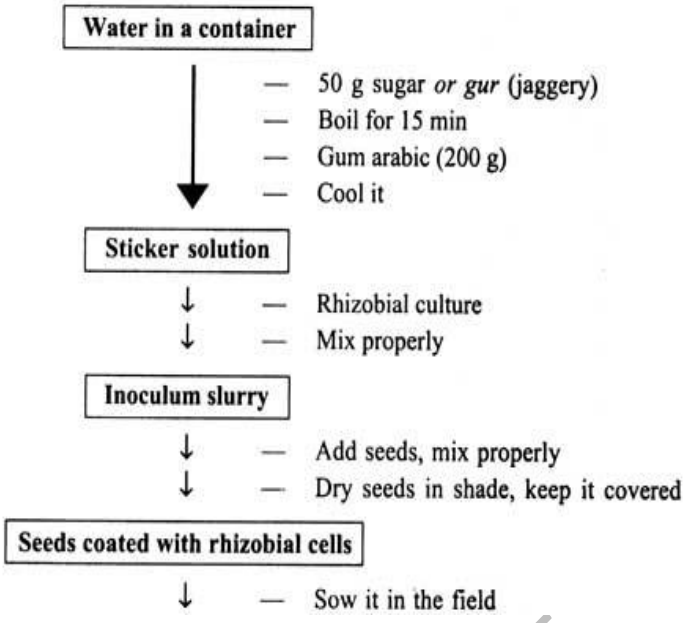
Thus biofertilizer is prepared, filled in plastic bags and stored in cool place.



FIELD APPLICATIONS:

a. Seed inoculation:

- On the basis of efficiency of *Azotobacter*, other micro-organisms present in the soil, benefits obtained from biofertilizer and expenditure it has been fixed to use Azotobacter - bio-fertilizer at the rate of 250 g biofertilizer for 10-15 kg
- If one knows this proportion then take a definite quantity of seed to be inoculated
- The required quantity of fresh biofertilizer is secured and slurry is made by adding adequate, quantity of water
- This slurry is uniformly applied to seed, seed is then dried in shed and sown
- Some stickers are used in order to adher biofertilizer to seeds. Viz. Jaggery or gum arebia.



b. Seedling inoculation:

- ❖ This method of inoculation is used where seedlings are used to grow the crop
- ❖ In this method, seedlings required for one acre are inoculated using 4-5 packets (2-2.5 kg)
- ❖ For this, in a bucket adequate quantity of water is taken and biofertilizer from these packets is added to bucket and mixed properly
- ❖ Roots or seedlings are then dipped in this mixture so as to enable roots to get inoculums. These seedlings are then transplanted e.g. Tomato, Rice, Onion, Cole, Crops, flowers.

c. Self inoculation or tube inoculation:

- ➢ In this method 50 litres of water is taken in a drum and 4-5 kg of Azotobacter biofertilizer is added and mixed properly
- ➢ Sets are required for one acre of land are dipped in this mixture. Potato tubers are dipped in the mixture of biofertilizer and planting is done.

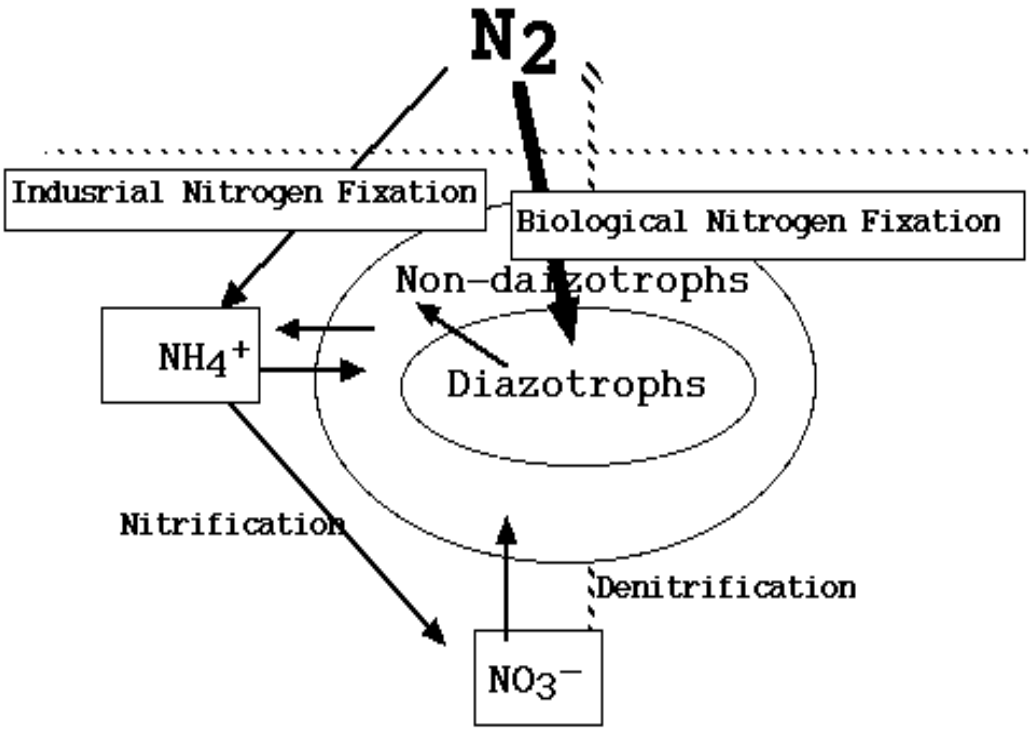
d. Soil application:

This method is mostly used for fruit crops, sugarcane, and trees. At the time of planting fruit tree 20 g of biofertilizer mixed with compost is to be added per sappling, when trees became matured the same quantity of biofertilizer is applied.

***Advantages of* Azotobacter:**

1. Azotobacter contributes moderate benefits
2. Azotobacter is heaviest breathing organism and requires a large amount of organic carbon for its growth.
3. It is poor competitor for nutrients in soil and hence its growth promoting substances, fungistatic substances.
4. It can benefit crops by Nitrogen fixation, growth promoting substances, fungi static substances.
5. Azotobacter is less effective in soils with poor organic matter content.
6. It improves seed germination and plant growth
7. It thrives even in alkaline soils.
8. Azotobacter is tolerant to high salts

NITROGEN FIXATION / METABOLISM:



Nitrogen cycle in biosphere

Diazotrophs = Nitrogen fixing organisms

- *Azotobacter* has generated a good deal of interest in the scientific community because of their unique mode of metabolism, by which they can fix nitrogen aerobically
- The cells' uniquely high respiration rates allow the normally oxygen-sensitive nitrogenase to experience limited oxygen exposure
- *Azotobacter* is also capable of producing a protein which protects the nitrogenase from sudden oxygen-provoked stress
- Another individualistic trait of *Azotobacter* is their ability to synthesize not just one, but three nitrogenases
- Specific genes are used to synthesize each nitrogenase. *Azotobacter*'s cells are large rods, at least 2 microns in diameter
- They can live singly, in chains, or in clumps, and may or may not be mobile by flagella
- Their resting stage is spent as a thick-walled cyst, which protects the organism from harsh climates

Associative Nitrogen Fixing Bacteria:

Associative nitrogen fixing bacterium is defined as the bacterium that not only lives on rhizospherial environment, but also fixes N2 from the atmosphere and contributes passively to the plant growth. Nitrogen fixing bacteria in rice root system were classified as in Table1.

The common associative N2 fixing bacteria in rice rhizosphere are *Alcaligenes faecalis, Enterobacter cloacae, Klebsiella oxytoca, Klebsiella planticola, Azospirillum brasilense* and *Azospirillum lipferum*.

Some strains have been isolated from *Alcaligenes faecalis* and *Azospirillum brasilense*. But only *Azospirillum, Enterobactercloacae, Alcaligenes faecalis* and *Klebseilla pneumonia* have been proved as safe strains and used for biofertilizer.

Generally, the number of associative N2 fixing bacteria in paddy is large r than that in dry land, as large as 103 – 107 cells / g soil. Associative N2 fixing bacteria live mainly in the rhizosphere.

Table 1 Classification of associative nitrogen fixing bacteria in rice root system

1. Autotroph	*Rhodobacter(rhodopseudomonas)*
-Photosynthetic N-fixing bacteria	*Rhodospirillum*
2. Heterotroph	
-Autofixing Bacteria/aerobic	*Azotobacter, Azotomonas*
-Autofixing Bacteria/ slightly aerobic	*Derxia, Methylomonas*
-Autofixing Bacteria/ anaerobic-aerobic	*Bacillus*
-Autofixing Bacteria/anaerobic	*Clostridium, Desulfotomaculum,Desulfovibrio*
-Associative nitrogen fixing bacteria/aerobic	*Beijerinckia*
-Associative nitrogen fixing bacteria/slightly anaerobic	*Alcaligenes, Arthrobater,Azospirillum, Flavobacterium, Pseudomonas*
-Associative nitrogen fixing bacteria/oxidative-reductive	*Enterobacter, Klebsiella*

Types and number of soil microorganisms depend primarily on the components of root exudates and chemical characteristics of root residues. In terms of rhizobacteria, the carbon sources needed for survival of rhizobacteria must be provided by plant roots because the capacity of degrading organic matter for rhizobacteria is very weak. In addition, there are some growth regulators and antibiotics in root exudates, which regulate the growth of associative N2 fixing bacteria.

On the other hand, plant roots have selectivity for types of microbes, such an effect is surveyed on different varieties of rice and difference in nitrogen fixation activity of one strain of associative nitrogen fixing bacteria inoculated to different varieties of rice is found. Rice root exudates and residues supply associative nitrogen fixing bacteria with organic acids and sugars for carbon source and growth regulators such as GA3. While, IAA is determined if rice roots were incubated with associative nitrogen fixing bacteria.

In general, either beneficial or adverse effect of rhizobacteria on plant root is surveyed. Associative N2 fixing bacteria belong to PGPR (Plant Growth-Promoting Rhizobacteria)

- (1)Associative N2 fixing bacteria provided rice with N sources such as ammonia exudates
- (2) Associative N2 fixing bacteria enhance the growth of rice roots by exudation of growth regulators such as GA3 and IAA
- (3) Most associative N2 fixing bacteria have nitratase, which goes into plant roots after inoculation and assist in nitrate reduction in plant and increase the N level, hence enhancing N2 fixation
- (4) Associative N2 fixing bacteria can enhance plant mineral uptake

- (5) Associative N2 fixing bacteria can enhance the growth of lateral roots

Phosphate Biofertilizers:

- **Phosphate solubilizing microbes** (PSB) are an aggregation of helpful microscopic organisms capable of hydrolysing natural and inorganic phosphorus from insoluble compounds.
- Phosphorus (P) is one of the major fundamental macronutrients for plants and is applied to soil as **phosphate biofertilizer**

Phosphate solubilizing bacteria

- **Phosphate solubilizing bacteria (PSB)** are beneficial bacteria capable of solubilizing inorganic phosphorus from insoluble compounds
- P-solubilization ability of rhizosphere microorganisms is considered to be one of the most important traits associated with plant phosphate nutrition
- It is generally accepted that the mechanism of mineral phosphate solubilization by PSB strains is associated with the release of low molecular weight organic acids, through which their hydroxyl and carboxyl groups chelate the cations bound to phosphate, thereby converting it into soluble forms
- Phosphorus (P) is one of the major essential macronutrients for plants and is applied to soil in the form of phosphate fertilizers. However, a large portion of soluble inorganic phosphate which is applied to the soil as chemical fertilizer is immobilized rapidly and becomes unavailable to plants
- Phosphate Solubilizing Bacteria are useful for all the crops i.e. Cereals, Cash crops. Leguminous crops. Horticultural crops. Vegetables etc.

Examples:

For example, those in *Bacillus, Pseudomonas, Erwinia, Agrobacterium, Serratia, Flavobacterium, Enterobacter, Micrococcus, Azotobacter, Bradyrhizobium, Salmonella, Alcaligenes, Chromobacterium, Arthrobacter, Streptomyces, Thiobacillus*

Uses

- Encourages early root development
- Fosfofix produce organic acids like malic, succinic, fumaric, citric, tartaric and alpha ketoglutaric acid which hastens the maturity and thereby increases the ratio of straw as well as the total yield.
- Increases compatibility of the other beneficial microbes with the plants
- Stimulates formation of fats and convertible starches

- Helps in rapid cell development in the plants and consequently increases the resistance towards disease

Pikovskayas Agar M520

Pikovskayas Agar is recommended for detection of phosphate-solubilizing soil microorganisms

Composition**

Ingredients		Gms / Litre
Yeast extract	-	0.500
Dextrose	-	10.000
Calcium phosphate	-	5.000
Ammonium sulphate	-	0.500
Potassium chloride	-	0.200
Magnesium sulphate	-	0.100
Manganese sulphate	-	0.0001
Ferrous sulphate	-	0.0001
Agar	-	15.000

**Formula adjusted, standardized to suit performance parameters

Directions:

Suspend 31.3 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

ISOLATION OF PSBs:

✪ **Soil Sample Collection**

The soil samples are collected from the fields of maize, onion, jasmine, and tomato rhizosphere

🡻

Rhizosphere soil was collected in polythene covers and refrigerated

✪ **Serial Dilution** 🡻

Samples serially diluted 10^{-1} to 10^{-9}

✪ **Homogenization of soil samples** 🡻

Soil samples were homogenously suspended in double distilled water in the ratio of 1:2 (wet w/v)

✪ **Isolation of PSBs**

After homogenization, 1 g of each soil samples were serially diluted up to 10^{-4} dilution

Then, 1 ml from each solution of dilution 10^{-3} and 10^{-4} were plated on Pikovskaya's Agar Medium by using Pour Plate Method

Pikovskaya's Agar Medium is a selective medium for isolating PSB Species

The inoculated plates were incubated aerobically at 32°C for 9 to 10 days

Screening and selection of phosphate-solubilizing bacteria

After incubation, the colonies showing clear zone of phosphate solubilization were counted and expressed as colony forming unit (cfu) per gram of soil

Single, well-separated colonies, from each sample, which grew on plates showing clear zones were picked and restreaked onto fresh Pikovskaya's solid medium using quadrant streak method

This procedure was repeated until pure culture with high P solubilization or mineralization was obtained

The strains which showed clear zones were inoculated into nutrient broth and incubated at 28°C ± 2 for 72 h at 100 rpm

Characterization of PSBs (Morphological and biochemical)

The bacterial species form characteristic colonies on Pikovskaya's Agar Media (Cultural characters like size, colour and shape of the colonies)

Morphology of the isolates was studied by Gram staining, acid –fast staining, capsular staining, spore staining, flagellar staining etc using kit by the standard procedure

The stained cells were observed under compound microscope

The Gram reaction and cell morphology for efficient PSB strains were recorded

↓

The isolates were examined for biochemical tests such as indole, methyl red, VP, Citrate Utilization, Urease, Oxidase,Starch hydrolyses and Catalase etc

Mass Cultivation of Phosphate Solubilizing Bacteria:

Following are the steps of mass cultivation of *PSBs.*

(a) Sterilize the growth medium and inoculate with broth of mother culture prepared in advance

↓

(b) Incubate for 3-4 days at 30 - 32°C

↓

(c) Test the cultures for its purity and transfer to a large fermenter, wait for 4-9 days for bacterial growth (for good bacterial growth make the device for its aeration)

↓

(d) Allow to grow the bacteria either in a large fermenter containing broth or in small flasks as per demand

↓

(e) Check the quality of broth

↓

(f) Blend the broth with sterile carrier *e.g.* peat, lignite, farmyard manure and charcoal powder

↓

(g) Pack the culture in polyethylene bags and keep at 25°C

↓

(h) Check the quality of carrier culture

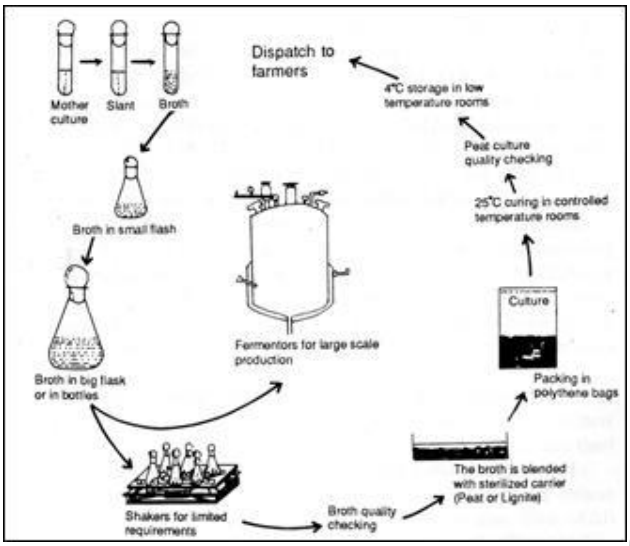
↓

(i) Store at 4°C in a controlled-temperature room

↓

(j) Supply to farmers.

(During the blending of broth a variety of carriers are used, for example, peat, lignite, farmyard manure, charcoal powder, etc. In India powdered farmyard manure and charcoal powder are good carrier and an alternative to peat and lignite)



N_2 fixing bacteria *(A. chroococcum)* and phosphate solubilizing bacteria *(B. megaterium* and *P. striata)* and their effect on sorghum and rice crops in a green house experiment by dual inoculation of sorghum with *A. brassilens* and *P. striata.* They found a significant increase in root nitrogenase activity, dry matter and seed yields as compared to single inoculation of both the organisms and control. Similarly, nutrient uptake and yield of rice increased when inoculated together with *A. chroococcum* and *P. striata* as compared to uninoculated control or single inoculated experiments

Active ingredient:

ENFOSFO is available in

- Bacillus (Bacillus polymyxa or Bacillus megaterium) and
- Pseudomonas (Pseudomonas striata) Etc.

Formulation - Talc /Dextrose/Liquid
CFU Count - NLT $1x10^{-7}/1x10^{-9}$ /$1x10^{-9}$

Shelf Life

- Talc Base formulation : Six months
- Dextrose / Liquid : One year

Packing

500gm in bilaminate pouches. 30 packs further packed in 7 ply carton. Other packing is available on

request.

FIELD APPLICATION

1. Seed Treatment

- Prepare a solution of 125 gm. of jaggery (gurh) in one litre of water and mix one packet (200gm) Phosphate Solubalizing Bacteria to form a slurry
- Sprinkle this slurry over the seeds required for one acre of crop
- Mix thoroughly and shade dry the seeds before sowing

2. Seedling treatment

- Prepare a thick mixture of 2 pockets (400 gm) of Phosphate Solubalizing Bacteria in 20-25 litre of water in big flat container
- Dip the roots of seedlings in it for 1/2 hr and then transport them in field.

3. Soil Treatment

- Mix 5 packets (1000gm) of culture powder in 50 kg. of F.Y.M./Vermicompost/Nadep compost/Soil spread in one acre field at the time of last ploughing before sowing or with the first irrigation.

SPECIAL FEATURES:

- Economic availability of phosphate is assured to plants.
- Metabolism of carbohydrates, fats and proteins in plants is increased.
- Savings in application of chemical phosphate fertilizer is 10-20%.
- Increase in crop yield is 15-20%

PRECAUTIONS:

- Treated Seeds should be dried under shade and should be sown within 2-3 hours of treatment.
- Entire packet should be used in one time.
- If the seed is to be treated with the pesticides it should be treated in order of fungicide, insecticide and then culture.

ADVANTAGES

- The effective strain of Phosphate Solubilized Bacteria used, increase the level of available P_2O_5 in the soil.
- With the increase in available P_2O_5 level, overall plant growth can be increased.
- In certain condition they also exhibit anti-fungal activities and thereby fungal diseases may be controlled indirectly.
- About 10 to 15% increase of crop yield can be achieved with the use of this culture.

Mechanism of Action

Phosphorus solubilizing activity is determined by the ability of microbes to release metabolites such as organic acids, which through their hydroxyl and carboxyl groups chelate the cation bound to phosphate, the latter being converted to soluble forms.

 Phosphate solubilization takes place through various microbial processes / mechanisms including organic acid production and proton extrusion.

 Phosphorus solubilization is carried out by a large number of saprophytic bacteria and fungi acting on sparingly soluble soil phosphates, mainly by chelation-mediated mechanisms.

 Inorganic P is solubilized by the action of organic and inorganic acids secreted by PSB in which hydroxyl and carboxyl groups of acids chelate cations (Al, Fe, Ca) and decrease the pH in basic soils.

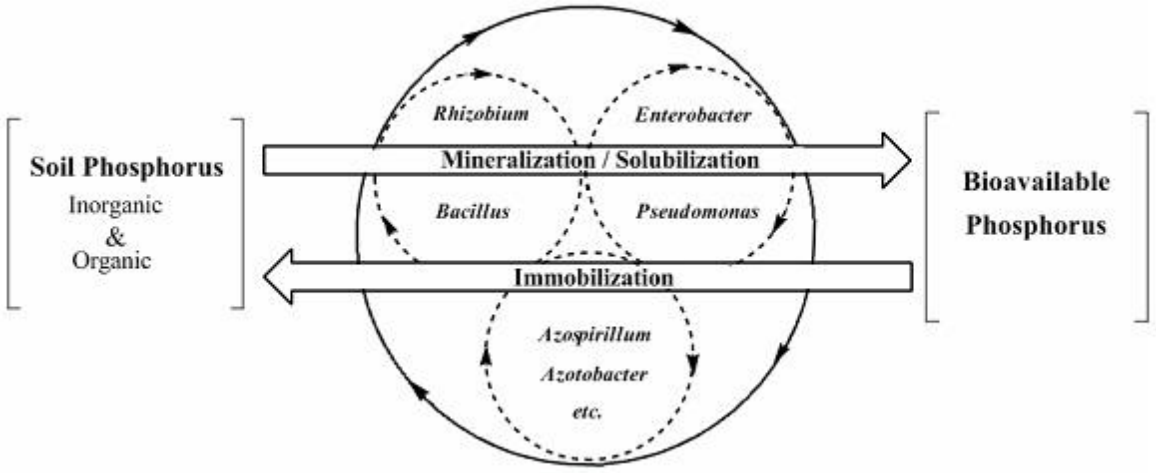
 The PSB dissolve the soil P through production of low molecular eight organic acids mainly gluconic and keto gluconic acids, in addition to lowering the pH of rhizosphere.

 The pH of rhizosphere is lowered through biotical production of proton / bicarbonate release (anion / cation balance) and gaseous (O2/CO2) exchanges.

- Phosphorus solubilization ability of PSB has direct correlation with pH of the medium.
- Release of root exudates such as organic ligands can also alter the concentration of P in the soil solution.
- Organic acids produced by PSB solubilize insoluble phosphates by lowering the pH, chelation of cations and competing with phosphate for adsorption sites in the soil.
- Inorganic acids e.g. hydrochloric acid can also solubilize phosphate but they are less effective

compared to organic acids at the same pH.

- In certain cases phosphate solubilization is induced by phosphate starvation.



Schematic diagram of soil phosphorus mobilization and immobilization by bacteria

UNIT V: Plant diseases and its control:

Plant Diseases:

Study of diseases in plants caused by pathogens and environmental conditions. Organisms that cause infectious disease include fungi, oomycetes, bacteria, viruses, viroids, virus-like organisms, phytoplasmas, protozoa, nematodes and parasitic plants.

Bacterial Diseases:

- aster yellows
- bacterial wilt
- blight
    - fire blight
    - rice bacterial blight
- canker
- crown gall
- rot
    - basal rot
- scab

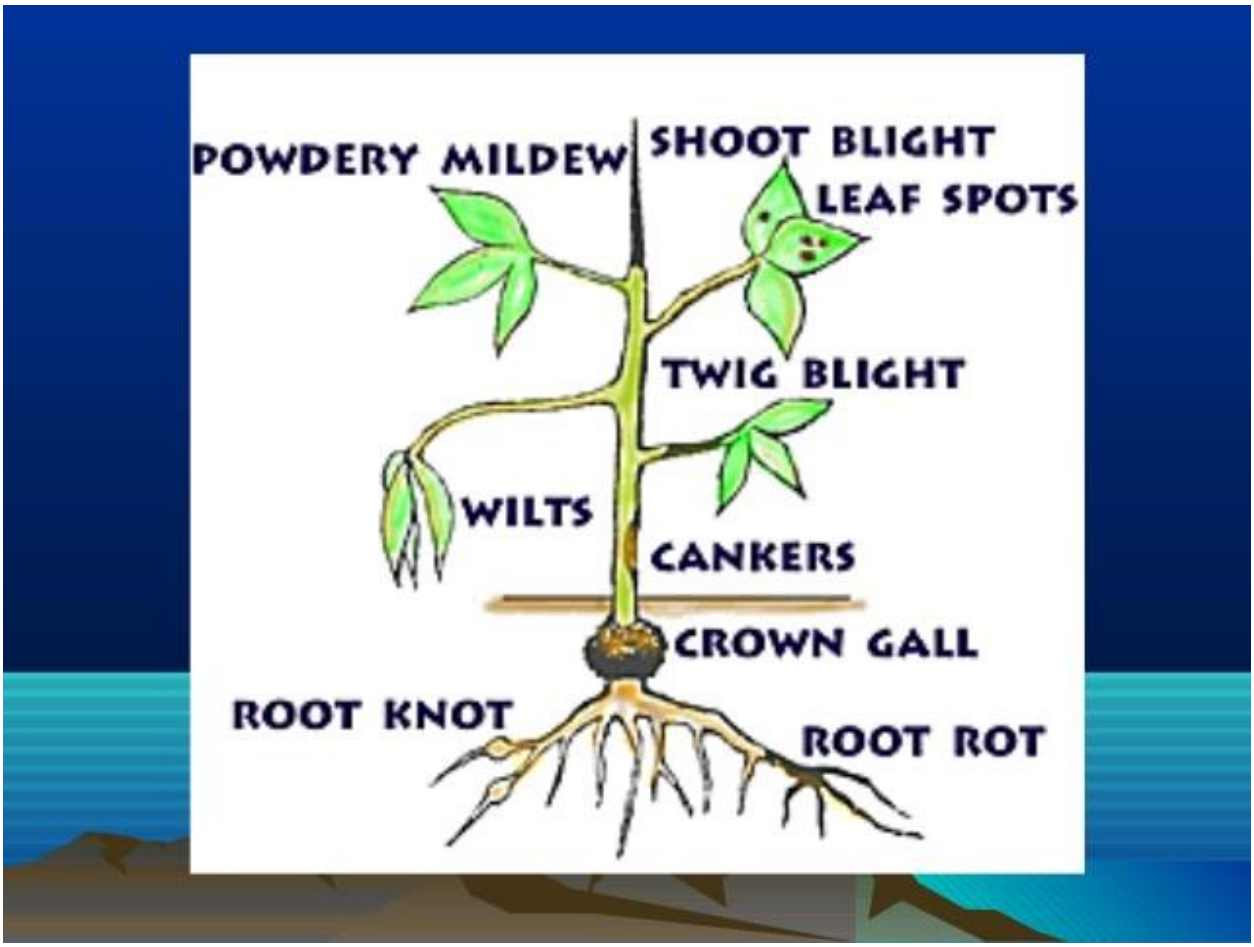
Fungal Diseases:

- anthracnose
- black knot
- blight
    - chestnut blight
    - late blight
- canker
- clubroot
- damping-off
- Dutch elm disease
- ergot
- Fusarium wilt
    - Panama disease
- leaf blister
- mildew
    - downy mildew
    - powdery mildew
- oak wilt
- rot
    - basal rot
    - gray mold rot
    - heart rot
- rust
    - blister rust
    - cedar-apple rust
    - coffee rust
- scab
    - apple scab
    -

- smut
    - bunt
    - corn smut
- snow mold
- sooty mold
- *Verticillium* wilt

Viral Diseases:

- curly top
- mosaic
- psorosis
- spotted wilt



Transmission of Plant Disease::-

For classifying the methods of disease transmission in relation to the methods of suitable control measures, the following two groups can be conveniently recognized.

1. **Direct transmission:** - Disease transmission where the pathogen is carried externally or internally on the seed or planting material like cuttings, sets, tubers, bulbs etc.

2. **Indirect transmission:-** The pathogen spreading itself by way of its persistent growth or certain structures of the pathogen carried independently by natural agencies like wind, water, animals, insects, mites, nematodes, birds etc. are the different methods of indirect transmissions.

Direct transmission: -

1. **Internal transmission through seed or planting material:-** False smut disease as well as Helminthosporin Blight disease of wheat are the common examples of fungal diseases carried internally through apparently healthy seed. Ring rot and Brown rot of potato caused by bacteria are carried internally through the tubers. The well known whip smut and red rot of sugarcane are fungal diseases carried internally in the planting sets. Mosaic and leaf roll of potato which are viral diseases are also carried inside the infected tubers.
2. **External transmission through seed or planting material:-** In this mode of transmission the pathogen is carried externally over the surface of seed or vegetatively propagated plant parts like sets, tubers, bulbs etc. or may even be carried as a physical mixture of fungal structures with the seed. The common grain smut of jowar is an example of the former type while the fungal structures called 'sclerotia' having the size of a grain or slightly bigger in case of the Ergot disease of bajra are often likely to be transmitted in the form of physical mixture with the seed.

Indirect transmission: -

1. **Autonomous transmission:-** It takes place by continuous and persistent growth of the threads or 'hyphae' of the causal fungi in soil, characteristic of several wood rotting fungi attacking forest trees and some fruit plants. Some root rotting fungi infecting certain seasonal crops also are transmitted by this method. The autonomous dispersal of such soil fungi may range from few cm. To several (8 to 10) meters in a single season. Some plant parasitic nematodes also exhibit active but limited mobility in the soil.
2. **Wind dispersal: -** Fungal spores produced externally on host surfaces are most easily carried by wind currents and this is the most dangerous mode of transmission of plant pathogenic fungi like those causing powdery and downy mildews, leaf spots, blasts, blights and rust diseases. The black stem rust disease of wheat in India perpetuates on wild grasses in the Nilgiri hills in the south India from where the rust spores are carried to south, central & then to north India by wind currents every year. Spores may be carried from low to very high altitudes of 12,000 to 14,000 feet and from short distances to very long distances of several hundred kilometers.

Extensive and severe epidemics of plant diseases are mostly the results of wind transmission of the pathogens. Wind dissemination involves four stages relating to the spores viz. Production of countless spores, their liberation in the wind currents, dispersal alongwith the wind and deposition on new susceptible host surfaces where they cause infection under favourable climatic conditions. Apart from spores, bits of fungal threads and nematode cysts are also amenable to wind transmission in certain cases.

3. **Water dissemination:** Disease transmission through the agency of water in different ways is comparatively less important as compared to the wind transmission. Splashing rain drops mostly transmit the foliar diseases from leaf to leaf, from shoot to shoot and even from plant to plant in case of closely spaced crops. Such transmission is usually accompanied by wind dispersal as well. Plant pathogens requiring high humidity conditions like the fungi causing downy mildew diseases or bacteria causing canker of citrus are well adapted to this kind of short distance water dispersal.

   Certain soil inhabiting pathogenic fungi and bacteria causing root and collar rots, wilts, foot, rots, etc are likely to be transmitted to much longer distances through the agencies like irrigation water, streams and rivers, etc. It is also an important agency in transmission of seeds of higher flowering parasites like dodder and striga.

4. **Animals:** Farm animals serve as disease transmitting agents in some cases. They are likely to carry the pathogen externally on their body surface, particularly on legs and hoofs, etc. or internally through their intestinal tract. Commonly, the soil inhabiting fungi causing rots and wilts are carried externally while certain smut fungi causing diseases to grain crops are transmitted through the intestinal tract.
5. **Birds:** Although birds play a very minor role in disease transmission, in cases of dispersal of seeds of higher flowering parasite. Loranthus sp. Parasitising certain trees like mango, etc. their role is of great significance. They transmit loranthus both externally and internally.
6. **Implements and Tools:** Farm implements used for cultivation of soil are often likely to transmit plant pathogens from one place to another. The pathogens in this case are usually carried in the form of bits of plant disease debris lying in the soil. Similarly tools used for carrying out operations like cutting, pruning, budding, grafting, thinning,etc. also help in the transmission of certain diseases from plant to plant. Several viral diseases are disseminated through the budding and grafting operations.
7. **Insects:** Most of the viral diseases of plants are transmitted through the agency of different insects. Both types of insects viz. sucking and chewing or/biting are capable of transmitting viral diseases. The transmission may be simply `mechanical' or it may be `biological'. In the latter case the specific insect and the specific viral pathogen have some kind of association or relationship between the two.

Insects in such cases are called the `vectors' for the particular viral pathogen. In case of mechanical transmission the pathogen is simply carried externally or internally by the insect.

Viruses carried `biologically' by the insect vectors are of two types:

1. Non-persistent-viral pathogen requiring no latent or incubation period in the insect body.
2. Persistent: viral pathogens requiring certain incubation period inside the vector body before they are inoculated or transmitted to healthy host.The insects responsible for transmission of viral diseases belong to the species of aphids, jassids (leaf hoppers), white flies, mealy bugs, etc. Certain bacterial and several fungal pathogens are also known to be carried by insects.

1. **Mites:** Mites in contrast to insects are wingless anthropods resembling ticks and having four pairs of legs and no antennae. It is suspected that some viral diseases of chillies, tomato, brinjal, etc. have vector relationship with mites.
2. **Nematodes:** Nematodes have been observed to transmit viral, bacterial and fungal plant diseases. Nematodes feeding externally on host plant roots cause injuries to roots which become the avenues for entrance of fungal and bacterial pathogens infecting plant roots. The Fan-leaf virus of grapevine is a well known example of transmission through a species of nematodes.
3. **Biological transmission**: Dodder which is higher flowering parasite is known to transmit certain viral diseases which remain `persistent' in the dodder plant. The flowering parasite after acquiring the virus from infected plant does not show any symptom itself but remains capable of transmitting the virus to healthy hosts.
4. **Human dispersal**: Man is often responsible for transmission of plant diseases in two ways viz.

    1. Workers handling seedlings, other planting material or fruits are likely to get personally in contact with plant pathogens like fungi or bacteria. While handling the diseased material and unknowingly and indirectly transmit the pathogens to healthy seedlings or plant parts through his contaminated hands. This is a kind of `continuous' mode of transmission.
    2. The other or`discontinous' mode of transmission for which only man is responsible is the most efficient and equally dangerous phenomenon of transmission of plant diseases between distant geographical areas often separated by physical barriers like oceans, mountains or deserts, etc. Such long distances transmission of a disease to an area or country hitherto free from the disease is usually accomplished by the transport of infected

seed, nursery stock or timber, etc. Thus it is a kind of direct transmission through propagating material.

Control of Plant Disease:

There are six basic principles of plant disease management.

1) Avoidance

2) Exclusion

3) Eradication

4) Protection

5) Resistant Varieties

6) Therapy

1. Avoidance:

Avoiding disease by planting at time, or planting in areas where inoculum is ineffective due to environmental condition or absent. This is achieved by

A. Choice of Geographical Areas

B. Selection of Field

C. Selection Of Seed And Planting Material

D. Choice of Time Of Showing

E. Disease Escaping Varieties

A) Choice of Geographical Area: Many fungal and bacterial diseases are more severe in wet areas than in dry areas.

B) Selection of Field: Selection of field will help in the management of many diseases, especially the soil borne diseases. Raising of a particular crop year after year in the same field makes the soil sick, where disease incidence and severity may be more.

C) Selection of Seed and Planting Material: Selection of seed and seedling material from healthy sources will effectively manage the diseases.

D) Choice of Time Of Sowing: Generally pathogens are able to infect the susceptible plants under certain environmental conditions. Alteration of date of sowing can help in avoidance of favorable conditions for pathogen.

E) Disease Escaping Varieties: Certain varieties of crops escape the disease damage because of their growth characteristics.

2. Exclusion:

Preventing the inoculum from entering or establishing in the field or area where it does not exist. It is achieved by

A . Seed Certification

B . Plant Quarantine

A) Seed Certification: Necessary precautions are taken to remove the diseased plants in early stages, and then the crop is certified as disease free. This practice will help in the prevention of spread of seed borne diseases.

B) Plant Quarantine: Plant quarantine is defined as a legal restriction on the movement of agricultural commodities for the purpose of exclusion, prevention or delaying the spread of the plant diseases in uninfected areas. Regulations are controlling the import and export of plants to prevent spread of disease.

3. Eradication:

Population of some plant pathogens can be reduced by eradication of infected plant or destruction and inactivation of inoculum or vector of disease. This is achieved by

A. Rouging

B. Eradication Of Alternate Host

C. Crop Rotation

D. Crop Sanitation

A) Rouging: Removal of diseased plants or their affected organs from the field, which prevent the dissemination of plant pathogens.

B) Eradication of Alternate Host: Eradication of alternate host will help in management of many plant diseases. Remove or burn all of the infected host plant or plant parts

C) Crop Rotation: Continuous cultivation of the same crop in the same field helps the perpetuation of pathogen in the soil. crop rotation should be adopted.

D) Crop Sanitation: Collection and destruction of plant debris from soil will help in the management of soil borne. Collection and destruction of plant debris is an important method to reduce the primary inoculum.

4. Protection:

Preventing infection by creating a chemical toxic barrier between the plant surface and pathogens.

- Chemical treatment
- Chemical control of insect vector

5. Resistant Varieties: Preventing infection or reducing effect of infection by managing the host through improvement of resistance by genetic manipulation or by chemotherapy. Using resistance varieties is one of the best disease control practice

6. Reducing severity of disease in an infected individual by chemicals or treating of healthy plants before it becomes diseased.

- Chemotherapy
- Tree Surgery
- Heat Therapy

Several bacterial diseases are characterized in the table.

Some bacterial diseases of plants				
disease	**causative agent**	**hosts**	**symptoms and signs**	**additional features**
Granville wilt	*Pseudomonas solanacearum*	tobacco, tomato, potato, eggplant, pepper, and other plants	stunting, yellowing, and wilting of parts above ground; roots decay and become black or brown	occurs in most countries in temperate and semitropical zones; causes crop losses of hundreds of millions of dollars
fire blight	*Erwinia amylovora*	apple and pear	blossoms appear water-soaked and shrivel; spreads to leaves and stems, causing rapid dieback	first plant disease proved to be caused by a bacterium
wildfire of tobacco	*Pseudomonas syringae*	tobacco	yellowish green spots on leaves	wildfire of tobacco occurs worldwide; causes losses in seedlings and field plants

Some bacterial diseases of plants				
blight of beans	*Xanthomonas campestris*	beans (common blight)	yellowish green spots on leaves	most phytopathogenic xanthomonads and pseudomonads cause necrotic spots on green parts of susceptible hosts; may be localized or systemic
	Pseudomonas syringae	beans (brown spot)	small water-soaked spots on lower side of leaves enlarge, coalesce, and become necrotic	
soft rot	*Erwinia carotovora*	many fleshy-tissue fruits—e.g., cabbage, carrot, celery, onion	soft decay of fleshy tissues that become mushy and soft	occurs worldwide; causes major economic losses
crown gall	*Agrobacterium tumefaciens*	more than 100 genera of woody and herbaceous plants	initially a small enlargement of stems or roots usually at or near the soil line, increasing in size, becoming wrinkled, and turning brown to black	the conversion of a normal cell to one that produces excessive cell multiplication is caused by a plasmid (a small circular piece of DNA) carried by the pathogenic bacterium
aster yellows	Mycoplasma-like organism (MLO)	many vegetables, ornamentals, and weeds	chlorosis; dwarfing malformations	greatest losses suffered by carrots; transmission by leafhoppers
citrus stubborn disease	*Spiroplasma citri* (MLO)	citrus and stone fruits and vegetables	chlorosis, yellowing of leaves, shortened internodes, wilting	first MLO pathogen of plant disease cultured

Several fungal diseases are characterized in the table.

Some fungal diseases of plants				
disease	**causative agent**	**hosts**	**symptoms and signs**	**additional features**
late blight of potato	*Phytophthora infestans*	potato	water-soaked dark green to black or purplish lesions with pale green margins on lower leaves, white mildew at edge of lesions	responsible for Irish famine; caused starvation and death and mass migration of population
chestnut blight	*Endothia parasitica*	chestnut tree	yellowish to reddish brown patches appear on bark; lesions spread quickly and girdle twigs or limbs, which die	disease accidentally imported from Asia; first observed in New York in 1904 and rapidly spread across the United States, practically eliminating native American chestnuts

Some fungal diseases of plants				
Dutch elm disease	*Ceratocystis ulmi*	elm tree	leaves wilt, turn dull green to yellow or brown, and drop off; branches die	the causative fungus is believed to have entered Europe from Asia during World War I and was later transported to the United States (1930) on elm burl logs imported for furniture veneer; elm bark beetles spread the pathogen in the United States
black stem rust of wheat	*Puccinia graminis*	wheat; many grasses	on wheat, rust-coloured pustules with spores, chlorosis of surrounding tissue, followed by development of black teliospores; on barberry, chlorosis and hypertrophy of infected tissue, orange spore masses	disease occurs wherever wheat is grown; in 1935 it destroyed about 60 percent of the total hard red spring wheat crop in Minnesota and South Dakota; fungus has a complex life cycle, partly on wheat and partly on the barberry plant; eradication of the barberry plant is an important control measure
coffee rust	*Hemileia vastatrix*	coffee	orange-yellow powdery spots on lower side of leaves; centres turn brown and leaves fall	most destructive disease of coffee; has caused devastating losses in all coffee-producing countries
white-pine blister rust	*Cronartium ribicola*	white pine tree	small, discoloured, spindle-shaped cankers surrounded by narrow band of yellow-orange bark; blisters exude secretion followed by bright orange pustules	one of the most important forest diseases in the United States; currant is the alternate host, and its eradication is an important control measure
corn smut	*Ustilago maydis*	corn	minute galls form on young corn seedlings; on older plants, large galls are produced on the silk of ears and on tassels, leaves, and stalks	occurs wherever corn is grown; may cause serious crop damage
loose smut	*Ustilago nuda*	barley, oats, wheat	infected heads are covered with masses of olive-green spores	worldwide occurrence; destroys kernels of the infected plant
downy mildew	many species of the family Peronosporaceae	many types of plants: grapes, grasses, vegetables, and others	yellow irregular spots appear on upper leaf surface; downy fungus growth appears on underside; leaves die	one of the first plant diseases controlled by a fungicide—i.e., Bordeaux mixture, a mixture of lime and copper sulfate used on grapes
powdery mildew	many species of the family Erysiphaceae	many types of plants: grasses,	spots of powdery mildew growth that enlarge to cover leaves or other	one of the most common and widely spread plant diseases

Some fungal diseases of plants				
		vegetables, shrubs, and trees	plant organs	
apple scab	*Venturia inaequalis*	apple	small olive-coloured areas appear on young leaves, later turn black, and may coalesce; black circular spots appear on fruit	occurs almost everywhere apples are grown; infection reduces fruit size and quality
black spot of rose	*Diplocarpon rosae*	rose	large circular black lesions on leaves; leaves turn yellow and fall off	classified as an anthracnose, which affects leaves, stems, and fruits of many plants
anthracnose of grape	*Elsinae ampelina*	grape	(as above)	(as above)
nectria canker	*Nectria galligena*	apple and pear and many hardwood forest trees	initially small circular brown areas that enlarge and become depressed with raised edges; callus tissue produced around canker	one of the most important diseases of pear, apple, and hardwood forest trees
black knot of plum and cherry	*Plowrightia morbosum*	plum and cherry	small black knotty swellings on twigs and branches	occurs primarily in the eastern half of the United States and New Zealand
brown rot	*Monilinia fructicola*	stone fruits	brown spots on blossoms; twigs develop small sunken brown cankers; fruit develops brown spots that spread rapidly	worldwide occurrence; can cause heavy losses both in orchards and in shipment
soft rot	*Rhizopus* species	flowers, fruits, and vegetables with fleshy organs	tissues become soft with water-soaked appearance that often spreads rapidly, followed by development of fuzzy gray mycelium and black spores	infection develops most rapidly on ripe fruits with favourable conditions (moderate temperature and high humidity)
fusarium wilt of tomato	*Fusarium oxysporum*	tomatoes	leaves are bent down, growth is stunted, plant dies; dark streaks appear in vascular tissue	one of the most destructive diseases of tomato; entire fields can be destroyed
wilts of vegetables, flowers, and some trees	*Verticillium* species	cotton, potato, tomato, alfalfa, shade trees, and others	similar to fusarium wilts; develops primarily in seedlings that die shortly after infection; older plants also are attacked	worldwide distribution; the fungus infects hundreds of species of plants

Viral diseases

Introduction:

This page provides an overview of viral diseases in vegetable crops. The related tools listed at the end of the page provided detailed information about the identification, symptoms, and management of viral diseases. It is important to have a plant diagnostics laboratory confirm the pathogen causing any diseases in a crop so that the disease can be appropriately managed.

Viruses cause major damage to many Australian vegetable crops. They are immobile and are usually transmitted from one plant to another by a living organism called a vector or carrier. The most significant vectors of plant viruses include aphids, whiteflies, thrips, and leafhoppers, which have piercing sucking mouthparts that allow the insects to access and feed on the contents of the plant cells. Viruses can also be transmitted by other insects, mites, nematodes, fungi, infected pollen or vegetative propagating material, contact between plants, and infected or contaminated seeds.

The virus is transmitted by sap-sucking insects in two ways: persistent transmission and non-persistent transmission, which relates to the time taken by an insect to acquire and transmit the virus.

Viruses, crops affected, and damage caused:

Means of transmission: Aphid

Virus	Host plants	Primary damage
Bean common mosaic virus	Beans.	Mottling, curling, and malformation of leaves and a general stunting of the plant.
Turnip mosaic virus	Brassicas.	Mottling and black necrotic spots in cabbage, cauliflower, and Brussels sprouts; mosaic with leaf distortion and stunting in turnip, radish, and Chinese cabbage.
Cucumber mosaic and potato mosaic virus	Capsicum; tomato; potato; celery.	Chlorosis and blistering mottle of leaves; plants are stunted.
Carrot virus Y	Carrot.	Severe root symptoms in carrots including shortened roots, knobbiness and severe distortion.
Papaya ringspot; Watermelon mosaic virus; Zucchini yellow mosaic virus	Cucurbits.	
Johnson grass mosaic virus	Sweet corn.	

Celery mosaic virus	Coriander; celery; parsley; parsnip.	Plants stunted with severe clearing on leaves, leaf-up curling and chlorosis.
Sweetpotato feathery mottle virus	Sweetpotato; peas.	
Subterranean clover stunt	Beans.	
Beet western yellow virus	Brassicas.	
Potato leafroll virus	Potato.	Stunted plants; lower leaves roll upwards at the margins, develop leathery texture and die prematurely.

Means of transmission: Thrips

Virus	**Specific vector**	**Host plants**	**Primary damage**
Tomato Spotted Wilt Virus (TSWV)	WFT, tomato thrips, and onion thrips.	Capsicum; tomato; eggplant; lettuce; celery; peas;potatoes; sweet basil.	Ringspots, line patterns, mottling, and chlorotic blotches on leaves.
Iris Yellow Spotted Virus (IYSV)	Only by onion thrips.	Onions; garlic; leeks; spring onions; herbs.	Eye-like or diamond-shaped spots on leaves and seed-stalk in onions; extensive chlorosis or yellowing.
Capsicum Chlorosis Virus (CaCV)	Melon thrips and tomato thrips.	Capsicum; tomato; chillies.	In capsicum: yellowing on leaf margins and between veins of young leaves; In tomato: chlorotic spots and blotches on leaves that become mottled.

Management:

Often the intricate relationship between the virus, host plants, and the vector or the carrier, creates problems in developing effective management systems. However, by using a combination of management options, or an Integrated Pest Management (IPM) approach, disease control can be successfully implemented.

- **Exclusion or avoidance** – quarantine; grow crops in regions where the virus seldom occurs or during periods when the virus or its vector are at a low activity level; and use virus-free seedling transplants.

- **Reduction in virus spreading sources** – control weeds and other virus hosts and insect vectors; destroy old crops promptly; separate new crops from maturing crops; and avoid overlapping crops, particularly year-round cropping.
- **Protection of the host plant** – plant virus-resistant varieties; use barrier crops to reduce insect vector activity in the crop; use insecticides to protect plants; and use highly reflective mulches and oil sprays to deter insects.

Plant protection:

Plant Protection continues to play a significant role in achieving targets of crops production.Plant Protection The major thrust areas of plant protection are promotion of Integrated Pest management, ensuring availability of safe and quality pesticides for sustaining crop production from the ravages of pests and diseases, streamlining the quarantine measures for accelerating the introduction of new high yielding crop varieties, besides eliminating the chances of entry of exotic pests and for human resource development including empowerment of women in plant protection skills.

Phenol compounds:

- The terms 'phenol' and 'polyphenol' can be defined chemically as substances that possess an aromatic ring bearing one (phenol) or more (polyphenol) hydroxyl substituents
- Synthesized via two different routes: Shikimate pathway (plants)¬ Acetate-melonate pathway (fungi and bacteria)
- Solubility:
  - Most of plant phenolics soluble in polar organic solvents
  - Phenolic glycosides are water soluble
  - Many serves as defence compounds against herbivore and pathogens
  - Attracts pollinators and seed dispersal
- **Phenolic compounds:-**
  - Widely distributed secondary metabolites,
  - Ubiquitously present in the plant kingdom.
  - Active and passive forms of defence.
  - **Functions in plants:** structure, protection and interaction with biotic and abiotic factors
- The first step of the defence mechanism in plants involves a rapid accumulation of phenols at the infection site.
- The role of phenolic compounds in defence is related to their antimicrobial, antinutritional or unpalatable properties.
- Environmentally induced as well as genetically controlled
- One mean of self-protection
- Classes of phenolic compounds

- ✓ Simple hydroxybenzoic acid
- ✓ Free and conjugated hydroxycinnamic acids,
- ✓ Coumarins
- ✓ Flavonoids and
- ✓ Stilbenes etc., involved in defence

❖ The types of phenol that are implicated in defence differ greatly and that depends on plant species

Occurrence of phenolics:

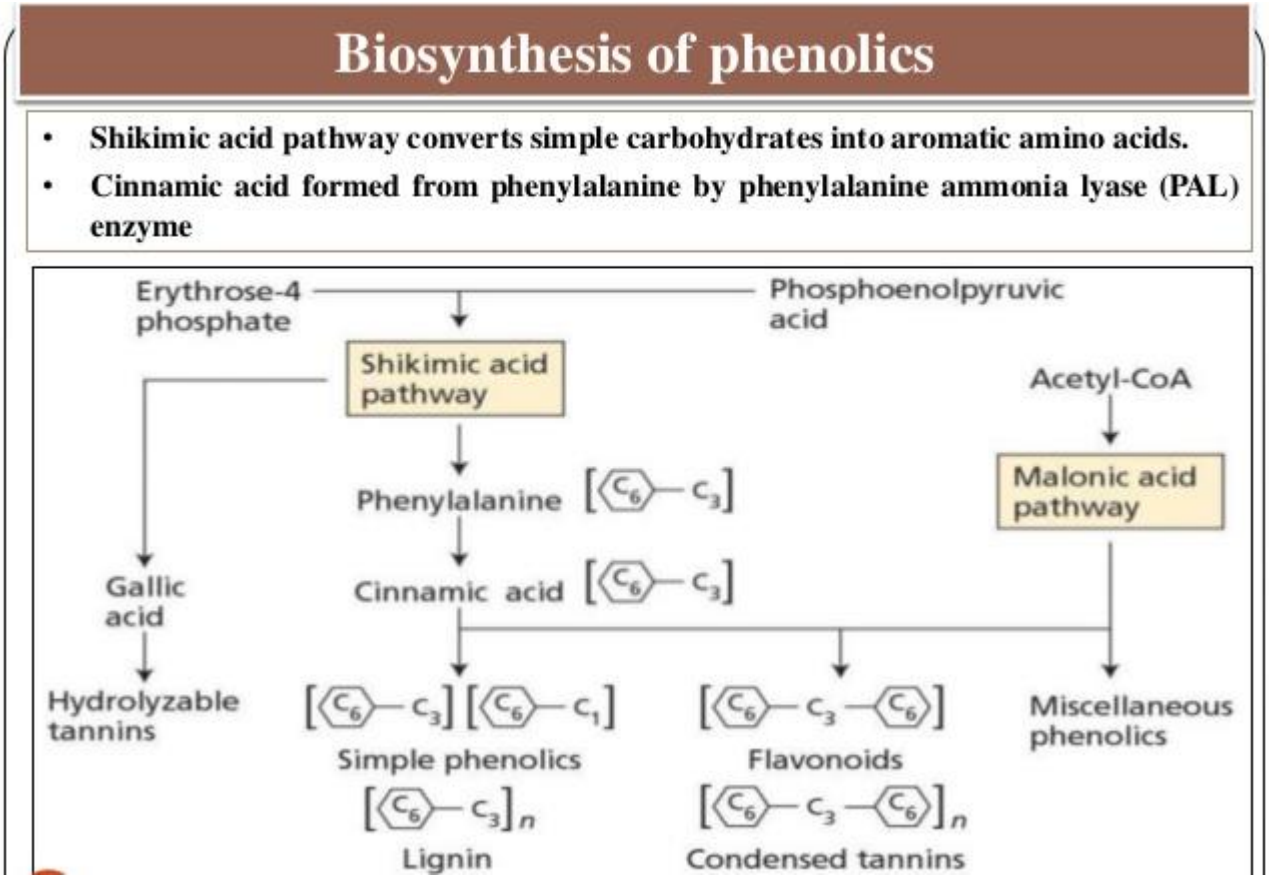
❖ They are usually found as esters or glycosides rather than as free compounds.

❖ Polyphones (relatively hydrophilic) accumulate in

- Central vacuoles of guard cells,
- Epidermal cells and
- Subepidermal cells of leaves and shoots

❖ Some are found covalently linked to the plant cell wall (lignin) and some found in waxes (related to lipidic structures) or on the external surfaces (cuticle) of plant organs

❖ Biosynthesis of phenolic compounds occurs at various sites in plant cells, such as the chloroplasts and endoplasmic reticulum membrane.



Important enzymes

Polyphenol oxidases	• Oxidises phenols to quinones- bactericidal and fungicidal • Oxidative detoxification of pathogen phytotoxins
Peroxidases	• Increases polymerization of the phenols into lignins- the complex phenols
PAL (Phenylalanine Ammonia Lyase)	• Key enzyme for the synthesis of phenols, phytoalexins and other defence related chemicals.

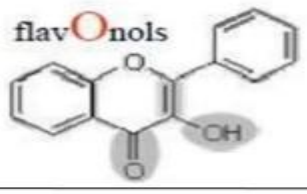
Significance of phenolic compounds

- **UV sunscreens**
- **Signal compounds**
- **Pigmentation**
- **Plant growth**
- **Plant defence**

UV sunscreens

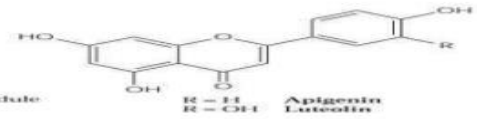
➢ Phenolic compounds act as a screen inside the epidermal cell layer by making adjustments to the antioxidant systems at both cell and whole organism level.

➢ **Flavonols** are involved in UV screening due to their strong absorbance in UV-A (325-400nm) and UV-B(280-325nm) wavelengths.



Phenolics as signal compounds

- Polyphenols enter the soil mainly as leachates - directly affect the rates of decomposition and nutrient cycling.
- **Allelopathic effects** -Examples:
  1. p-Hydroxy benzoic acid , p-coumaric acid (present in leaves),
  2. Quercetin, juglone (present in leaves, bark and root exudates)
  3. Catechin , sorgoleone (found in rhizosphere and root exudates)
- **Symbiosis**– Legumes & Rhizobium nodulation by flavonoid Apigenin, luteolin



Phenolics as pigments

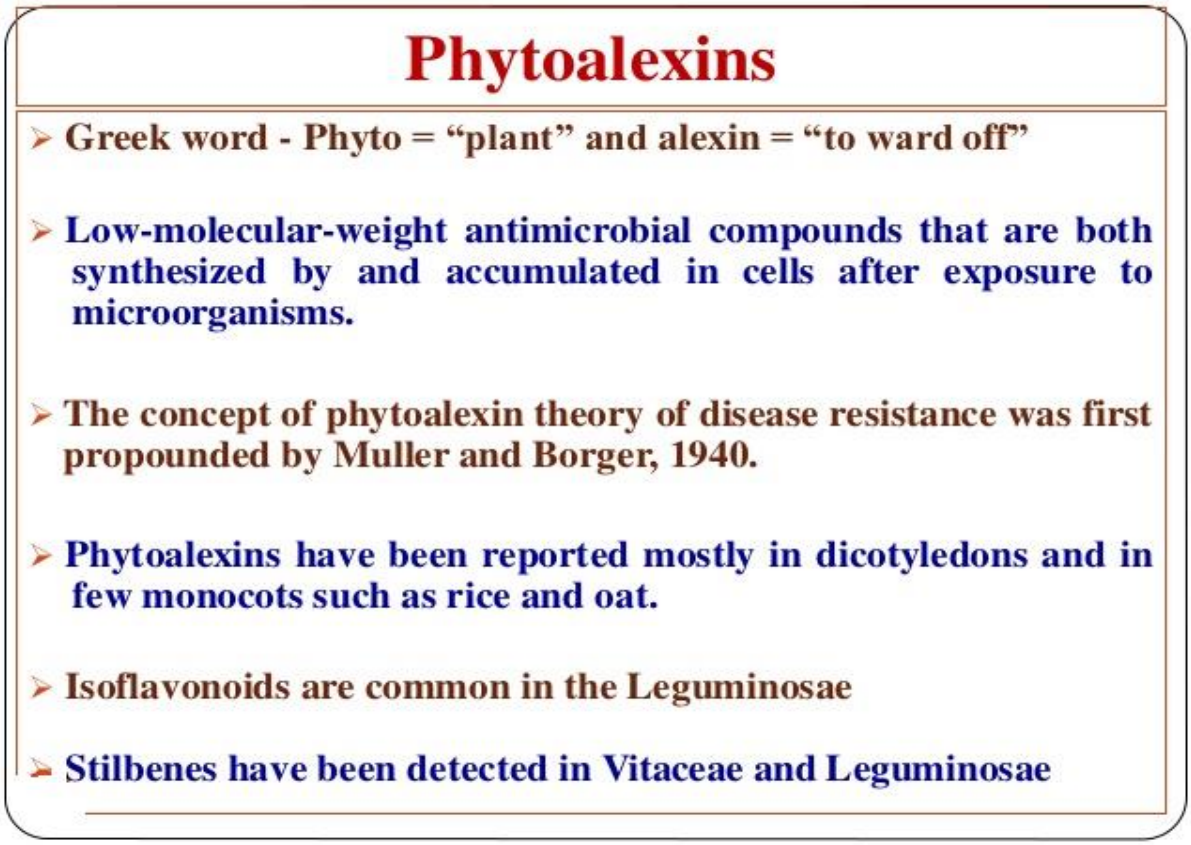
- **Role of flavonoids is to serve as visual signals by**
  - **Acting as pigments in fruits and flowers**
  - **To attract animals as pollinators in flowers,**
  - **To attract animals to eat the fruits and help in seed dispersal**
- **Anthocyanins and anthocyanidins** - red, blue and purple colours in plants.
- **Chalcones** and **aurones** are two classes of flavonoids that contribute to yellow flower colour in a number of plants.
  Eg: yellow carnation (chalcone isosalipurposide).

Carnation

Phenolics and plant growth

- **Cell wall integrity and shape** – wall bound phenolic acids like *p*-coumaric and ferulic acid acts as reservoir of phenylpropanid units for lignin biosynthesis.
- Flavanoids have role in functional **pollen development** in petunia plants.
- **Phenolic turgorins -** gallic acid and gentisic acid found in the pulvini in *Mimosa pudica* L.
  -Nyctinastic leaf movement

- **Seed germination and dormancy**
  Ferulic acid inhibits germination of seeds of *Raphanus sativus*
  Coumarinic acid found more in rapidly germinating *Melilotus alba*



PESTICIDES:

- A pesticide is a substance or mixture of substance intended for preventing, destroying, repelling or lessening the damage caused by the pest.
- A pesticide can be a insect, plantϖ pathogen, weed, bacteria, bird etc. That competes with the human for food, destroy property, and spread disease.
- A pesticide can be a chemical, biological agent, antimicrobial, disinfectant etc.
- Many chemical pesticides are poisonous toϖ human and animals.

Classification of pesticides:

1. **Herbicide**-These are the chemicals used to kill weeds (i.e., unwanted plants) e.g. Borax, Nitrofen.
2. **Insecticide**-These are used to kill insect. E.g. DDT, BHC.
3. **Rodenticide**-These are used to kill rodents. e.g. Warfarin, Zinc phosphide.
4. **Nematicide**-These are used to kill namatodes e.g. DBCP, Phorate
5. **Molluscicide**-These ar used to kill molluscs e.g Sodium pentachloridephenate.
6. **Fungicides**-These are used to kill fungus e.g. Bordeaux mixture
7. **Algaecides**-These are used to kill algae e.g. Copper sulphate, Endothal
8. **Bactericide**-These are used to kill bacteria e.g. Dichlorophen,Oxolinic acid
9. **Piscicides**-These are used to kill fishes e.g. Trifloro methyl nitrophenol(TFM)

Chemical or Synthetic Pesticides:

- ❖ Organochlorenes-These are non-biodegradable and persist in soil for long time e.g., DDT, BHC, Endosulfan, Aldrin.
- ❖ Organophosphate-These are esters of alcoholsϖ with phosphoric acid or with some other acids. These are very toxic acetyl-cholinesterase inhibitors as a result of which the breakdown of acetyl choline stops. The accumulation of acetyl choline resulting in convulsion paralysis and death e.g.,Malathion.
- ❖ Carbamates-They are derived from carbamic acid. Mode of action of carbamates is almost similar to organo- phosphates e.g.,Carbaryl, Dimetilan.

Hazards of pesticides:

1. The pesticide industries cause pollution of soil, water and air. The pesticidal residue washed along with rain water, is added to the nearby water resources making it unfit for drinking.

2. They enter the food chain chain and cause problem of bioaccumulation or biomagnification.

3. They are not target specific hence also kills non-pest insects. It adversely affect the mechanism of entomophily.

4. Continuous and indiscriminate use of pesticides may develop resistance in insect pest like superpest and superbugs.

5. They are non-biodegradable and affect the balance of ecosystem.

6. They are highly toxic in nature and if not handled carefully, they can cause serious health problems like cancer, deformities and disease.

7. Accidents in pesticides manufacturing units cause great loss of human life e.g., Bolsover

The Bhopal Gas Tragedy: Pesticides in our midst

- **The worst industrial disaster in the history of the world is related to pesticide production. This Occurred at Union Carbide Factory in Bhopal, India Dec. 3, 1984.**
- **In this incident, Methyl Isocyanide (MIC) – an ingredient in the production of the insecticide Carbaryl , escaped into the atmosphere killing more than 3,000 people within a few hour.**
- **The insecticide, Carbaryl, itself is a highly toxic chemical and carcinogen (cancer causing agent) to humans.**
- **The tragedy occurred due to lack of adequate safeguards in the storing the chemical and lack of adequate warning to the public.**

(Top) Survivors of the tragedy lineup outside the factory awaiting treatment. Pesticides such as Lindane (middle) and Sevin (bottom) are still being stored in unsafe manner in the now abandoned the factory.

Biopesticide:

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**.

Classes of biopesticides

1. Biochemical pesticides:

- Naturally occurring substances that control pests by non-toxic mechanisms

- Includes substances that interfere with mating, such as insect sex pheromones, as well as various scented plant extracts that attract insect pests to traps

2. Microbial biopesticides:

- Consist of a microorganism (e.g., a bacterium, fungus, virus or protozoan) as the active ingredient.

- Active ingredient is relatively specific for its target pest

- Eg: some Bt ingredients control moth larvae found on plants, other Bt ingredients are specific for larvae of flies and mosquitoes.

3. Plant – incorporated protectants (pips):

- Pesticidal substances that plants produce from genetic material that has been added to the plant.

- Eg: 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.

Advantages of biopesticides:

- Less toxic than conventional pesticides.
- Effect only the target pest and closely related organisms
- whereas conventional pesticides are broad spectrum pesticides.
- Effective in very small quantities and often decompose quickly, resulting in lower exposures and largely avoiding pollution problems caused by conventional pesticides.
- When used as a component of Integrated Pest Management programs, biopesticides can greatly reduce the use of conventional pesticides while crop yields remain high.

BACTERIAL BIOPESTICIDES:

- Mainly 4 categories:

1.Crystalliferous spore formers (Bacillus thuringiensis)

2.Obligate pathogens (Bacillus papiliae)

3.Potential pathogens (Serratia marcesens)

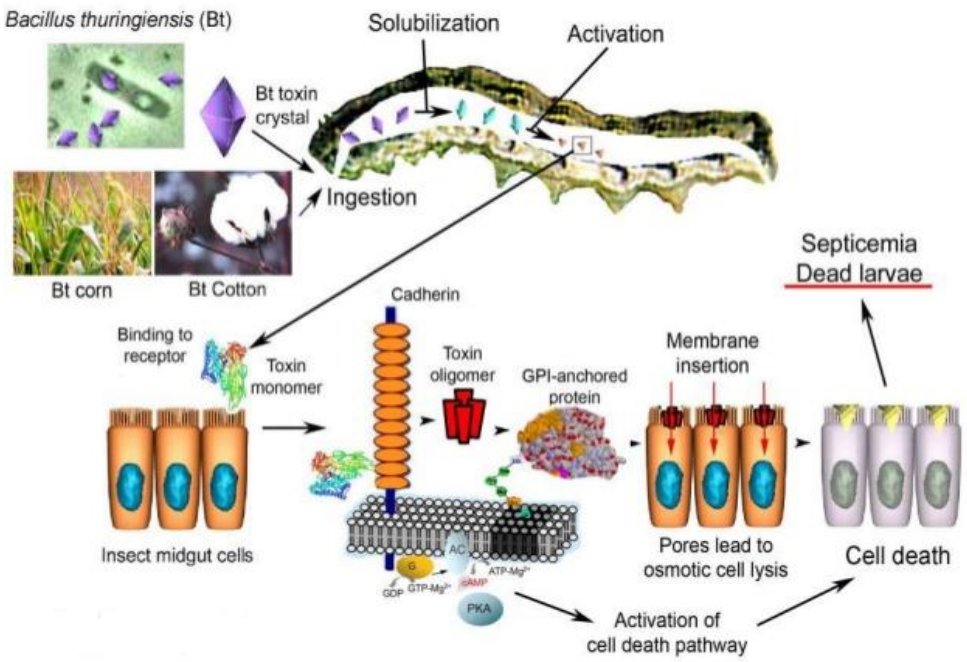
4.Facultative pathogens (Pseudomonas aeruginosa) Out of these four, 1 and 2 are important biopesticides.

Bacillus thuringiensis

- Gram positive, spore forming, facultative bacterium with nearly 100 subspecies and varieties divided into 70 serotypes.
- Specific, safe and effective tool for insect control.
- Insecticidal property resides in Cry family of crystalline proteins that are produced in the parasporal crystals and are encoded by the cry genes

***B.thuringiensis* CRY proteins:**

- Cry proteins are globular molecules with 3 structural domain connected by single linkers.
- This 3 domain family is characterised by protoxins of two different lengths, one being longer with C – terminal extension necessary for toxicity.
- This extension also has a characteristic role in crystal formation within the bacterium.
- Cry proteins are responsible for feeding cessation and death of the insect.



Mechanism of Cry Protein:

- Cry protoxins are ingested and then solubilised, releasing a protease resistant biologically active endotoxin, before it is being digested by protease of the gut to remove amino acids from its C and N terminal ends.
- The C terminal domain of the active toxin binds to the specific receptors on brush border membranes of the midgut.
- It is followed by the insertion of the hydrophobic region of the toxin into the cell membrane

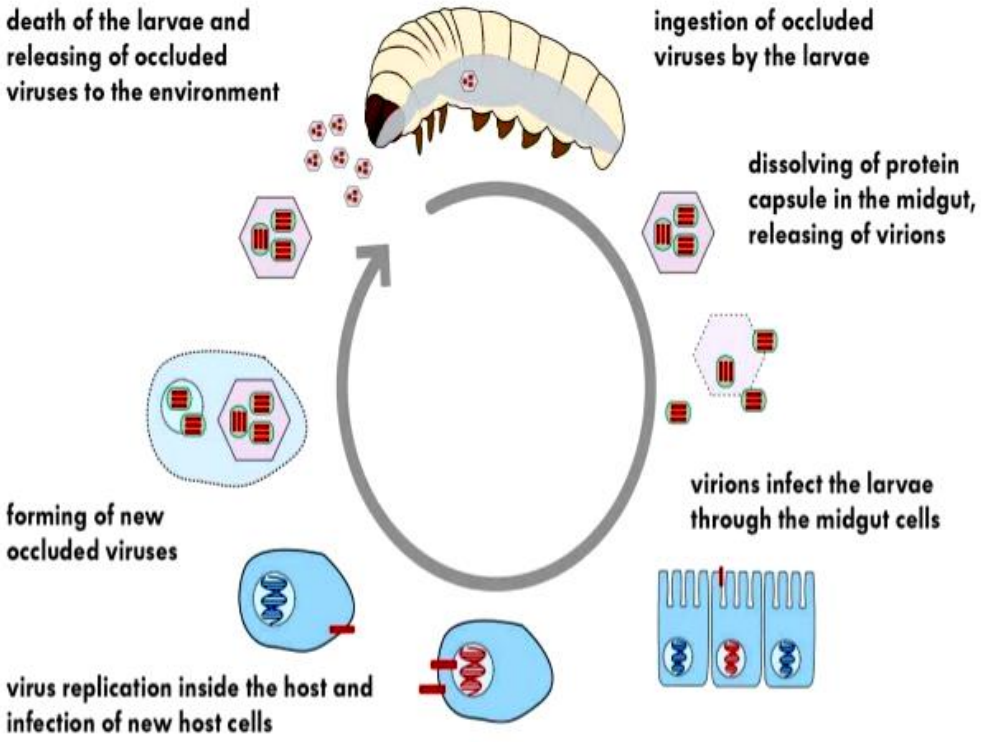
- This creates a disruption in the osmotic balance because of the formation of transmembrane pores.
- Ultimately cell lysis occurs in the gut wall leading to leakage of gut contents.
- This induces starvation and lethal septicaemia of the target pest.

Pseudomonas fluorescens (Phenazine):

- This bacteria is used to control damping off caused by *Pythium sp., Rhizoctonia solani, Gaeumannomyces graminis.*
- It has ability to grow quickly in the rhizosphere

VIRAL BIOPESTICIDES:

- The viruses used for pest control are: DNA containing baculoviruses (BVs) Nucleopolyhedrosis viruses (NPVs) Granuloviruses (GVs) Acoviruses Parvoviruses Polydnaviruses Pox viruses RNA containing recoviruses Cytoplasmic polyhedrosis viruses Nodaviruses Picorna like viruses Tetraviruses
- They are narrow spectrum
- After application to plant surface, baculovirus occlusion bodies (OBs) are rapidly inactivated by solar UV radiation (280 – 320nm).
- Efficacy can be improved by the use of formulations that include stilbene derived optical brighteners, which increase susceptibility to NPV infection.
- UV inactivation can be controlled by creating systems which filter UV radiation such as plastic greenhouse structures



Mechanism of Viral Biopesticide:

- ❖ Replication of virus occurs in the nuclei or cytoplasm of the target cell
- ❖ The expression of viral proteins occurs in 3 phases:
  - Early phase ie, 0-6 hr post infection
  - Late phase ie, 6-24 hr post infection
  - Very late phase ie, upto 72 hr post infection
- ❖ It is at the late phase that the virions assemble as the 29kDa occlusion body protein is synthesised.
- ❖ Virions of NPVs are occluded within each occlusion body to develop polyhedra whereas the GV virion is occluded in a small occlusion body to generate granules
- ❖ Infected nuclei can produce 100s of polyhedra and 1000s of granules per cell.
- ❖ These can create enzootics, deplete the pest populations and ultimately create significant impact on the economic threshold of the pest.