SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – I –Soil & Agricultural Microbiology– SMB2201

Distribution of microbes is nature

Soil is a dynamic habitat for an enormous variety of life-form. Soils give a mechanical support to plants from which they extract nutrients. soil provides shelters for many animal types, from invertebrates such as worms and insects up to mammals like rabbits, moles, foxes and badgers. It also provides habitats colonised by a staggering variety of microorganisms.

All these forms of life interact with one another and also interact with the soil to create continually changing conditions. This allows an on-going evolution of soil habitats. 2. Distribution of Microorganisms Microorganisms constitute < 0.5% (w/w) of the soil mass yet they have a major impact on soil properties and processes. 60-80 % of the total soil metabolism is due to the microflora.

These are the smallest organisms (<0.1 mm in diameter) and are extremely abundant and diverse. They include algae, bacteria, cyanobacteria, fungi, yeasts, myxomycetes and actinomycetes that are able to decompose almost any existing natural material. Micro- organisms transform organic matter into plant nutrients that are assimilated by plants. Soil organisms represent a large fraction of global terrestrial biodiversity.

Soil microorganisms, is an important component in a forest ecosystem, are sensitive to environmental change and represent the key factor affecting the decomposition of soil organic matter, nutrient cycling, and biogeochemical cycles . The biogeochemical distribution of soil microbial communities helps to explore the ecological process more deeply, but also has important implications for the protection of microbial resources and the management of terrestrial forest ecosystems

Soil organisms can be grouped on the basis of: Size:how big they are –Species: who they are related to –Function: how they make their living.

One gram of topsoil may contain:

a) as many as one billion bacteria

b) up to 100 million actinomycetes

c) one million fungi

d) 100 nematodes

Importance of Soil Organisms

1. Responsible for cycling of C, N and other nutrients
2. Increase soil aeration and penetrability
3. Maintain soil quality and health
4. Relocate and decompose organic materials
5. Enhance soil structure
6. Involved in disease transmission and control

Essential functions performed by different members of soil organisms

Functions	Organisms involved
Maintenance of soil structure	**Bioturbating invertebrates and plant roots, mycorrhizae and some other micro-organisms**
Regulation of soil hydrological processes	**Most bioturbating invertebrates and plant roots**
Gas exchange and carbon sequestration (accumulation in soil)	**Most micro-organisms and plant roots, some C protected in large compact biogenic invertebrate aggregates**

Soil detoxification	**Most micro-organisms and plant roots, some C protected in large compact biogenic invertebrate aggregate**
Nutrient cycling	**Most micro-organisms and plant roots, some soil- and litter-feeding invertebrates**
Decomposition of organic matter	**various saprophytic and litter-feeding invertebrates (detritivores), fungi, bacteria, actinomycetes and other micro-organisms**
Suppression of pests, parasites and diseases	**Plants, mycorrhizae and other fungi, nematodes, bacteria and various other micro-organisms, collembola, earthworms, various predators**
Sources of food and medicines	**Plant roots, various insects (crickets, beetle larvae, ants, termites), earthworms, vertebrates, micro-organisms and their by-products**
Symbiotic and asymbiotic relationships with plants and their roots	**Rhizobia, mycorrhizae, actinomycetes, diazotrophic bacteria and various other rhizosphere micro-organisms, ants**
Plant growth control (positive and negative)	**Direct effects: plant roots, rhizobia, mycorrhizae, actinomycetes, pathogens, phytoparasitic nematodes, rhizophagous insects, plant-growth promoting rhizosphere micro-organisms, biocontrol agents Indirect effects: most soil biota**

Rhizosphere

- The rhizosphere is the region of soil immediately adjacent to and affected by plant roots. It is a very dynamic environment where plants, soil, micro-organisms, nutrients and water meet and interact. The rhizosphere differs from the bulk soil because of the activities of plant roots and their effect on soil organisms.

Microbial communities in soil

Microorganisms affect the structure and fertility of different soils and contribute to nutrient availability in soil(OM decomposition, humus formation, N-fixation, seed germination) manage soil stability by different biochemical processes Degrade pesticides and chemicals in soil Contribute the growth and success of the plants and overall ecosystem of a soil environment.

Types of microorganisms present in the soil are

1. Bacteria
2. Actinomycetes
3. Fungi
4. Algae
5. Protozoa

BACTERIA

It's the smallest organisms in the soil .It is a Prokaryotic (simple cell structure with no internal organelles) Bacteria are the most abundant microorganisms in the soil .Perform many biochemical processes such as Nitrogen fixation (Nitrobacter sp.),Degradation of hydrocarbon,used for remediation(Pseudomonas sp).e.g., *Azotobacler*, *Rhizobium, Bacillus and Xanthomonas*

Function of Bacteria: Bacteria bring about a number of changes and biochemical transformations in the soil and thereby directly or indirectly help in the nutrition of higher plants growing in the soil. The important transformations and processes in which soil bacteria play vital role are:

1. Decomposition of cellulose and other carbohydrates,
2. Ammonification (proteins ammonia),
3. Nitrification (ammonia-nitrites-nitrates),
4. Denitrification (release of free elemental nitrogen),
5. Biological fixation of atmospheric nitrogen (symbiotic and non-symbiotic)
6. Oxidation and reduction of sulphur & iron compounds

ACTINOMYCETES

Actinomycetes are clubbed with bacteria, the class of Schizomycetes and confined to the order Actinomycetales. They are unicellular like bacteria, but produce a mycelium which is non-septate (coenocytic) and more slender, like true bacteria they do not have distinct cell-wall and their cell wall is without chitin and cellulose (commonly found in the cell wall of fungi).

Actinomycetes are numerous and widely distributed in soil and are next to bacteria in abundanceThey are heterotrophic, aerobic and mesophilic (25-30c) organisms and some species are commonly present in compost and manures are thermophilic growing at 55-65° c
temperature (e.g. Thermoatinomycetes, Streptomyces). Actinomycetes belonging to the order of Actinomycetales are grouped under four families: Mycobacteriaceae Actinomycetaceae Streptomycetaceae Actinoplanaceae. In the order of abundance in soils, the common genera of actinomycetes are Streptomyces (nearly 70%), Nocardia and Micromonospora although Actinomycetes, Actinoplanes, Micromonospora and Streptosporangium are also generally encountered.

Functions of Actinomycetes

1. Degrade/decompose all sorts of organic substances like cellulose, polysaccharides, protein fats, organic- acids etc.
2. Organic residues / substances added soil are first attacked by bacteria and fungi and later by actinomycetes, because they are slow in activity and growth than bacteria and fungi.
3. They decompose / degrade the more resistant and indecomposable organic substance/matter and produce a number of dark black to brown pigments which contribute to the dark colour of soil humus.
4. They are also responsible for subsequent further decomposition of humus (resistant material) in soil.
5. They are responsible for earthy / musty odour / smell of freshly ploughed soils.
6. Many genera species and strains (e.g. Streptomyces if actinomycetes produce/synthesize number of antibiotics like Streptomycin, Terramycin, Aureomycin etc.
7. One of the species of actinomycetes Streptomyces scabies causes disease "Potato scab" in potato.

FUNGI

Fungi in soil are present as mycelial bits, rhizomorph or as different spores. □Soil fungi possess filamentous mycelium composed of individual hyphae. The fungal hyphae may be aseptate /coenocytic (Mastigomycotina and Zygomycotina) or septate (Ascomycotina, Basidiomycotina & Deuteromycotina). Most commonly encountered genera of fungi in soil are;

Alternaria, Aspergillus, Cladosporium, Cephalosporium Botrytis, Chaetomium, Fusarium, Mucor, Penicillium, Verticillium, Trichoderma, Rhizopus, Gliocladium, Monilia, Pythium, etc. Soil fungi are aerobic and heterotrophic, they require abundant supply of oxygen and organic matter in soil. Fungi are dominant in acid soils, because acidic environment is not conducive / suitable for the existence of either bacteria or actinomycetes. The optimum PH range for fungi lies-between 4.5 to 6.5. They are also present in neutral and alkaline soils and some can even tolerate PH beyond 9.0

Functions of Fungi

1. Fungi plays significant role in soils and plant nutrition.
2. They plays important role in the degradation / decomposition of cellulose, hemi cellulose, starch, pectin, lignin in the organic matter added to the soil.
3. Lignin which is resistant to decomposition by bacteria is mainly decomposed by fungi.
4. They also serve as food for bacteria.
5. Certain fungi belonging to sub-division Zygomycotina and Deuteromycotina are predaceous in nature and attack on protozoa & nematodes in soil and thus, maintain biological equilibrium in soil.
6. They also plays important role in soil aggregation and in the formation of humus.
7. Some soil fungi are parasitic and causes number of plant diseases such as wilts, root rots, damping-off and seedling blights e.g. Pythium, Phyiophlhora, Fusarium, Verticillium etc.
8. Number of soil fungi forms mycorrhizal association with the roots of higher plants (symbiotic association of a fungus with the roots of a higher plant) and helps in mobilization of soil phosphorus and nitrogen. e.g. Glomus, Gigaspora, Aculospora, (Endomycorrhiza) and Amanita, Boletus, Entoloma, Lactarius (Ectomycorrhiza).

ALGAE

Algae are present in most of the soils where moisture and sunlight are available. Their number in soil usually ranges from 100 to 10,000 per gram of soil. They are photoautotrophic, aerobic organisms and obtain CO_2 from atmosphere and energy from sunlight and synthesize their own food. They are unicellular, filamentous or colonial.

Soil algae are divided in to four main classes or phyla as follows:

1. Cyanophyta (Blue-green algae)
2. Chlorophyta (Grass-green algae)
3. Xanthophyta (Yellow-green algae)

4. Bacillariophyta (diatoms or golden-brown algae)
Out of these four classes / phyla, blue-green algae and grass-green algae are more abundant in soil. The **green-grass algae** and diatoms are dominant in the soils of **temperate regi**on while **blue-green algae** predominate in **tropical soils**.

Green-algae prefer acid soils while blue green algae are commonly found in neutral and alkaline soils. The most common genera of green algae found in soil are: *Chlorella, Chlamydomonas, Chlorococcum, Protosiphon* etc. and that of diatoms are *Navicula, Pinnularia, Synedra, Frangilaria*.

Blue green algae are unicellular, photoautotrophic prokaryotes containing phycocyanin pigment in addition to chlorophyll. They are common in neutral to alkaline soils. The dominant genera of BGA in soil are: *Chrococcus, Phormidium, Anabaena, Aphanocapra, Oscillatoria* etc. Some BGA posses specialized cells known as "Heterocyst" which is the sites of nitrogen fixation. BGA fixes nitrogen (non-symbiotically) in puddle paddy/water logged paddy fields (20-30 kg/ha/season). There are certain BGA which possess the character of symbiotic nitrogen fixation in association with other organisms like fungi, mosses, liverworts and aquatic ferns Azolla. e.g. Anabaena-Azolla association fix nitrogen symbiotically in rice fields.

Functions of Algae or BGA

1. Plays important role in the maintenance of soil fertility especially in tropical soils.
2. Add organic matter to soil when die and thus increase the amount of organic carbon in soil. 3. Most of soil algae (especially BGA) act as cementing agent in binding soil particles and thereby reduce/prevent soil erosion.
4. Mucilage secreted by the BGA is hygroscopic in nature and thus helps in increasing water retention capacity of soil for longer time/period.
5. Soil algae through the process of photosynthesis liberate large quantity of oxygen in the soil environment and thus facilitate the aeration in submerged soils or oxygenate the soil environment.
6. They help in checking the loss of nitrates through leaching and drainage especially in un-cropped soils.
7. They help in weathering of rocks and building up of soil structure

PROTOZOA

Soil protozoa belonging to the class ciliate / ciliophora are characterized by the presence of cilia (short hair-like appendages) around their body, which helps in locomotion. Protozoa are abundant in the upper layer (15 cm) of soil. Protozoa can be split up into three categories: flagellates, amoebae, and ciliates.

The important soil inhabitants of this class are *Colpidium, Colpoda, Balantiophorus, Gastrostyla, Halteria, Uroleptus, Vortiicella, Pleurotricha* etc. Soil moisture, aeration, temperature and pH are the important factors affecting soil protozoa.

Function of Protozoa

1. Most of protozoans derive their nutrition by feeding or ingesting soil bacteria belonging to the genera *Enterobacter, Agrobacterium, Bacillus, Escherichia, Micrococcus*, and *Pseudomonas* and thus, they play important role in maintaining microbial / bacterial equilibrium in the soil.
2. Some protozoa have been recently used as biological control agents against phytopathogens. 3. Species of the bacterial genera viz. *Enterobacter* and *Aerobacter* are commonly used as the food base for isolation and enumeration of soil protozoans.
4. Several soil protozoa cause diseases in human beings which are carried through water and other vectors, e.g. Amoebic dysentery caused by *Entamoeba histolytica*

Factor influencing the microbial communities in soil

Soil biodiversity reflects the mix of living organisms in the soil. These organisms interact with one another and with plants and small animals forming a web of biological activity. ... These organisms improve the entry and storage of water, resistance to erosion, plant nutrition, and break down of organic matter. The soil environment, soil microorganisms play key roles in ecosystem functioning. They are known to be influenced by biotic and abiotic factors, such as plant cover or edaphic parameters. Major factors affecting microbial communities are

1. pH
2. Temperature
3. Moisture.
4. Soil mineralogy.
5. Light.
6. Organic and Inorganic Chemicals
7. Soil Organic Matter
8. Types of Vegetation and its Growth Stages
9. Different Seasons

Among the edaphic parameters, pH is the factor that most strongly influences soil bacterial communities.

Autochthonus and Zymogenous

On the basis of ecological characteristics classified soil microorganisms in general and bacteria in particular into two broad categories:

1. **Autochthonous** (Indigenous species)
   Autochthonous bacterial population is uniform and constant in soil, since their nutrition is derived from native soil organic matter. E.g. Arthrobacter and Nocardia .
2. **Zymogenous** (fermentative).

Zymogenous bacterial population in soil is low, as they require an external source of energy. E.g. *Pseudomonas* & *Bacillus*. The population of Zymogenous bacteria increases gradually when a specific substrate is added to the soil. They are cellulose decomposers, nitrogen utilizing bacteria and ammonifiers. Most of the bacteria which are predominantly encountered in soil are taxonomically included in the three orders, Pseudomonadales, Eubacteriales and Actinomycetales of the class Schizomycetes. The most common soil bacteria belong to the genera *Pseudomonas, Arthrobacter, Clostridium, Achromobacter, Sarcina, Enterobacter* etc.

Microbial Association

The inter- and intra-relationships between various microorganisms. This can include both positive (like Symbiosis) and negative (like Antibiosis) interactions.

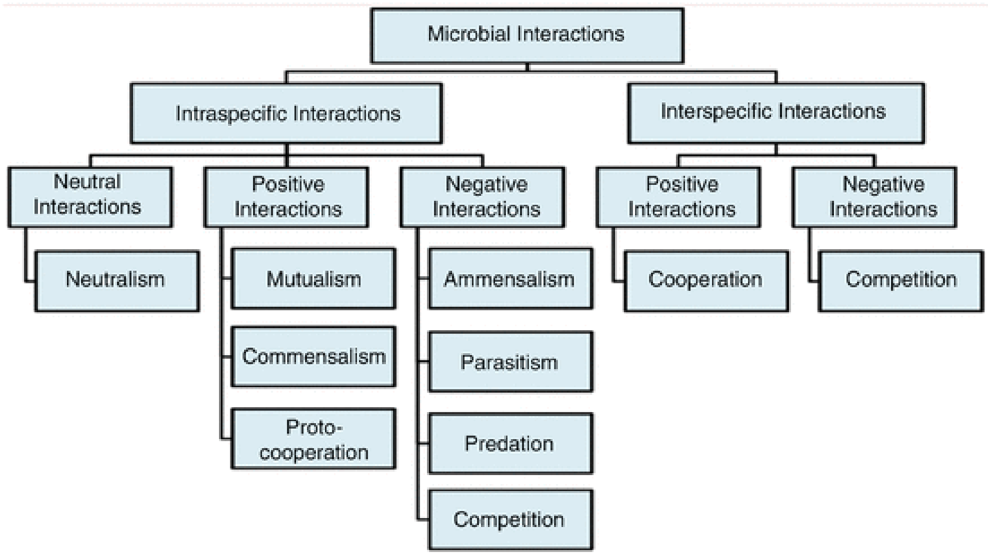
Microorganism plays very important role in agriculture because the soil microbes (bacteria and fungi) are essential for decomposing organic matter and recycling old plant material. Some soil bacteria and fungi form relationships with plant roots that provide important nutrients like nitrogen or phosphorus.

Plant—microbe association can be friendly or hostile

Soil microorganisms play a crucial role in plant growth and plant exudates. Microorganisms can affect exudation by affecting the permeability of root cells and root metabolism. Microorganisms can also absorb certain compounds in root exudates and excrete other compounds.Densely colonized soil contains beneficial mycorrhizal fungi and rhizobia, which associate with roots and provide plants with mineral nutrients and fixed nitrogen, respectively, in exchange for carbon.

Types of Microbial Association

1. Positive interaction: Mutualism, Syntrophism, Proto-cooperation, Commensalism.
2. Negative interaction: Ammensalism (antagonism), parasitism, predation, competition



Mutualism

Mutualism describes the ecological interaction between two or more species where each species has a benefit. It is a symbiotic relationship in which two different species interact with and, totally rely on one another for survival.

Organisms live in mutualistic relationships for a number of important reasons, including a need for shelter, protection, and nutrition, as well as for reproductive purposes.

Mutualistic relationships can be categorized as either obligate or facultative. In obligate mutualism, the survival of one or both organisms involved is dependent upon the relationship. In facultative mutualism, both organisms benefit from but are not dependent upon their relationship for survival.

A number of examples of mutualism can be observed between a variety of organisms such as bacteria, fungi, algae, plants, and animals.

Common mutualistic associations occur between organisms in which one organism obtains nutrition, while the other receives some type of service. Other mutualistic relationships are multifaceted and include a combination of several benefits for both species

Examples of Mutualism.

1. ocellaris clownfish are hiding in an anemone. ...

Plant Pollinators and Plants- Flowering plants rely heavily on insects and other animals for pollination Insects and animals play a vital role in the pollination of flowering plants. While the plant-pollinator receives nectar or fruit from the plant, it also collects and transfers pollen in the process.

2. Ants and Aphids- ant species herd aphids in order to have a constant supply of honeydew that the aphids produce. In exchange, the aphids are protected by the ants from other insect predators.

3. Oxpeckers and Grazing Animals- Oxpeckers are birds that eat ticks, flies, and other insects from cattle and other grazing mammals. The oxpecker receives nourishment, and the animal that it grooms receives pest control

4. Clownfish and Sea anemones- Clownfish live within the protective tentacles of the sea anemone. In return, the sea anemone receives cleaning and protection.

5. Sharks and Remora Fish- Remora are small fish that can attach to sharks and other large marine animals. Remora receive food, while the shark receives grooming.

6. Lichens- Lichens result from the symbiotic union between fungi and algae or fungi and cyanobacteria. The fungus receives nutrients obtained from the photosynthetic algae or bacteria, while the algae or bacteria receive food, protection, and stability from the fungus

7. Nitrogen-Fixing Bacteria and Legumes- Nitrogen-fixing bacteria live in the root hairs of legume plants where they convert nitrogen to ammonia. The plant uses the ammonia for growth and development, while the bacteria receive nutrients and a suitable place to grow.

8. The protozoan-termite relationship- flagellated protozoa live in the gut of termites and wood roaches. The protozoa engulf wood particles, digest the cellulose, and metabolize it to acetate and other products. Termites oxidize the acetate released by their flagellates.

Symbiosis:


An association of two or more different species.Two types of symbiosis are Ectosymbisis -One organism can be located on the surface of another, as an ectosymbiont. In this case, the ectosymbiont usually is a smaller organism located on the surface of a larger organism. E.g Thiothrix species, a sulfur-using bacterium, which is atached to the surface of a mayfly larva and which itself contains a parasitic bacterium.

Endosymbiosis-one organism can be located within another organism as an endosymbiont. Example-Fungi associated with plant roots (mycorrhizal fungi) often contain endosymbiotic bacteria, as well as having bacteria living on their surfaces microorganisms live on both the inside and the outside of another organism

Protocooperation

It is a relationship in which an organism in an association is mutually benefited with each other. This interaction is similar to mutualism but the relationships between the organisms in protocooperation are not obligatory as in mutualism.

Example:

A Marine Worm-Bacterial Protocooperative relationship the worms secrete mucous from tiny glands on their backs to feed the bacteria, and in return they are protected by some degree of insulation.

Amensalism :

A relationship in which the product of one organism has negative effect on other organism.

Example:

The production of antibiotics that can inhibit or kill a susceptible microorganism

- Bacteriocins (Proteinaceous toxins produced by bacteria with antimicrobial toxicity. Most bacteriocins target other strains of the same species as the producing organism, but some are more broad-spectrum)

Commensalism :

It is a relationship in which one symbiont, the commensal, benefits while the other is neither harmed nor helped (neutral). Commensalistic relationships between microorganisms include situations in which the waste product of one microorganism is the substrate for another species.

Examples of Commensalism

- Intestinal microorganisms: in the human colon, when oxygen is used up by the facultatively anaerobic E. coli, obligate anaerobes such as Bacteroides are able to

grow in the colon.

• Microbial succession during spoilage of milk- fermenting bacteria promote growth of acid tolerant species

• Formation of biofilms– initial colonizer helps other microorganisms attach

• Skin or surface microbes on plants or animals– host plant or animal releases volatile, soluble, and particulate organic compounds used by commensals.

Predation

When one organism, the predator, engulfs and digests another organism as its prey. The prey can be larger or smaller than the predator, and this normally results in the death of the prey.

Examples

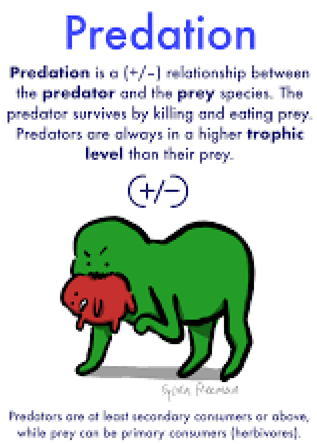
a) *Bdellovibrio*, a periplasmic predator that penetrates the cell wall and grows outside the plasma membrane.

b) *Vampirococcus* with its unique epibiotic mode of attacking a prey bacterium

c) *Daptobacter* showing its cytoplasmic location as it attacks a susceptible bacterium.

It has beneficial effects:

• Digestion e.g The microbial loop
• Protection and increased fitness
• Survival and increased pathogensity e.g, The intracellular survival of Legionella ingested by ciliates.



Parasitism

The population that benefits, the parasite, drives its nutritional requirements from host, which is harmed

• It can involve physical maintenance in or on the host.

• Characterized by relatively long period of contact.

E.g fungal parasite of plant.

SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – II –Soil & Agricultural Microbiology– SMB2201

Microbial Decomposition

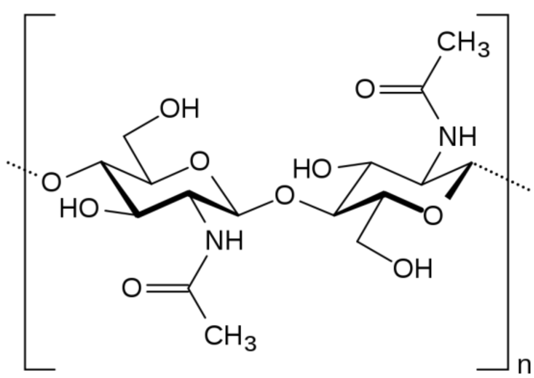
Microbiology of decomposition is the study of all microorganisms involved in decomposition, the chemical and physical processes during which organic matter is broken down and reduced to its original elements. In these processes, bacteria, fungi, molds, protozoa, actinomycetes, and other saprophytic organisms feed upon decaying organic materials initially, while in the later stages of decomposition mites, millipedes, centipedes, springtails, beetles and earthworms further breakdown and enrich the composting materials.

Plant cell wall polysaccharides are the most abundant organic compounds found in nature.
1. Cellulose, 2. Hemicelluloses, 3.Lignin, 4. Pectin

CHITIN

Chitin is a natural polysaccharide in nature and it's the 2nd abundant organic source on earth is one .Chitosan is the N-deacetylated derivative of chitin. Chitin is mainly produced by fungi, arthropods and nematodes. In insects, it functions as scaffold material, supporting the cuticles of the epidermis and trachea as well as the peritrophic matrices lining the gut epithelium

Structure of Chitin



The structure of chitin is similar to that of cellulose, but with one hydroxyl group on each monomer replaced with an acetyl amine group, which allows for better hydrogen bonding, making the overall matrix stronger. Chitin is covalently linked to other polysaccharides, such as glucans, and forms chitin-glucan complex Chitin is strong, flexible, and translucent. The main role of Chitin is to provide structure and support.

Occurence

Chitin present as exoskeleton of arthropods, shells of mollusks, spines of diatoms, cellwall of fungi, mold and yeast. The chitin content in fungi varies from 0.5% in yeast to 50% on filamentous fungi species

Decomposition

Chitin is degraded by the joint action of chitinase, which yields oligomeric fragments, and exochitinase, or β-N-acetylglucosaminidase, which hydrolyzes terminal polymers or dimers.

Classification of Chitinase

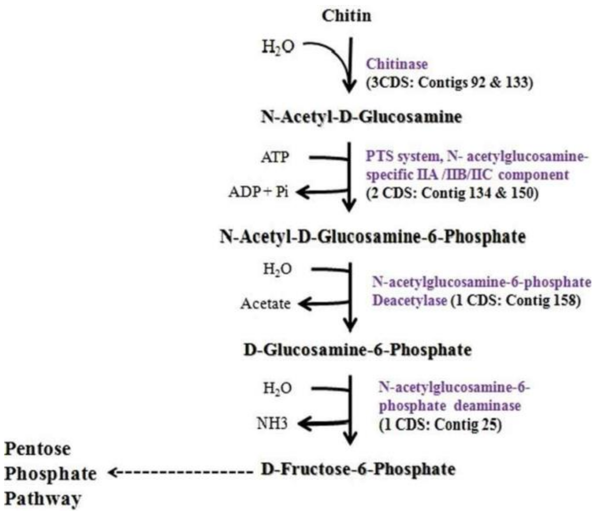
1.Endochitinases (EC 3.2.1.14) randomly split chitin at internal sites of the chitin microfibril, forming soluble, low molecular mass multimer products. The multimer products includes di-acetylchitobiose, chitotriose, and chitotetraose, with the dimer being the predominant product.

2.Exochitinases have also been divided into two sub categories

a)Chitobiosidases - act on the non-reducing end of the chitin microfibril, releasing the dimer, di-acetylchitobiose, one by one from the chitin chain.

b)β-1,4- N-acetylglucosaminidases - split the multimer products, such as di-acetylchitobiose, chitotriose, and chitotetraose, into monomers of N-acetylglucoseamine

Chitinases also occur naturally in many common foods. Bananas, chestnuts, kiwis, avocados, papaya, and tomatoes.



Applications

Bio-conversion of chitin to useful products such as

- Fertilizer
- Enhancement of insecticides and fungicides
- The production of non-allergenic, non-toxic, biocompatible products

Biomedical application

- Wound dressings are used to protect wound skin form insult, contamination and infection
- Chitin-based wound dressings - Increase dermal regeneration – Accelerate wound healing
- Prevent bacteria infiltration
- Avoid water loss
- Chitin surgical threads - strong, flexible, decompose after the heals
- Chitosan wound dressings
- Contact lens

PECTIN

Pectin is a natural fibre found in plant cell walls and most concentrated in the skin of fruits.It is a naturally occurring substance (a polysaccharide) found in berries, apples and other fruit. When heated together with sugar, it causes a thickening that is characteristic of jams and jellies.

Pectin is a complex polysaccharide consisting mainly of esterified D-galacturonic acid resides in an alpha-(1-4) chain. The acid groups along the chain are largely esterifed with methoxy groups in the natural product. ... Pectin is also known to contain other neutral sugars which are present in side chain.

Microbial Degradation of Pectin

Pectinase is an enzyme that breaks down pectin, a polysaccharide found in plant cell walls. Commonly referred to as pectic enzymes, they include pectolyase, pectozyme, and polygalacturonase, one of the most studied and widely used commercial pectinases.
Various fungal, bacterial, and yeast strains degrades pectin.E.g Aspergillus and Penicillium ,Bacillus etc.,

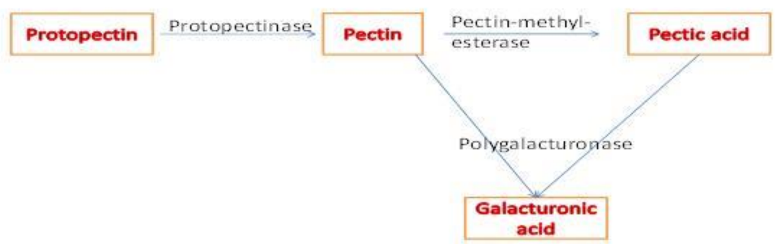


Fig: Degradation of Pectin

Application

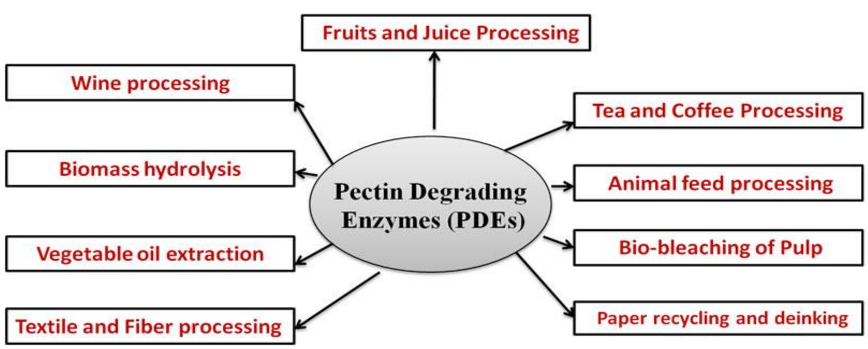
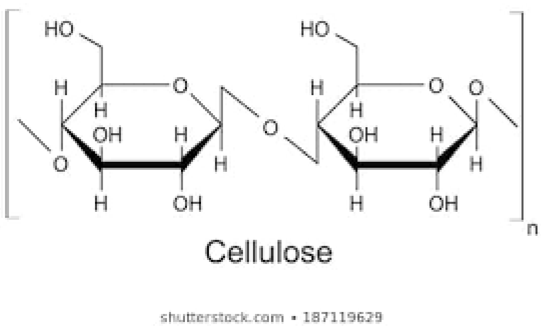


Figure 4: A drawing highlighting the applications of pectin degrading enzymes (PDEs) in different industries.

CELLULOSE

Cellulose is an important structural component of the primary cell wall of green plants, many forms of algae and the oomycetes. Some species of bacteria secrete it to form biofilms. Cellulose is the most abundant organic polymer on Earth. Cellulose can be also produced from corn cobs or stalks, soybean hulls, sugar cane stalks, oat hulls, rice hulls, wheat straw, sugar beet pulp, bamboo, jute, flax and ramie.

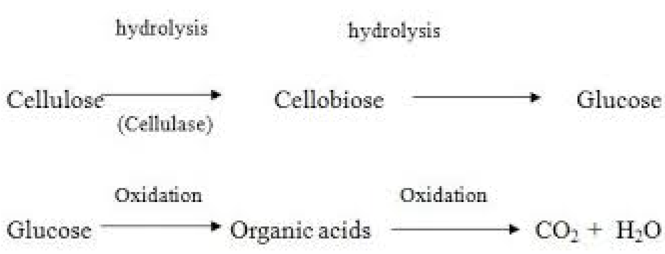
Structure

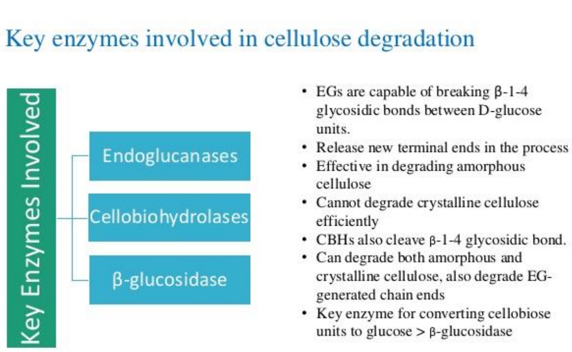


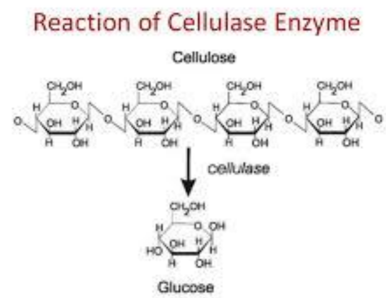
Decomposition

Plants typically contain up to 60% cellulose the decomposition of cellulose is a key activity of soil bacteria and is vital to the energy flow through soils and the cycling of N, P, and S, where immobilization generally accompanies cellulose decomposition.

Cellulase is the enzyme that hydrolyzes β-1,4-glucoside linkages of cellulose chain and roughly grouped into endolytic enzymes (endo-β-1,4-d-glucanase) and exolytic enzymes(cellobiohydrolase).







Cellulase producing microorganisms

Bacteria-*Bacillus subtilis, B.licheniformis,Cellulomonas sp., Pseudomonas sp.,Geobacillus* sp.,

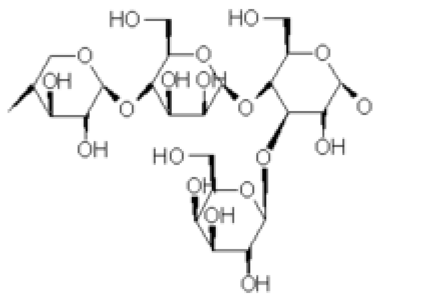
Fungi-*Trichoderma viridae,Trichoderma sp., Aspergillus fumigates, Aspergillus niger., A.flavus*

Application

Cellulase is used for many purposes in the industrial sector. Especially for textile wet processing, biofuel production, biostoning of denim fabric, biopolishing of textile fibers, softening of garments, and removal of excess dye from the fabrics are some of the major applications of this enzyme in the industry.

HEMICELLULOSE

Hemicellulose is a branched polymer of pentose and hexose sugars, found in the plant cell wall. The uronic acid composition is mainly d-glucuronic acid and 4-O-methyl-d-glucuronic acid. It is a smaller branched carbohydrate which can be made of different monosaccharides, but cellulose is made of very long unbranched fibrils composed exclusively of glucose, held together by hydrogen bonding.



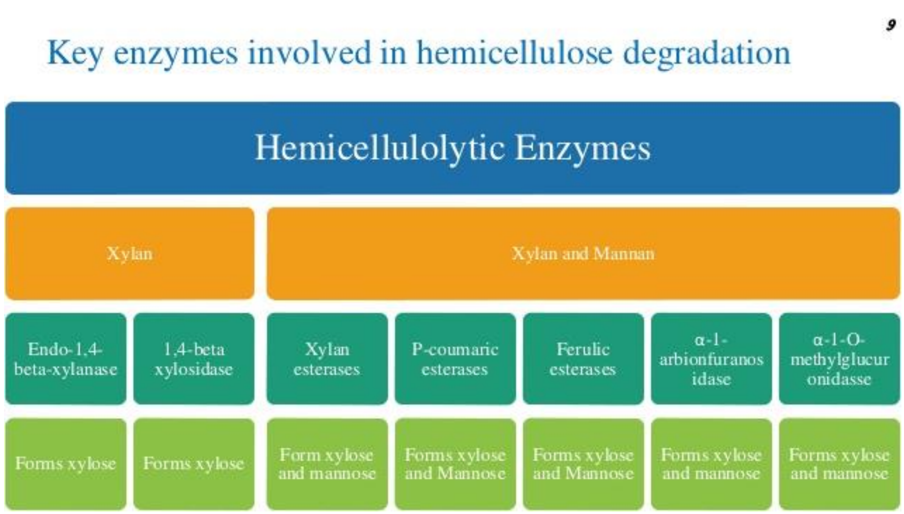
- Xylose - ß(1,4) - Mannose - ß(1,4) - Glucose -
- alpha(1,3) - Galactose

Hemicellulose

Microbial Decompostion of Hemicellulose

Hemicellulase is a type of enzyme that degrades the plant cell wall polymer hemicelluloses. The bioconversion of lignocellulosic biomass in various industrial processes, such as the production of biofuels, requires the degradation of hemicellulose. *Clostridium stercorarium* is a thermophilic bacterium, well known for its outstanding hemicellulose-degrading capability.

Monomeric units of hemicellulose are D- xylose, D-mannose, D-galactose, D- glucose, L-arabinose, 4-O-methyl- glucuronic, D-galacturonic and D- glucuronic acid.Its less resistant againt biodegradation.



Important microorganisms in hemicellulose degradation

White-rot fungus - *Phanerochaete chrysosporium* which produces xylanases, a major component of hemicellulolytic enzymes,

Thermomonospora(produce-thermostable xylanase) and *Actinomadura, Streptomyces viridosporus, T. reesei, P. chrysosporium, B. stearothermophilus* and *Butyrivibrio fibrisolvens*

LIGNIN

Lignin is a class of complex organic polymers that form key structural materials in the support tissues of vascular plants and some algae. Lignin cross-links different plant polysaccharidesand it is a tasteless, insoluble, fibrous, hydrophobic , amorphous heteropolymer •

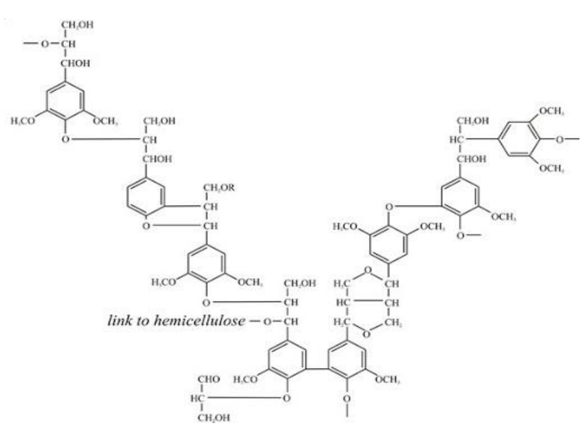
The Structure consists of three methoxylated monolignols precursors

• P-coumaryl alcohol (1)

• Coniferyl alcohol (2)

• Sinapyl alcohol (3) and

• 4-hydroxyphenylpropanoids are formed with syringyl, p-hydroxyphenyl, and guaiacyl • It provides structural strength, impermeability, microbial-attack-resistant, and oxidative stress



Important microorganisms in lignin biodegradation

Phanerochaete chrysosporium are capable of degrading wood-lignin

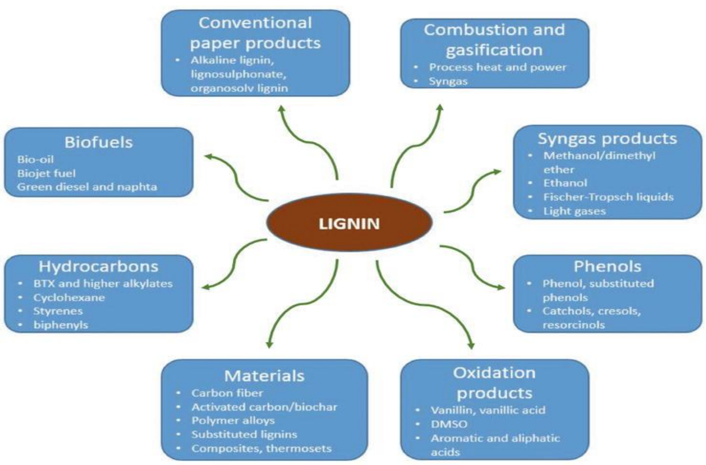
Aspergillus, Myceliophora thermophila, and *Chaemotium thermophilum*

Actinobacteria and Streptomyces.

Key Enzymes Involved in Degradation of Lignin

- Ligninolysis Peroxidases
- Lignin Peroxidase Manganese-
- dependent peroxidase
- Versatile Peroxidase
- Dye- decolorizing peroxidase
- Laccase

Application

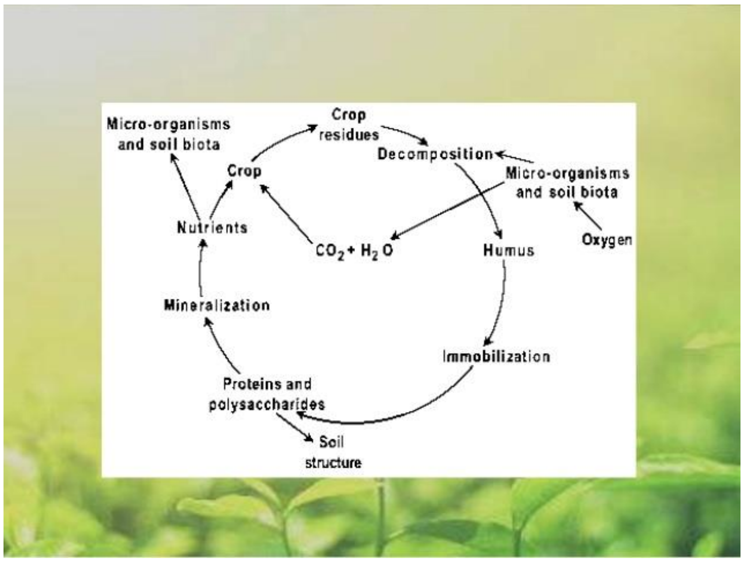


BIOCONVERSION OF ORGANIC WASTE

Organic waste, or green waste, is organic material such as food, garden and lawn clippings. It can also include animal and plant based material and degradable carbon such as paper, cardboard and timber.

Many organic wastes are used in biotechnological products such as enzymes,single-cell proteins,fuels,chemicals,biodegradable plastics,and pharmaceutical products.inedible parts of plants,agricultural residues from the harvest and food-processing wastes can be used as

raw materials. These organic wastes contain valuable components such as starch in potato and cassava pulp,pectin in apple pomace,sucrose in molasses,cellulose and hemicellulose in wood,garden waste,and rice hull.



Different organic residues contain different group of organic compounds ,so different

groups of compounds have their different case of decomposition.

1. Very slowly decomposed – lignin, Fat, Waxes, Cellulose and Hemicellulose.
2. Rapidly decomposed – Crude proteins, simple proteins, sugar, starch.

An enzymes is a substance, composed of protein, that is capable of lowering the activation energy of other selective compounds , enough to allow the breaking of a particular bond, under a particular environment so much reactions influenced by enzymes are called Biological

reaction.Urease – Breakdown urea in Ammonium carbamate,Protease – By involving water, it the bond linking amino acid to form separate amino acid. Cellulase – Breaks cellulose which are long chains sugar units important in decomposition of organic matter. Lignase – Degrades lignin compounds of decomposed organic residues. Hemicellulase – break hemicellulose compound or organic material.

Factors affecting decomposition of organic matter

1. Temperature: Cold periods retard plant growth and OM decomposition. Warm summers may permit plant growth and humus accumulation
2. Soil moisture: Extremes of both arid and anaerobicconditions reduce plant growth and microbialdecomposition. Near or slightly wetter than field capacity moisture conditions are most favorable for both processes.
3. Nutrients: Lack of nutrients particularly N slows decomposition.

4. Soil PH: Most of the microbes grow best at pH 6 – 8, but are severely inhibited below pH 4.5 and above pH 8.5.
5. Soil Texture: Soils higher in clays tend to retain larger amounts of humus.
6. Other Factors: Toxic levels of elements (Al, Mn, B, Se, Cl), excessive soluble salts, shade and organic phytotoxins in plant materials

CONVERSION OF COIR PITH

The largest by products of coconut is coconut husk from which coir fibre is extracted. This extraction process generates a large quantity of dusty material called coir dust or coir pith. Large quantity of coir waste of about 7.5 million tones is available annually form coir industries in India. In Tamil Nadu state alone 5 lakh tons of coir dust is available.

Coir pith has gained importance owing to its properties for use as a growth medium in Horticulture. Because of wider carbon and nitrogen ratio and lower biodegradability due to high lignin content, coir pith is still not considered as a good carbon source for use in agriculture. Coir pith is composted to reduce the wider C:N ratio, reduce the lignin and cellulose content and also to increase the manorial value of pith. Composting of coir pith reduces its bulkiness and converts plant nutrients to the available form.

Coir pith composting technology

Coir pith is collected from the coir industry without any fiber. If fibrous materials are present, it is removed by sieving at the source itself. Otherwise, it has to be removed at the end of composting at the compost yard. These fibrous materials will not get composted and it will hinder with composting process. It is advisable to bring fibre free coir pith for composting.

Site selection for composting

A separate area should be earmarked for composting. It is better to have an elevated place for composting. In between coconut trees, shade under any tree is good for composting. The shady area conserves the moisture in the composting material. The floor of the compost making area should be levelled. If earthen floor is available the floor can be made to hard by hard pressing and also by applying cow dung slurry. Presence of roof over the composting material is advantageous, since it protects the material from rain and severe sunshine.

Coir pith is an aerobic composting. So it should be heaped above the soil. There is no need for pit or cement tub to make the compost. Coir pith should be spread to the length of 4 feet and breadth of 3 feet. Initially coir pith should be put up for 3 inch height and thoroughly moistened. After moistening, nitrogenous source material should be added. The nitrogenous source may be in the form of urea or fresh poultry litter. If urea is applied, it is recommended that 5 kg urea is required for one ton of coir pith. This 5 kg equally divided into five portions and in alternative layer of coir pith one kg of urea should be applied. If fresh poultry litter is applied, it is recommended @ 200 kg for one ton of coir pith.

One has to proportionally divide and put the required amount of poultry litter over the coir pith. For example if one ton coir pith is divided into 10 portions, in the first layer, 100 kg poultry litter is added. After adding, the nitrogen source, the microbial inoculums Pleurotus and biomineralizer (2%) are added over the material. Over this one portion of coir pith is added and the same input mentioned above should be added. It is advisable to make a heap up to minimum of 4 feet height. But beyond 5 feet, it requires machinery to handle the materials. The increase in height retains the temperature generated in the coir pith compost process. If the height is low, whatever the heat generated will be dissipated easily.

Turning of material

The compost heap should be turned once in 10 days to allow the stale air trapped inside the compost material to go out and fresh air will get in. The composting process is an aerobic one, the organism performing the composting require oxygen for its metabolic activity. This turning of material indirectly aerates the substrate. The other way of giving aeration is inserting perforated unused PVC or iron pipe in the composting material both vertically and horizontally.

Moisture maintenance

Maintaining optimum moisture is the pre-requisite for uniform composting or waste material. Sixty percent moisture is to be maintained 60 % moisture is, the compost material should be always wet. But excess water should not be drained form the waste material is to take a handful of composting material and put in between the palms and squeeze it. If no water is coming out of the material, that moisture status is ideal for composting.

Compost maturity

The period of composting vary from substrate to substrate. If all the above said conditions are maintained in the composting, it will take sixty days (60 days) for some of the physical parameters to be observed in the compost. First observation is volume reduction of waste material. When the waste material is composted, the compost heap height will be reduced by 30 %. The second observation is waste materials are turned to black in colour and the waste particle size is reduced. The third observation is that composted material emits earthy odour. The chemical observation for compost maturity is to be analysed in the laboratory. The chemical observations are narrower C:N ratio (20:1), less oxygen uptake, less number of microorganism, more amount of available nutrients and highly cation exchange capacity.

Compost harvest

The composted material which is obtained from sieving is ready for use. If the composition is not used immediately, it should be stored in a open, cool place, to retain the moisture, so that the beneficial microorganism present in the compost will not die. Once in a month, water is sprinkled over the compost material to maintain the moisture.

Benefits of composted coir pith

- The addition of composted coir dust improves soil texture, structure and tilth, sandy soil become more compact and clayey soil become more arable.
- It improves the soil aggregation
- It improves the water holding capacity (more than 5 times its dry weight) contributing towards increased soil moisture.
- The bulk density of both the sub surface (15-30 cm) sioil is reduced to considerable extent with the application composted coir pith.
- Composted coir dust contains all plant nutrient elements and it can provide a supplemental effect along with inorganic fertilizers.
- There is improvement in cation exchange capacity of soils, where composted coir pith is applied.

Coir pith compost application increased the soil native microflora because of addition of humic materials.Ammonification, nitrification and nitrogen fixation are increased due to improved microbiological activity.

Application of coir pith compost

It is recommended that 5 tons of composted coir pith per hectare of land irrespective of the raised.

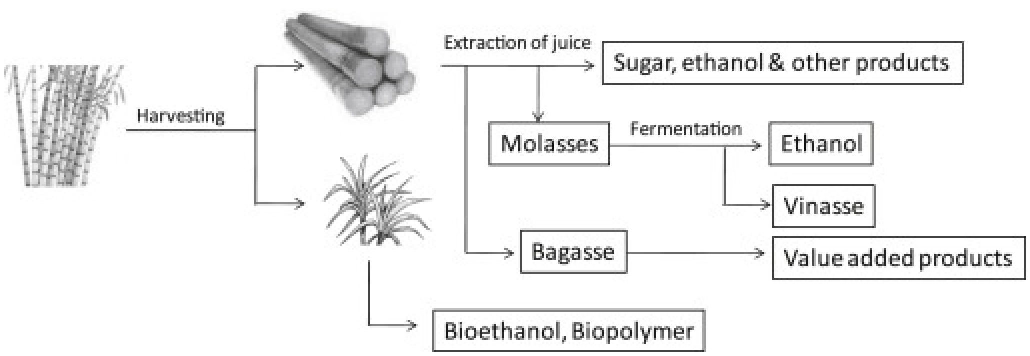
Is advised that composted coir pith should be applied basally before take up the sowing.

For nursery development in poly bags and in mud pots, while preparing the potting mixture 20 % of composted coir pith can be mixed with the soil and sand before filing it in the poly bag or mud pot

BIOCONVERSION OF SUGARCANE WASTE

The sugarcane is basically consisted of stem and straw. Sugarcane bagasse (SB) and straw (SS) are normally burned in industries to supply all the energy required in the process Bagasse is the fibrous residue left over after milling of the Cane, with 45-50% moisture content and consisting of a mixture of hard fibre, with soft and smooth parenchymatous (pith) tissue with high hygroscopic property. Bagasse contains mainly cellulose, hemi cellulose, pentosans, lignin, sugars, wax, and minerals. The quantity obtained varies from 22 to 36% on Cane and is mainly due to the fibre portion in Cane and the cleanliness of Cane supplied, which, in turn, depends on harvesting practices.

Bagasse is usually combusted in furnaces to produce steam for power generation. 2. Bagasse is also emerging as an attractive feedstock for bioethanol production.It is also utilized as the raw material for production of paper and as feedstock for cattle**.**



SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – III–Soil & Agricultural Microbiology– SMB2201

ROLE OF ENZYMES AND TOXINS IN PATHOGENESIS

A plant pathogen is an organism that causes a disease on a plant. Although relatives of some plant pathogens are human or animal pathogens, most plant pathogens only harm plants. The most common plant pathogens are fungi, bacteria, mollicutes, parasitic higher plants, parasitic green algae, nematodes, protozoa, viruses, and viroids.

There are many ways in which plant disease pathogens can affect plants

1. By utilizing host cell contents
2. By killing host or by interfering with its metabolic processes through their enzymes, toxins etc.
3. By weakening the host due to continuous loss of the nutrients.
4. By interfering with the translocation of the food, minerals land water.

Mode of pathogenesis of organisms are

- Enzymes
- Toxins
- Growth regulators
- polysaccharides

All plant pathogens except viruses and viriods can probably produce enzymes growth regulators and polysaccharides. They don't produce substances themselves but induce the host
to produce certain substances.

Routes of entry of pathogens are as follows

- First contact of pathogen with host –on plant surface
- Aerial plant part surfacesconsist primarily of cuticle and/or cellulose
- Root cell wall surfaces that consist only of cellulose.
- Cuticle consists primarily of cutin
- Lower layer consisting predominantly of pectic substances
- Polysaccharides of various types are often found in cell walls.
- epidermal cell walls may also contain suberin and lignin.
- Complete plant tissue disintegration involves the breakdown of lignin.
- Pathogen attack the plant by degrading these substances by several enzymes

ENZYMES IN PLANT DISEASES

Enzymes are large protein molecules that catalyse all the interrelated reactions in a living cell. Some enzymes are present in cells at all times

(constitutive). Many are produced only when they are needed by the cell in response to internal or external gene activators (induced)
Cutinases, cellulases, pectinases and lignases are often secreted by the pathogenic organism. Fungi, nematodes and bacteria are all known to produce one or more of the above. Enzymes in specific pathogen-host combinations.Viruses and viroids are generally not considered to secrete enzymes

Enzymes Degrading Cutin

Cutin is the main component of cuticle. Many fungi and bacteria produce cutinase- degrade cutin Fungi constantly produce cutinase in small amount come in contact with cutin and release small amount of monomers.These enter the pathogen cell and trigger the expression of cutinase gene.Presence of glucose suppresses the expression of cutinase gene. Enzymes reaches the higher concentration at penetration point of germtube and infection peg of appressorium forming fungi. Pathogens produce higher amount of cutinase seems to be more pathogenic than others. Eg: *Fusarium,Penicillium spinulosum*(rotting of leaves).

Enzyme Degrading Pectin

Main component of middle lamella-intracellular cement are pectin. The enzyme degrade pectic substances are pectinases or pectolytic enzymes
Examples

- Pectin methyl esterases- remove small branches of pectic chains and promote the attack of chain splitting pectinases
- Polygalacturonases – split the pectic chain by adding a molecule of water
- Pectin lyases – split the chain by removing a molecule of water
- Pectinase: 2 types such as Endopectinase : break pectin chains at random sites and Exopectinase :only break terminal linkage

Pathogen produce a small amount of pectin all the time. When contact with the pectin in the host they produce small amount of monomers, dimers or oligomers which induce the pectinase production in large amount.Pectin degradation produce many diseases particularly those characterised by soft rotting of tissues. Pectic enzymes are produced by germinating spores and act together with other pathogen enzymes – helps in penetration of host. Pectin degradation results in liquefaction of pectic substances that hold plant cells together- lead to weakening of cell wall – leads to tissue maceration and Weakening of plant tissue and maceration lead to inter and intra cellular invasion of tissue by pathogen. E.g– Brown rot (*Monilinia fructicola*) in plum– Bacterium *Erwinia sp*.– *Pseudomona*s.

Enzyme Degrading Cellulose

Cellulose is a polysaccharide consists of chains of glucose molecules and its degraded by cellulase. Soluble sugars serve as the food for pathogens and cellulases produced by several phytopathogenicfungi, bacteria, nematodes and parasitic higher plants.

- Softening and disintegration of cell wall
- Liberating large molecules to transpiration stream which interfere with normal movement of water
- Eg:
  Corn stalk rot fungus-*Fusarium*
  Rhizoctonia solani
  Fusarium moniliforme
  Sclerotium rolfsii

Enzyme Degrading Hemicellulose

Hemicellulose are polymers include: xyloglucan,glucomannan, galactomannans etc

• Hemicellulases degrade hemicellulose
• Depending on the monomer released after degradation they are called

- Xylanase
- Glucanase
- Galactanase
- Arabinase
- Mannase

Fungal pathogens produce enzymes and oxidative agents (such as activated oxygen and
other radicals) to degrade hemicellulose
Eg.
– *Sclerotium rolfsii*
– *Sclerotinia sclerotiorum*
– *Diploidia viticola*

Enzyme Degrading Lignin

Found in middle lamella, secondary cell wall of xylem vessels and the fibers that strengthen
the plants. Only a small group of microorganism is capable of degrading lignin-more resistant to enzymatic degradation than other plant substances
• Ligninase degrade lignin

• Brown rot fungi degrade lignin but not utilise it
• White rot fungi degrade and utilise lignin
• Wood rotting basidiomycetes, several ascomycetes,imperfect fungi and some bacteria produce small amount of lignin degrading enzymes and cause soft rot cavities in wood they colonise
• Eg.
– *Ceratocystis paradoxa* (stem bleeding in coconut)
– *Polyporus versicolor*
–*Fusarium spp*
– *Phellinus spp*

TOXINS IN PATHOGENESIS

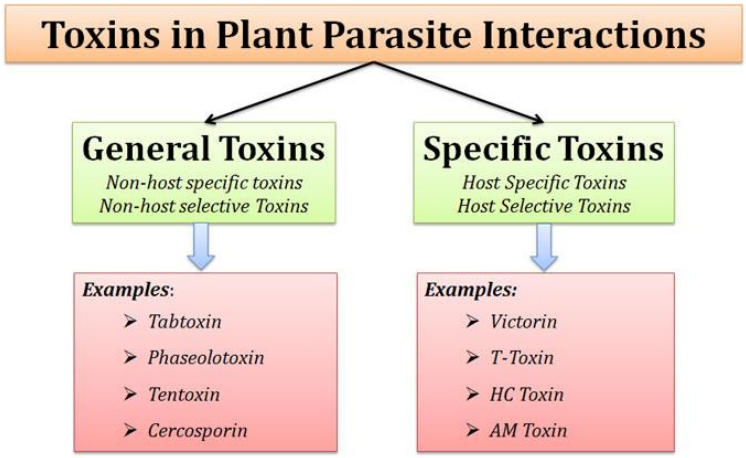
Microorganisms are pathogenic only if they are toxicogenic: in other words, the agents responsible for diseases can damage their hosts only if they form toxins-microbial poisons that penetrate into host tissue.

Pathogenesis refers to the origin, development and resultant effects of a disease, from the initial appearance of disease all the way to its end stages. The pathogenesis can also describe the origin and development of the disease and whether it is acute, chronic or recurrent.

Toxin hypothesis

1. A toxin should produce all symptoms characteristic of the disease.
2. Sensitivity to toxin will be correlated with susceptibility to pathogen.
3. Toxin production by the pathogen will be directly related to its ability to cause disease**.**

Classification of toxins



Source of Origin

- **Phytotoxin-** Phytotoxins are substances that are poisonous or toxic to the growth of plants. ... Most examples pertaining to this definition of phytotoxin are members of various classes of secondary metabolites, including alkaloids, terpenes, and especially phenolics, though not all such compounds are toxic or serve defensive purposes. E.g **Alternaric acid and lycomarasmin**
- **Vivotoxin-** Vivotoxin is a substance produced in the infected plant by the pathogen and or its host which functions in production of disease **E.g Fusaric acid and piricularin**
- **Pathotoxin-** A chemical of biological origin, other than an enzyme, that plays an important causal role in a plant disease. ... Some pathogen-produced pathotoxins are highly selective in that they cause severe damage and typical disease symptoms only on plants susceptible to the pathogens that produce them. **E.g Tabtoxin**

Based On Producing Organisms

Toxins are classified based on organism as follows

Fungal toxins:

- **Mycotoxin –** Aflotoxin, fumonisin
- **Mushroom toxin-***Amanitia muscaria, A. phallaoid*

Bacterial toxin

- **Syringomycin-***Pseudomonas syringae pv.*
- **Phaseolotoxin-** *Pseudomonas phaseolicola*

FUNGAL DISEASES OF PLANTS

Fungi and FLOs are eukaryotic organisms that lack chlorophyll and thus do not have the ability to photosynthesize their own food. They obtain nutrients by absorption through tiny thread-like filaments called hyphae that branch in all directions throughout a substrate Mycelia are the key diagnostic sign associated with diseases caused by fungi and FLOs.

Fungi and FLOs (indeed all pathogens) can be grouped into the following four categories based on their preference for surviving on dead or decaying organic matter versus living tissue:

1. **Obligate saprophytes**—always a saprophyte. These organisms can only survive or are obliged to gain nourishment by colonizing dead or decaying organic matter. They are not parasites.
2. **Obligate parasite**s—always a parasite. Can only grow as a parasite on or in a living host.
3. **Facultative parasites**—usually survive as a saprophyte but have the ability to parasitize and cause disease under certain conditions. Examples include *Pythium* species and many bacterial pathogens.
4. **Facultative saprophytes**—usually survive as a parasite but have the ability to live on dead and decaying organic matter under the right conditions. Examples include *Phytophthora and Botrytis* species.

RUST OF WHEAT

Wheat leaf rust is a common fungal disease in wheat, rye and barley. This disease affects, the stems, leaves and grains of these plants. The pathogen that causes the wheat leaf rust is the Puccinia rust fungus

Leaf rust, caused by ***Puccinia triticina***, is the most common rust disease of wheat. The fungus is an obligate parasite capable of producing infectious urediniospores as long as infected leaf tissue remains alive.

Stem rust (also known as black stem rust) is caused by ***Puccinia graminis*** .It is primarily a disease on wheat, though it can also cause minor infections on certain cultivars of barley and rye.

Mode of Transmission

Leaf rust spores are spread by wind and splashing water. Optimal environmental conditions for development of infection are temperatures ranging from 59 to 68 degrees F and at least six hours of moisture on the leaf surface.

Symptoms- dusty, reddish-orange to reddish-brown fruiting bodies that appear on the leaf surface

Treatment

- Select rust-resistant plant varieties when available.
- Pick off and destroy infected leaves and frequently rake under plants to remove all fallen debris.
- Water in the early morning hours — avoiding overhead sprinklers — to give plants time to dry out during the day.

RUST OF LINSEED

Rust of linseed is a common disease of flax growing areas of the world. *Melampsora lini* is a species of fungus and plant pathogen found in Ireland and commonly known as flax rust. The disease causes disastrous effects on the plants. Once the disease sets in a field, most of the linseed fields in the locality get affected within no time. The disease generally appears in epidemic form.

Rust of linseed is a common disease of flax growing areas of the world. In India flax is a major oilseed crop cultivated in almost all the states. The crop is generally cultivated during October-April. The disease generally appears in February or later but Butler (1918) has observed the disease in central India in early November. At the time of crop harvesting the affected plants get a fired appearance due to the presence of telial sori.

The disease causes disastrous effects on the plants. Once the disease sets in a field, most of the linseed fields in the locality get affected within no time. The disease generally appears in epidemic form.Severely infected plants get mostly defoliated due to which starch formation is also reduced.

Symptoms of the Rust of Linseed:

The leaves are the first to show the symptoms and gradually all the aerial parts of the plant get infected. Large, orange coloured pustules generally appear on the leaves. Small pustules are initially surrounded by chlorotic areas. Little necrosis of the leaves is at first observed but it grows, becomes more general and the leaves prematurely die.

The pustules on the leaves are uredopustules containing uredospores. Uredopustules may also appear on stems. While the uredopustules on leaves are round and small, those on stems are elongated and irregular.

Treatment and control

- Use disease resistant var NP(RR)
- Seed treatment to kill Seed treatment to kill the teleutospores in hills has also been suggested.
- Avoidance of excessive nitrogenous manures is recommended

LATE BLIGHT OF POTATO

Late blight is caused by the fungus like oomycete pathogen *Phytophthora infestans*. This potentially devastating disease can infect potato foliage and tubers at any stage of crop development.

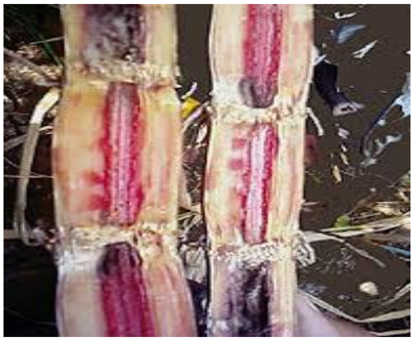
Symptoms

The first symptoms of late blight in the field are small, light to dark green, circular to irregular-shaped water-soaked spots (Figure 1). These lesions usually appear first on the lower leaves. Lesions often begin to develop near the leaf tips or edges, where dew is retained the longest. Late blight, also called potato blight, disease of potato and tomato plants that is caused by the water mold Phytophthora infestans. The disease occurs in humid regions with temperatures ranging between 4 and 29 °C (40 and 80 °F)

Treatment and Control

- Use potato tubers for seed from disease-free areas to ensure that the pathogen is not carried through seed tuber.
- The infected plant material in the field should be properly destroyed.
- Grow resistant varieties like Kufri Navtal**.**
- Fungicidal sprays on the appearance of initial symptoms

RED ROT OF SUGARCANE



Red rot is a very serious disease of sugarcane. ... The symptom of the disease is the reddening of the internal internodal tissues with crossbars of white patches in the reddened area. This red colour is caused by a dye which is secreted by the host and is antagonistic to the red rot fungus.

Symptoms

- Stop & sugar formation start
- Third leaf from top start - yellowing
- Later infected cane – shriveled
- Rind shrink
- Cane light in weight & easily broken
- Reddening of pith with clear area (white) running across the width of the cane

Causative organism

Causal Organism :- *Colletrotrichum falcatum*
Perfect stage:- *Glomerella tucumanensis*
The fungi causing the disease occur in two stages.

- Asexual stage, which is called anamorph or imperfect stage .
- Perfect stage :-which is called teleomorph or the sexual stage.

Treatment and control

- Use Always Disease-free seed treatment – Carbendazim @ 2.5 gm/lit. of water for 30 minutes
- Hot water treatment – 52° C for 8 hrs or 54° C for 2 hrs
- Hot air treatment - 54° C for 6 hrs
- Removal of infected stools
- Crop rotation 2-3 yrs
- Use disease Resistant. Varieties.- Cos -767

BACTERIAL DISEASES OF PLANT

Plant pathogenic bacteria cause many different kinds of symptoms that include galls and overgrowths, wilts, leaf spots, specks and blights, soft rots, as well as scabs and cankers. In contrast to viruses, which are inside host cells, walled bacteria grow in the spaces between cells and do not invade them.

CITRUS CANKER

Citrus canker it is a disease affecting Citrus species caused by the bacterium *Xanthomonas axonopodis*. Infection causes lesions on the leaves, stems, and fruit of citrus trees, including lime, oranges, and grapefruit.

Causative organism

Citrus canker is caused by ***Xanthomonas citri***.

It is a gram negative, monotrichous, capsulated bacillus which is strictly aerobic in nature.

It is 1.5-3 micrometer in length and 0.5-1.5 microm eter in breadth.The bacteria does not produce endospores and can not reduce nitrates.

Symptoms

Citrus canker causes premature leaf and fruit drop, twig dieback, general decline, and blemished fruit Blister-like lesions on leaves and fruit start small and expand as the disease progresses. These lesions may darken to tan or black and develop a water-soaked margin with a yellow halo surrounding it.

The center of the lesion on leaves as well as on stems and twigs can appear raised and corky or scabby surrounded by a water-soaked margin.

Mature lesions on older symptomatic leaves may have a shot-hole look (Fig. 2) and these lesions eventually die and fall out.

Treatment and control

1. Complete destruction of diseased plant by burning them.
2. Use of disease free nursery stock for planting.
3. Spraying the plant before planting with 1% Bordeaux mixture.
4. Antibiotic spray with streptomycin, phytomycin.
5. Spraying leaves with neem-cake (160 lbs/acre).
6. Disease resistant varieties.

BLIGHT OF RICE

Causative organism

Rice bacterial blight, also called bacterial blight of rice. It is a deadly bacterial disease that is among the most destructive afflictions of cultivated rice (*Oryza sativa* and *O. glaberrima*).Major rice cultivating countries are: China, India, Indonesia, Bangladesh,Vietnam, Thailand,Myanmar and Philippines.

Xanthomonas oryzae pv. Oryzae is the Causative organism and it causes wilting of seedlings, yellowing and drying of leaves.

Transmission:

- The disease usually occurs during the early stage of planting from maximum tillering to panicle initiation
- Older plants are more resistant to the disease.
- The disease is transmitted through seeds
- Infection occur through wounds and stomata
- Infected seeds present in the water or those surviving in the debris left after harvest, are also sources of inoculums in the next planting season.
- The disease also disperse and spread by wind and rain water.
- High dose of nitrogenous fertilizer, excessive shade and close planting favour the disease

Symptoms

- On seedling, the infection appears tiny water soaked spot at the margin on the leaves.
- On leaf blade, the infection begins at the margin as water socked stripe.
- Resulting in the wavy margin and yellow leaf within few days.
- The lesion can cover the whole blade, turn white and later grayish contaminated with various saprophytic fungi.
- A turbid ooze of the bacterium, streaming from the vascular bundle can be observed on dipping the cut end of affected leaves in clear water.
- Symptoms observed at the tillering stage.
- Seedling wilt can be observed in 1-3 weeks after transplanting.

Treatment and control

- Planting resistant varieties is efficient way to control bacterial blight.
- Use balanced amounts of plant nutrients, especially nitrogen.
- Ensure good drainage of fields and nurseries.
- Keep fields clean. Remove weed hosts and plow under rice stubble , straw which can serve as hosts of bacteria.
- Allow fallow fields to dry to suppress disease agents in the soil and plant residues.
- Seed treatment with bleaching powder (100g/ml) and zinc sulphate (2 per cent) reduce bacterial blight.
- Copper compounds and antibiotics.
- Application of streptocycline @.6% for spray

VIRAL DISEASES OF PLANTS

Plant viruses consist of a nucleoprotein that multiplies only in the living cells of a host. The presence of viruses in host cells often results in disease.400 or more viruses are known to attack plants .viruses are generally specific, what infects a plant does not cause disease in an animal, and vice versa. The first record of a disease that was later found to be caused by a plant virus was on tulips in the 17th century in the Netherlands. First experimental demonstration of the infectious nature of viral disease was recorded by Lawrence, who described the transmission of a disease of jasmine by grafting.

Properties of plant viruses

Noncellular, ultramicroscopic particles, that multiply only in living cells. very, very small! (size measured in nanometers). Most plant viruses consist of protein shells surrounded by a core of positive-stranded nucleic acid (normally ssRNA - nucleotides , but sometimes these viruses contain dsRNA or dsDNA. 5-40% of virus is nucleic acid 60-95% is protein. Protein coats or shells can be different shapes, but are normally rod, filamentous, isometric, quasi-isometric/bacilliform or variants of these structures.

Transmission

- Mechanical transmission through sap by plants touching one another
- Through root grafts, and manhandling.
- Vegetative propagation and grafting.
- Seed, pollen, mites, nematodes, dodder,
- Fungi (carried by zoospores and mycelium)
- Insects (aphids, leafhoppers, scale insects, thrips, grasshoppers, beetles, whiteflies).

LEAF CURL OF PAPAYA

Papaya leaf curl disease is caused by Papaya leaf curl virus (PaLCuV), a begomovirus naturally transmitted through whitefly (***Bemisia tabaci***). Main symptoms of papaya leaf curl disease are inward/outward curling of plant leaves, vein thickening, and stunted plant growth with small distorted fruits or no fruits. Papaya leaf curl was first reported in TamilNadu in 1939.

Causative organism- **Papaya leaf curl virus** (**PaLCuV**), is a DNA virus from the genus Begomovirus and the family Geminiviridae. PaLCuV causes severe disease in papaya (*Carica papaya*), but can sometimes infect other crops such as tobacco or tomato

Symptom

- The most prominent symptoms are the rolling of the leaves downward and inward in the form of an inverted cup and the thickening of veins.
- Sometimes all the leaves at the top of the plant are affected by these symptoms.
- In advanced stages of the disease, defoliation takes place and the growth of the plant is arrested.
- The production of flower and fruits are compromised.
- Fruits will be detorated in shape and fall immaturely.

Transmission

- Vector**:** Silver leaf whitefly **(Bemisia tabaci).**

Treatment and control

- Immediately after the disease is noticed, the plant must be uprooted and destroyed.
- Avoid growing alternative hosts such as tomato and tobacco near papaya.
- Spray insecticides for vector control.

VEIN CLEARING OF LADY'S FINGER

This is a viral disease occurring on bhendi (Okra/Lady's Finger). Yellowing of the entire network of veins in the leaf blade is the characteristic symptom. ... The veins of the leaves will be cleared by the virus and interveinal area becomes completely yellow or white. The veins become considerably thickened.

Causative organism

This disease is caused by a complex consisting of the monopartite Begomovirus **Bhendi yellow vein mosaic virus** (BYVMV, family: Geminiviridae) and a small satellite DNA beta component. BYVMV can systemically infect bhendi upon agroinoculation but produces only mild leaf curling in this host. Bhendi yellow vein mosaic was first reported in okra plants in 1924 in India and Sri Lanka.

Symptom

- Vein clearing and vein chlorosis of leaves.
- The complex network of interwoven yellow, thickened veins and veinlets encloses

islands of green tissue.

- In severe cases, the chlorosis may extend to the interveinal area and leaves may turn completely yellow.
- Fruits are dwarfed, malformed and yellow-green.

Transmission

The virus is transmitted by the whitely ***Bemisia tabaci***

Treatment and control

- Use resistant cultivar
- Sow disease free certified seeds
- Follow crop rotation
- Keep the field free from weeds
- Control vector with suitable insecticide

SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – IV–Soil & Agricultural Microbiology– SMB2201

Microorganisms in the Decomposition of Organic Matter

Organic matter-is a matter composed of organic compounds that have come from the remains of organisms such as plants and animals and their waste products in the environment. Organic molecules can also be made by chemical reactions that don't involve life. Such wastes can be produced by households, industry and agriculture.

Organic matter is broken down into carbon dioxide and the mineral forms of nutrients like nitrogen. It is also converted into fungi and bacteria through these organisms feeding on the organic material and reproducing.

Most soil organic matter originates from plant tissue. Plant residues contain 60-90 percent moisture. The remaining dry matter consists of **carbon (C), oxygen, hydrogen (H) and small amounts of sulphur (S), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg)**. Although present in small amounts, these nutrients are very important from the soil fertility.

Organic molecules are released directly from cells of fresh residues, such as **proteins, amino acids, sugars, and starches.** This part of soil organic matter is the active, or easily decomposed, fraction. This active fraction is influenced strongly by weather conditions, moisture status of the soil, growth stage of the vegetation, addition of organic residues, and cultural practices, such as tillage.

Carbohydrates occur in the soil in three main forms: free sugars in the soil solution, cellulose and hemicellulose; complex polysaccharides; and polymeric molecules of various sizes and shapes that are attached strongly to clay colloids and humic substances. The **simple sugars, cellulose and hemicellulose**, may constitute **5-25 percent of the organic matter** in most soils, but are easily broken down by micro-organisms.

Soil life plays a major role in many natural processes that determine nutrient and water availability for agricultural productivity. The primary activities of all living organisms are growing and reproducing. By-products from growing roots and plant residues feed soil organisms. In turn, soil organisms support plant health as they decompose organic matter, cycle nutrients, enhance soil structure and control the populations of soil organisms, both beneficial and harmful (pests and pathogens) in terms of crop productivity. Decompose organic matter towards humus.

The living part of soil organic matter includes a wide **variety of micro-organisms such as bacteria, viruses, fungi, protozoa and algae**. It also includes plant roots, insects, earthworms, and larger animals such as moles, mice and rabbits that spend part of their life in the soil. The living portion represents about 5 percent of the total soil organic matter. Micro-organisms, earthworms and insects help break down crop residues and manures by ingesting them and mixing them with the minerals in the soil, and in the process recycling energy and plant nutrients.

Classification of soil organisms involved in decomposition

Micro-organisms	Microflora	<5 µm	Bacteria Fungi
	Microfauna	<100 µm	Protozoa Nematodes
Macro-organisms	Meso-organisms	100 µm - 2 mm	Springtails Mites
	Macro-organisms	2 - 20 mm	Earthworms Millipedes Woodlice Snails and slugs
Plants	Algae	10 µm	
	Roots	> 10 µm	

Decomposition of Organic Matter by Microorganisms:

The organic carbon compounds that eventually are deposited in the soil are degraded by the activities of microorganisms which are mainly the bacteria and fungi. The CO2 is released into the air and soil.

(i) **Cellulose Decomposition**:

Cellulose is the most abundant organic material in plants. It is readily attacked by many species of fungi and bacteria.The fungi which decompose cellulose in soil are mainly Trichoderma, Aspergillus, Penicillium, Fusarium, Chaetomium, Verticillium. Rhizoctonia, Myrothecium, Merulius, Pleurotus, Fomes, etc.

The bacteria that bring about cellulose decomposition in soil consist mainly of the species of Clostridium, Cellulomonas, Streptomyces, Cytophaga, Bacillus, Pseudomonas, Nocardia, Micromonospora, Sporocytophaga, Polyangium, Cellfalcicula, etc.

(ii) **Hemicellulose Decomposition**:

Hemicelluloses are the polymers of simple sugars such as pentoses, hexoses and uronic acid. The decomposition of hemicelluloses by microorganisms takes place through the agency of extracellular enzymes called hemicellulases.

The fungi that degrade hemicelluloses in soil are *Chaetomium, Aspergillus, Penicillium, Trichoderma, Fusarium, Humicola, etc.* Bacteria that degrade hemicelluloses in soil *Bacillus, Pseudomonas, Cytophaga, Vibrio, Erwinia, Streptomyces, Actinomyces*.

(iii) **Lignin Decomposition**:

Lignin is the third most abundant constituent of the plants. It is highly resistant of microbial degradation. Fungi (*Aspergillus, Penicillium, Fusarium, Lenzites, Clavaria, Polyporus,* etc.) and bacteria (*Streptomyces, Nocardia, Flavabacterium, Xanthomonas, Pseudomonas, Micrococcus,* etc.) are known to degrade lignin at slow rates.

Significance of microbial decomposition of OM

- Retain N and other nutrients.
- Glue soil particles together for best structure.
- Protect roots from diseases and parasites.
- Make retained nutrients available to the plant.
- Produce hormones that help plants grow.
- Retain water.

CARBON CYCLE

Carbon is the most important element in the biological system and constitutes about 50% of all living organisms. Carbon dioxide present in the atmosphere or dissolved in water is the ultimate source of organic carbon compounds occurring in nature.

The cycle of carbon in nature comprises of two main processes**:**

1) **The conversion of oxidized form of carbon into reduced organic form** by photosynthetic organisms, CO_2 is reduced into organic carbon compounds mainly by the process of photosynthesis. Photosynthetic algae and higher plants are the most important agents of carbon dioxide fixation. In the ocean the major plant forms that fix carbon are the free floating microscopic algae called phytoplanktons

i) The carbon dioxide represents the sole source of carbon for autotrophic bacteria.

The latter fix CO_2 to carbohydrates by a reduction reaction.

$CO_2 + 2H_2 \rightarrow (CH_2O)_x + H_2O$

(ii) Heterotrophic bacteria fix carbon dioxide commonly.

$$\underset{\text{(Pyruvic acid)}}{CH_3COCOOH} + CO_2 \longrightarrow \underset{\text{(oxaloacetic acid)}}{HOOCCH_2.COCOOH}$$

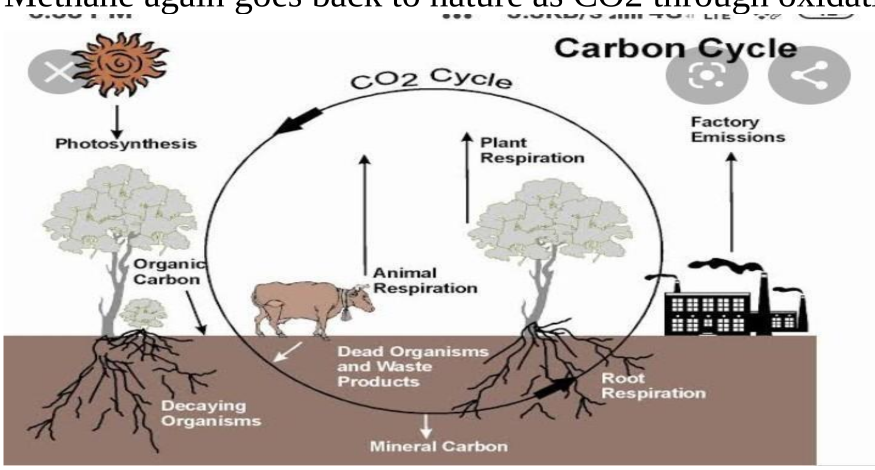
(2) **Restoration of original oxidized form through mineralization of the organic form by the micro-organisms.**

There are three different modes through which the organic matter is mineralizes and the CO_2 is released in the atmosphere

- Process of respiration,
- Accidental (forest fire) and intentional (fuel) burning.
- Decomposition of organic matter by microorganism

The process of respiration in plants and animals, and the accidental and intentional burning of plants and their parts result in the breakdown of organic carbon compounds releasing carbon dioxide in the atmosphere.

The decomposition of organic matters takes place by both aerobic and anaerobic condition. During aerobic degradation, 60-90% of carbon of organic residue is utilised as a source of energy, releasing CO2 in atmosphere. But in anaerobic condition, about 95-98% carbon is released as methane (CH4). Methane again goes back to nature as CO2 through oxidation.



The organic carbon compounds that eventually are deposited in the soil are degraded by the activities of microorganisms which are mainly the bacteria and fungi. The CO2 is released into the air and soil.

Decomposition of organic matter by microorganisms.

The process of respiration in plants and animals, and the accidental and intentional burning of plants and their parts result in the breakdown of organic carbon compounds releasing carbon dioxide in the atmosphere.The organic carbon compounds that eventually are deposited in the soil are degraded by the activities of microorganisms which are mainly the bacteria and fungi..

(i) Cellulose Decomposition**:** Cellulose is the most abundant organic material in plants. It is readily attacked by many species of fungi and bacteria**.**The process of cellulose decomposition to carbon dioxide can be summarized in the form of following reaction:

$$(i)\ \text{Cellulose} \xrightarrow[\text{cellulase}]{\text{enzyme}} \text{Cellobiose}$$

$$(ii)\ \text{Cellobiose} \xrightarrow[\beta\text{-glucosidase}]{\text{enzyme}} \text{Glucose}$$

$$(iii)\ \text{Glucose} \xrightarrow[\text{of many microbes}]{\text{enzyme system}} CO_2 + H_2O + \text{and/or other end products.}$$

The fungi which decompose cellulose in soil are mainly *Trichoderma*, *Aspergillus, Penicillium, Fusarium, Chaetomium, Verticillium. Rhizoctonia, Myrothecium, Merulius, Pleurotus*

The bacteria which decompose cellulose in soil are *Clostridium, Cellulomonas, Streptomyces, Cytophaga, Bacillus, Pseudomonas, Nocardia, Micromonospora, Sporocytophaga, Polyangium, Cellfalcicula*

(ii) Hemicellulose Decomposition**:** they are the polymers of simple sugars such as pentoses, hexoses and uronic acid. The decomposition of hemicelluloses by microorganisms takes place through the agency of extracellular enzymes called hemicellulases. The fungi that degrade hemicelluloses in soil *Chaetomium, Aspergillus, Penicillium, Trichoderma, Fusarium, Humicola* and bacteria which degrades hemicelluloses are *Bacillus, Pseudomonas, Cytophaga, Vibrio, Erwinia, Streptomyces, Actinomyces*

(iii) Lignin Decomposition: Lignin is the third most abundant constituent of the plants. It is highly resistant of microbial degradation. The fungi which decompose lignin *Aspergillus, Penicillium, Fusarium, Lenzites, Clavaria, Polyporus* and bacteria which decompose lignin are *Streptomyces, Nocardia, Flavabacterium, Xanthomonas, Pseudomonas, Micrococcus*.

NITROGEN CYCLE

Nitrogen is the fourth most prevalent element in living systems. It is a constituent of a number of organic compounds like amino acids, proteins, nucleotides, nucleic acid, hormones, chlorophyll, many vitamins. nitrogen is the most critical element. A regular supply of nitrogen to the plants is maintained through nitrogen cycle. Nitrogen cycle is regular circulation of nitrogen amongst living organisms, reservoir pool in the atmosphere and cycling pool in the lithosphere. Nitrogen compounds are obtained from reservoir pool through nitrogen fixation.

Nitrogen is replenished through the process of de-nitrification of nitrates and release of nitrogen from decaying organic matter. Cycling pool is augmented by ammonification and nitrification. Plants obtain nitrogen from soil as $NO_3^–$ (nitrate), NH_4^+ (ammonium) and $NO_2^–$ (nitrite) ions. Nitrate and nitrite are reduced to ammonium state which is then incorporated into amino acids, proteins and other organic substances.

There are two methods of nitrogen fixation—

- Abiological – industrial and natural
- Biological – symbiotic and freeliving bacteria

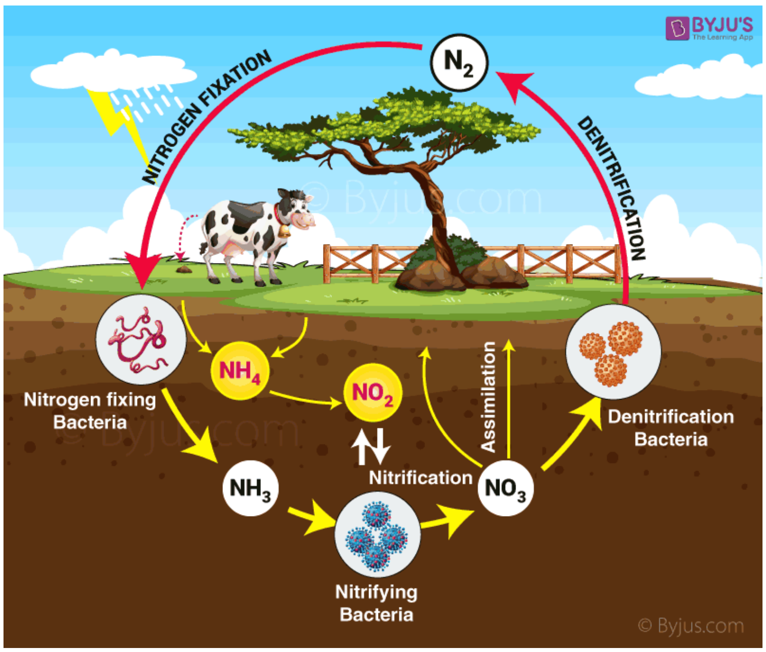
Natural abiological nitrogen Fixation: Atmospheric nitrogen combines with oxygen in the presence of electric discharges, ozonization and combustion. Different types of nitrogen oxides are produced. The nitrogen oxides dissolve in water and give rise to hyponitrous, nitrous and nitric acids. They enter soil along with rain water forming hyponitrites, nitrites and nitrates.

$$N_2 + O_2 \xrightarrow[\text{Discharges}]{\text{Electric}} \underset{\text{Nitrogen oxide}}{2NO}$$

$$2NO + 2[O] \xrightarrow[\text{Ozonization}]{\text{Electric Discharges}} \underset{\text{Nitrogen peroxide}}{2NO_2}$$

$$2NO + 3[O] \xrightarrow{\text{Ozonisation}} N_2O_5$$

Industrial abiological nitrogen fixation: Ammonia is produced industrially by direct combination of nitrogen with hydrogen (got from water) at high temperature and pressure. It is changed to various types of fertilizers including urea.



Biological Nitrogen Fixation:

It is the second most important natural process and the major source of nitrogen fixation which is performed by two types of prokaryotes, bacteria and cyanobacteria (= blue green algae).

They include both free living and symbiotic forms:

(a) Free Living Nitrogen Fixing Bacteria:

- Aerobic bacteria-***Azotobacter, Beijerinckia***
- Anaerobic bacteria- ***Bacillus, Klebsiella, Clostridium*** and saprotrophic bacteria that perform nitrogen fixation.
- **Desulphovibrio** is chemotrophic nitrogen fixing bacterium.
- ***Rhodopseudomonas, Rhodospirillum and Chromatium*** are nitrogen fixing anaerobic photoautotrophic bacteria.

- Free living nitrogen fixing bacteria add 10-25 kg of nitrogen/ha/annum.

(b) Free Living Nitrogen Fixing Cyanobacteria:

Many free living blue- green algae (BGA) or cyanobacteria perform nitrogen fixation.

They add 20-30 kg of nitrogen per hectare of soil and water bodies

E.g Anabaena, Nostoc, Calothrix, Lyngbia, Aulosira, Cylindrospermum, Trichodesmium.

Cyanobacteria are also important ecologically as they occur in water-logged soils where denitrifying bacteria can be active. *Aulosira fertilissima* is the most active nitrogen fixer in Rice fields while *Cylindrospermum* is active in Sugarcane and Maize fields.

(c) Symbiotic Nitrogen Fixing Cyanobacteria:

Anabaena and Nostoc species are common symbionts in lichens Anthoceros, Azolla and Cycad roots.

Azolla pinnata (a water fern) has *Anabaena azollae* in its ponds. It is often inoculated to rice fields for nitrogen fixation.

(d) Symbiotic Nitrogen Fixing Bacteria:

Rhizobium is nitrogen fixing bacterial symbiont of papilionaceous roots. Sesbania rostrata has Rhizobium in root nodules and Aerorhizobium in stem nodules.

Frankia is symbiont in root nodules of several nonlegume plants like Casuarina (Australian Pine), Myrica and Alnus (Alder).

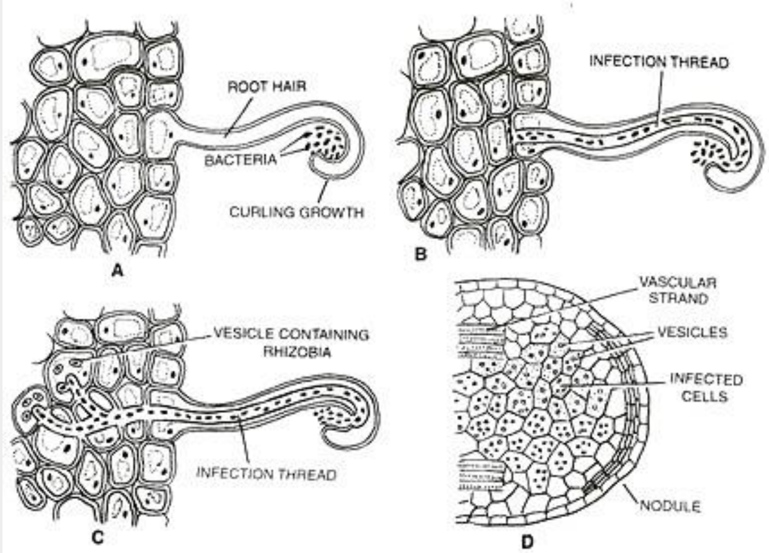
Xanthomonas and Mycobacterium form symbiotic association with the leaves of several members of rubiaceae and myrsinaceae (e.g., Ardisia).

Both Rhizobium and Frankia live free as aerobes in the soil but are unable to fix nitrogen. They develop the ability to fix nitrogen only as a symbiont when they become anaerobic. Rhizobium is rod-shaped bacterium while Frankia is an actinomycete.

Root nodule formation of Rhizobium

Rhizobium is the most important for crop lands because it is associated with pulses and other legumes of family fabaceae, e.g., Chick Pea or Gram (Cicer arietinum), Pigeon Pea or Red Gram (Cajanus cajan), Garden or Edible Pea (Pisum sativum), Soya bean (Glycine max), Lentil (Lens culinaris), Green Gram (Vigna radiata = Phaseolus aureus), Black Gram (Vigna or Phaseolus mungo), Sweet Clover, Sweet Pea, Alfalfa, Broad Bean, Clover Bean. Several species of the bacterium (e.g., Rhizobium leguminosarum, R. meliloti) live in the soil.

They are unable to fix nitrogen by themselves. Roots of a legume secrete chemical attractants (flavonoids and betaines). Bacteria collect over the root hairs, release nod factors that cause curling of root hairs around the bacteria, degradation of cell wall and formation of an infection thread enclosing the bacteria.



1. Fig. 12.11. Nodule formation in the root of a legume.

Infection thread grows along with multiplication of bacteria. It branches and its ends come to lie opposite protoxylem points of vascular strand. The infected cortical cells dedifferentiate and start dividing. It produces swellings or nodules. Nodule formation is stimulated by auxin produced by cortical cells and cytokinin liberated by invading bacteria. The infected cells enlarge. Bacteria stop dividing and form irregular polyhedral structures called bacteriods (Fig. 12.12). However, some bacteria retain normal structure, divide and invade new areas. In an infected cell bacteriods occur in groups surrounded by host membrane.

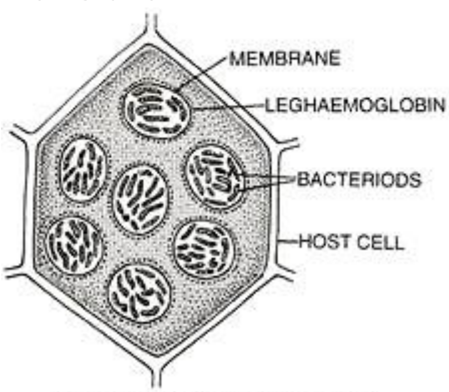


Fig. 12.12. Bacteriods in a nodule.

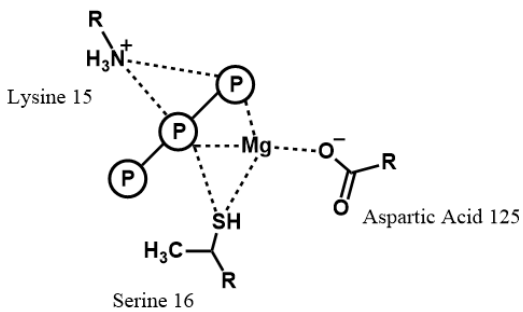
The host cell develops a **pinkish pigment called leg-haemoglobin (Lb**). It is oxygen scavenger and is related to blood pigment haemoglobin. It protects nitrogen fixing enzyme nitrogenase from oxygen. Symbiotic nitrogen fixation requires cooperation of **Nod genes of legume, nod, nif and fix gene clusters of bacteria**.

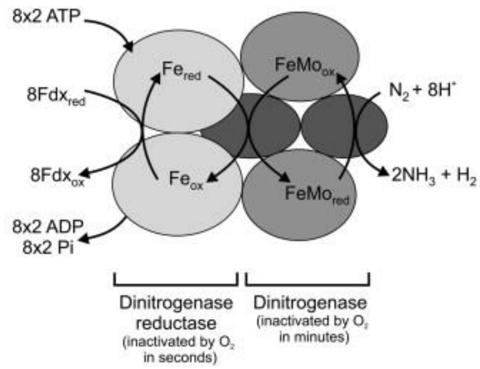
Mechanism of Nitrogen Fixation:

Nitrogen fixation requires (i) a reducing power like NADPH, FMNH2 (ii) a source of energy like ATP (iii) enzyme di-nitrogenase and (iv) compounds for trapping ammonia formed by the reduction of di-nitrogen. Enzyme nitrogenase (See the figure below)has iron and molybdenum. Both of them take part in attachment of a molecule of nitrogen (N2).

Bonds between the two atoms of nitrogen become weakened by their attachment to the metallic components. The weakened molecule of nitrogen is acted upon by hydrogen (Fig. 12.13) from a reduced coenzyme. It produces dimide (N_2H_2), hydrazine (N_2H_4) and then ammo-nia ($2NH_3$)

Ammonia is not liberated. It is toxic in even small quantities. The nitrogen fixers protect themselves from it by providing organic acids. The reaction between ammonia and organic acids gives rise to amino acids.





N5 + 8e– 8H++16ATP- di-nitrogenase → 2NH3 + 2H++ 16ADP + l6Pi

Ammonia + α-ketoglutarate + NAD(P)H- dehydrogenase → Glutamate + NAD(P)+ + H2O

Symbiotic nitrogen fixing organisms hand over a part of their fixed nitrogen to the host in return for shelter and food. Free living nitrogen fixers do not immediately enrich the soil. It is only after their death that the fixed nitrogen enters the cycling pool. It occurs in two steps, **ammonification and nitrification**.

Ammonification:

It is carried out by decay causing organisms. They act upon nitrogenous excretions and proteins of dead bodies of living organisms, e.g., *Bacillus* *ramosus, B. vulgaris, B. mesentericus, Actinomyces*. Proteins are first broken up into amino acids. The latter are deaminated. Organic acids released in the process are used by microorganisms for their own metabolism

$$\text{Proteins} \xrightarrow{+H_2O} \underset{\text{Amino acid}}{R-NH_2} \xrightarrow{H_2O} \underset{\text{Organic acid}}{ROH} + \underset{\text{Ammonia}}{NH_3}$$

Nitrification:

It is the phenomenon of conversion of ammonium nitrogen to nitrate nitrogen. It is performed in two steps as below

(a) Nitrite formation- ammonium ions are oxidised to nitrites by *Nitrosococcus, Nitrosomonas*

(b) Nitrate formation- Nitrites are changed to nitrates by *Nitrocystis, Nitrobacter*.

(c) Both the steps can be carried out by *Aspergillus flavus*.

$$2NH_3 + 3O_2 \xrightarrow[\textit{Aspergillus flavus}]{\textit{Nitrosococcus, Nitrosomonas}} 2\,NO_2^- + 2H^+ + 2H_2O + \text{energy}$$

Most of the bacteria performing nitrification are chemoautotrophs. They use the energy liberated during nitrification in synthesis of organic substances from CO_2 and a hydrogen donor. They are thus autotrophs which do not use solar energy for synthesis of food.

De-nitrification:

In this process, nitrates are reduced to gaseous compounds of nitrogen. The latter escape from the soil. Common bacteria causing de-nitrification of soil are *Pseudomonas denitrificans*, *Thiobacillus denitrificans*, *Micrococcus denitrificans*.

$$2\,NO_3' \longrightarrow 2\,NO_2' \longrightarrow 2NO \longrightarrow N_2O \longrightarrow N_2$$

Nitrogen oxides escaping into atmosphere or formed during abiological fixation can also be broken down by radiations to form molecular nitrogen. De-nitrification of soil not only depletes the soil of an important nutrient but also causes acidification which is equally harmful in solubilisation of harmful metals.

Nitrate Assimilation:

Nitrate is the most important source of nitrogen to the plants. It can accumulate in the cell sap of several plants and take part in producing osmotic potential. However it cannot be used as such by the plants. It is first reduced to level of ammonia before being incorporated into organic compounds. Reduction of nitrate occurs in two steps.

(i) Reduction of Nitrate to Nitrite:

It is carried out by the agency of an inducible enzyme called nitrate reductase.

The enzyme is a molybdoflavoprotein. It requires a re-duced coenzyme (NADH or NADPH) for its activity. The reduced coenzyme is brought in contact with nitrate by FAD or FMN.

$$NO_3 + NAD(P)H + H^+ \xrightarrow[FAD/FMN]{\text{Nitrate Reductase}} NO_2^- + H_2O + NADP^+$$

(ii) **Reduction of Nitrite**:

It is performed by enzyme nitrite reductase. The enzyme is a metalloflavoprotein which contains copper and iron. It occurs inside chloroplasts in the leaf cells and leucoplasts of other cells. In contrast nitrate reductase is found attached loosely to cell membrane. Nitrite reductase requires reducing power.

It is NADPH in illuminated cells and NADH in others. The process of reduction also requires ferredoxin which occurs in higher plants mostly in green tissues. Therefore, it is presumed that in higher plants either nitrite is trans-located to leaf cells or some other electron donor (like FAD) operates in un-illuminated cells. The product of nitrite reduction is ammonia.

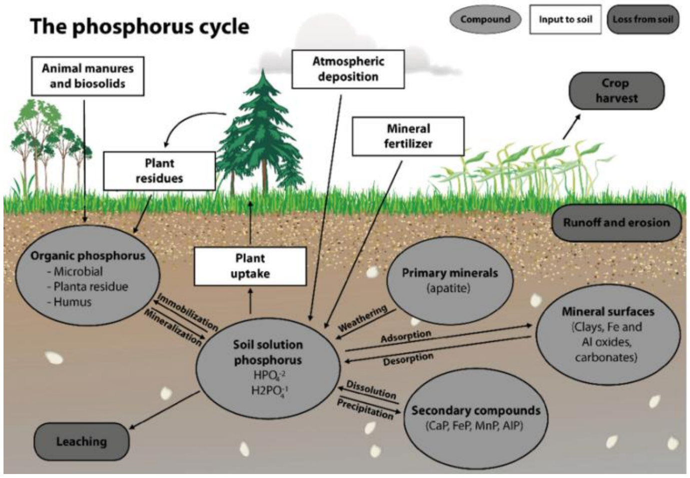
$$2\,NO_2^- + 7NAD(P)H + 7H^+ \xrightarrow[\text{Ferredoxin}]{\text{Nitrite reductase}} 2NH_3 + 4H_2O + 7NAD(P)^+$$

Ammonia is not liberated. It combines with some organic acids to produce amino acids. Amino acids then form various types of nitrogenous compounds.

PHOSPHORUS CYCLE

Phosphorus is one of the most important constituent of several important compounds always present in organisms. It occurs both in organic (nucleic acids, nucleoproteins, phospholipids, etc.) and inorganic (phosphate) forms in the living organisms. Animals possessing bones have large amount of phosphorus in its inorganic form.

Phosphorus is also added to soil through chemical fertilizers, excrete and organism- residues. Though there is plenty of phosphorus present in the soil in unavailable inorganic forms, most of the plants obtain it only as orthrophosphate ions (soluble inorganic forms). The symbiotic relationship of fungi (mycorrhizal association) helps the plants in obtaining phosphorus.



1. **Mineralization:** Conversion of organic phosphorus into insoluble inorganic phosphates**:**

Many soil microorganisms produce enzymes that attack many of the organic phosphorus compounds in the soil and release inorganic phosphate. This process is comparable to the mineralization of organic nitrogen compounds. The enzymes involved in these reactions are collectively called 'phosphatases' which have a broad range of substrate specificity**.**

2. **Solubilization:** Conversion of Insoluble Inorganic Phosphates into Soluble Inorganic Phosphates. The availability of phosphorus depends on the degree of solubilization by various organic and inorganic acids produced by microorganisms in soil.
3. Organisms involved produce substantial amounts of organic acids**:** The action of acids to convert insoluble phosphates into soluble ones is generally called 'solubilization' and particularly takes place in close proximity of the root surfaces where sugar from root-exudates are converted by the action of microorganisms into organic acids.

   Fungi- *Aspergillus*, *Penicillium*, *Fusarium* and **Bacteria-** *Bacillus, Pseudomonas*, *Micrococcus*, *Flavobacterium*

The overall conversion of insoluble inorganic phosphates into soluble inorganic phosphates by the action of acids can be exemplified via reactions as under:

(a.i) $$\underset{\text{(Insoluble calcium phosphate)}}{Ca_3(PO_4)_2} + \underset{\text{(Sulfuric acid)}}{H_2SO_4} \longrightarrow \underset{\text{(Calcium-monohydrogen phosphate fairly soluble in water)}}{Ca_2H_2(PO_4)_2} + CaSO_4$$

(ii) $$Ca_2H_2(PO_4)_2 + H_2SO_4 \longrightarrow \underset{\text{(Calcium-dihydrogen phosphate; highly soluble in water)}}{CaH_4(PO_4)_2} + CaSO_4$$

(b.i) $$\underset{\text{(Insoluble calcium phosphate)}}{Ca_3(PO_4)_2} + \underset{\text{(Nitric acid)}}{2HNO_3} \longrightarrow \underset{\text{(Calcium-monohydrogen phosphate; fairly soluble in water)}}{Ca_2H_2(PO_4)_2} + Ca(NO_3)_2$$

(ii) $$Ca_2H_2(PO_4)_2 + 2HNO_3 \longrightarrow \underset{\text{(Calcium-dihydrogen phosphate; highly soluble in water)}}{CaH_4(PO_4)_2} + Ca(NO_3)_2$$

BIOFERTILIZER

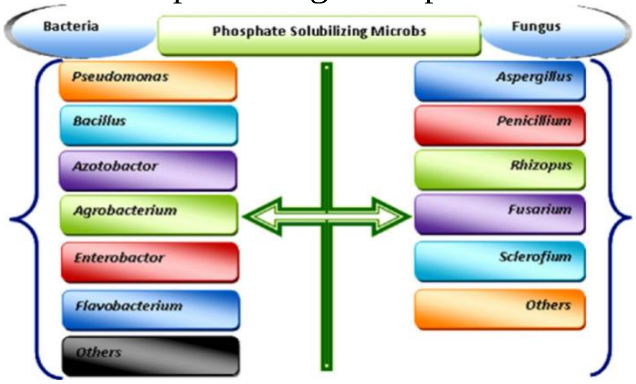
Biofertilizer is a type of fertilizer containing living microorganisms, that enrich the nutrient quality of the soil. The main sources of biofertilizers are bacteria, fungi, and cyanobacteria (blue-green algae). They form a symbiotic relationship with plants, in which the partners derive benefits from each other.
Biofertilizer are classified based on
1) For nitrogen -Rhizobium for legumes crops -Azotobacter/ Azospirilium for non legume crops -Acetobacter for sugarcane only. -Blue –Green Algae (BGA) and Azolla for low land paddy.
2) For phosphorus -Phosphate solubilizing Bacteria (PSB) for all crops to be applied with Rhizobium, Azotobacter, Azospirilium and Acetobacter
3) For enriched compost -Phosphate solubilizing Bacteria (PSB) and Azatobacter culture -Cellulolytic fungal culture

Biofertilizers in agriculture includes the following:
- Symbiotic nitrogen fixers, Rhizobium spp.;
- Non-symbiotic, free-living nitrogen fixers (Azotobacter, Azospirillum, etc.)
- Algal biofertilizers (blue-green algae or blue-green algae in association with Azolla)
- Phosphate-solubilising bacteria
- Mycorrhizae
- Organic fertilizers

PHOSPHATE SOLUBILIZER

Phosphate solubilizing bacteria (PSB) are beneficial bacteria capable of solubilizing inorganic phosphorus from insoluble compounds. They have the capability of dissolving soil phosphorus which have been adsorbed and can mineralize organic P to become inorganic P, hence increasing the avalibility of P in the soil.

Phosphate solubilizing bacteria such as *Bacillus, Rhizobium, and Pseudomonas, flavobacterium,micrococcus,Streptomyces* and fungi belongs to *Aspergillus,Penicillium, Trichoderma* are the potent genera which are efficient to hydrolyze the inorganic phosphorus into soluble form and easily made available to the plant for growth promotion and root formation.



Plants absorb phosphate in the form $H_2PO_4^-$ and HPO_4^{-2} ions.Plants absorb only 10-15% -rest remains in insoluble form of phosphate. **Phosphobacteria** secrete organic acids which dissolve this unavailable phosphate into soluble form and make it available to the plants

Phosphobacteria means microbial inoculants capable of phosphate solubilizing nature. Commonly used Phosphobacteria is Bacillus megaterium. Phosphobacteria is suitable for all crops. This has to be mixed with rhizobium.

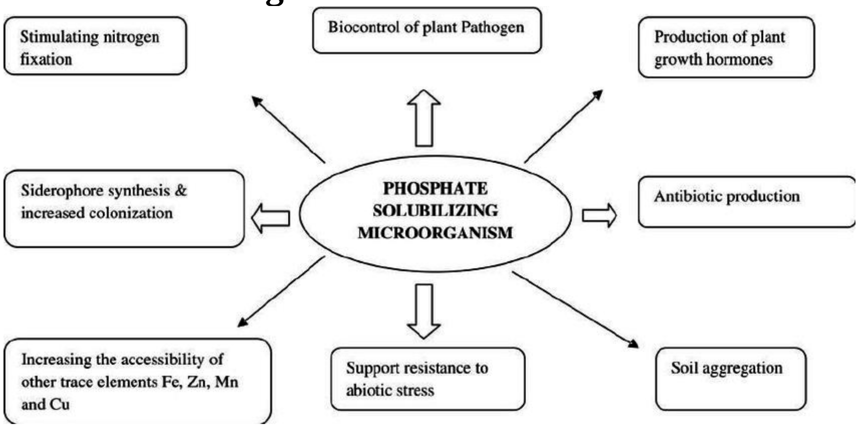
Media Preparation

Prepare appropriate media for specific to the bacterial inoculant in 250 ml, 500 ml, 3 litre and 5 litre conical flasks and sterilize. -The media in 250 ml flask is inoculated with efficient bacterial strain under aseptic condition Keep the flask under room temperature in rotary shaker (200 rpm) for 5- 7 days. -Observe the flask for growth of the culture and estimate the population, which serves as the starter culture. Using the starter culture (at log phase) inoculate the larger flasks (500 ml, 3 litre and 5 litre) containing the media, after obtaining growth in each flask. -The above media is prepared in large quantities in fermentor, sterilized well, cooled and kept it ready.

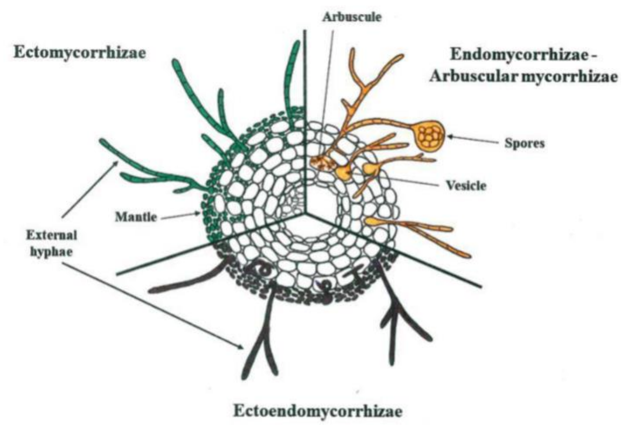
Inoculum preparation

The media in the fermentor is inoculated with the log phase culture grown in 5 litre flask. Usually 1 -2 % inoculum is sufficient, however inoculation is done up to 5% depending on the growth of the culture in the larger flasks. -The cells are grown in fermentor by providing aeration (passing sterile air through compressor and sterilizing agents like glass wool, cotton wool, acid etc.) and given continuous stirring. -The broth is checked for the population of inoculated organism and contamination if any at the growth period. -The cells are harvested with the population load of 109 cells ml-1 after incubation period. -There should not be any fungal or any other bacterial contamination at 10-6 dilution level -It is not advisable to store the broth after fermentation for periods longer than 24 hours. Even at 4^{o} C number of viable cells begins to decrease**.** PSB biofertlizer enhances the yield of wheat, rice and chickpea

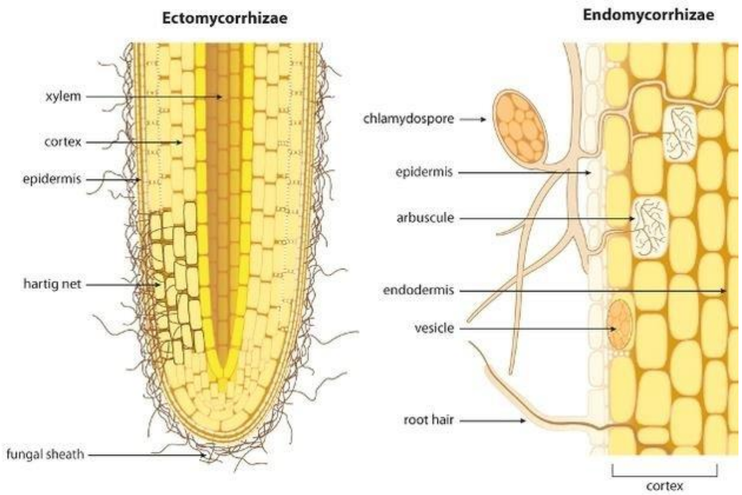
Functions and significance of PSB



MYCORRHIZAL ASSOCIATION



A **mycorrhiza** (the term 'mycorrhiza' comes from Greek – mycos meaning fungus and
rhiza meaning roots) 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.
Based on mycorrhizae association pattern of fungi with plants are broadly classified into
1.Ectomycorrhizae
2.Endomycorrhizae



Features of Mycorrhiza

(i) Absence of any phytopathological symptoms in the partners during the active phase of mutualism,

(ii) Presence of complex interfaces between cells of the partners with a predominant type of perisymbiotic membrane, sur-rounding intracellular symbionts,

(iii) Presence of various types of phagocyte--like structures during establishment of symbionts and during harvesting phase to control the symbiotic population by the host.

Types of Mycorrhiza:

There are seven types of mycorrhizae. These are

1. Ectomycorrhizae,
2. Vesicular-arbuscular mycorrhizae,
3. Ectendomycorrhizae (Arbutoid),
4. Ericoid mycorrhizae,
5. Arbutoid mycorrhizae
6. Orchidoid mycorrhizae, and
7. Monotropoid mycorrhizae.

Ectomycorrhizae:

Ectomycorrhiza is commonly called “sheathing mycorrhiza”. They occur in 3% of all seed plants in forests of tem-perate regions, especially on pine, beech, spruce, birch etc

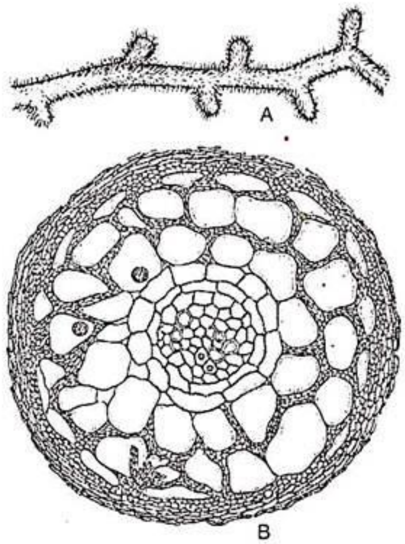


Fig. 4.103 : Ectomycorrhiza : A. *Pinus* root covered with mycorrhizal fungi, B. T.S. of *Pinus* root showing ectotrophic mycorrhizal fungi forming a mantle covering and growth of fungi between cortical cells

They cause exten-sive branching and growth of roots and modi-fication of branching pattern, such as racemose type in dicots (Fagus) and dichotomous in gym- nosperms (Pinus). In beech (Fagus) the ultimate lateral rootlets are differentiated into ‘long’ and ‘short’ roots.

The long roots show indefinite growth and their branches are the short roots that are thickened, forked and mycorrhizal. They appear in various colours like white, brown, yellow, black etc., depending on the colour of the fungus. The fungus enters the cortex forming ‘**Hartig net(is a network of inward growing hyphae, that extends into the root, penetrating between the epidermis and cortex of ectomycorrhizal plants.)**, but never goes inside the endodermis or stele. They form a mantle of varying thickness.

Vesicular-arbuscular mycorrhizae(VAM)

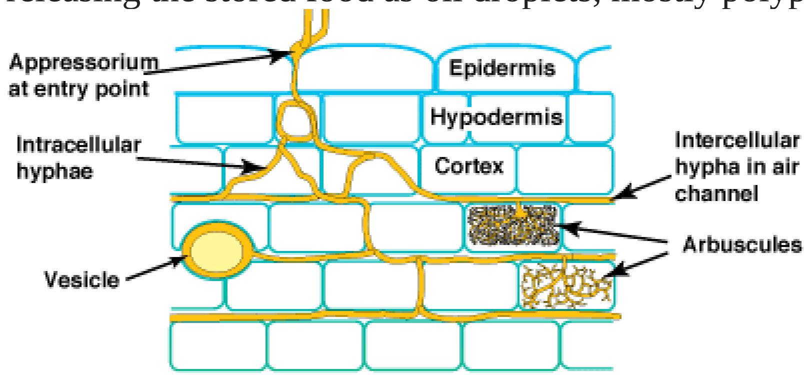
It is a type of endomycorrhizal asso-ciation, where both vesicles and arbuscles are developed together. VAM is by far the common-est of all mycorrhizae and has been reported in more than 90% of land plants.They are found in bryophytes, pteridophytes, gymnosperm (except Pinaceae) and most of angiosperms, commonly in Leguminosae (Fabaceae), Rosaceae, Gramineae (Poaceae) and Palmae (Arecaceae). VAM is not found in Ericaceae and Orchidaceae, where other type of association is available. VAM has even been reported in Lower Devonian plant, Rhynia.

VAM is produced by aseptate mycelial fungi belong to Endogonaceae under Mucorales of Zygomycotina and those members produced zygospores. The important genera involved in VAM are Glomus, Gyrospora, Acaulospora etc. Most of the members are not culturable

Ectendomycorrhizae (Arbutoid). The VAM is so named because of the presence of two characteristic structures namely

(i) **The vesicles** are thin or thick walled vesicular structures produced intra-cellularly and stored materials like poly-phosphate and other minerals and arbuscles.

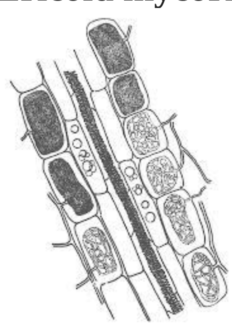
(ii) **The arbuscles** are repeated dichotomously branched haustoria which grow intracellularly The arbuscles live for four days and then get lysed releasing the stored food as oil droplets, mostly polyphosphate



There is no fungus mantle, but only a loose and very sparse network of septate hyphae spread into the soil. These hyphae bear different types of spores, chlamydospores, or aggregation of spores in sporocarp or zygospores. The super-ficial hyphae bear branches that penetrate the epidermis and then grow intercellularly only in cortex.

Intercellular hyphae form arbuscles inside the parenchyma of cortex by repeated dichotomous branching of the penetrating hyphae. The cell membrane of the penetrated cell is invaginated and covers the arbuscles. The hyphae also develop both inter- and intracellular thick-walled vesicles. The chlamydospores may germinate on nutrient agar, but the hyphae stop growing when food inside the spore is used up, thus they cannot be subcultured.

Ericoid mycorrhizae

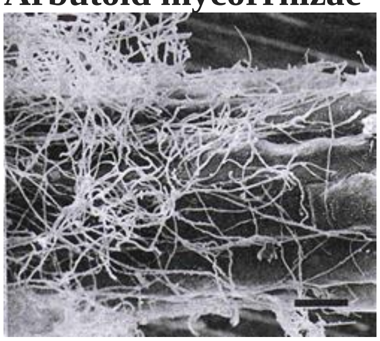


This is actually a type of endomycorrhiza. Ericoid mycorrhizae are found in the different members of Ericaceae like Erica, Calluna, Vaccinium, Rhododendron etc. The fungi are slow-growing, septate and mostly sterile. They are mostly culturable. Both *Pezizella ericae* (Ascomycotina) and *Clavaria vermiculata* (Basidiomycotina) have been iso-lated from Rhododendrons.

During this association the rootlets of the plants are covered by very sparse, loose, dark, septate hyphae that penetrate the cortex forming intercellular coils. (After 3-4 weeks the coils degenerate like arbuscles of vesicular- asbuscular mycorrhiza (VAM).

Most of the members of Ericaceae grow in acid soil with less amount of P and N nutrition. The fungus gets the photosynthate from the host and improves the mineral uptake and nutrition of the host, especially P and N. Many mycotrophs of Ericaceae show high resistance to metals like Zn and Cu. The mycorrhizal plants also show high tolerance to these metals, which is totally absent in non-infected plants.

Arbutoid mycorrhizae



It is a type of endomycorrhizal fungi that look similar to ectomycorrhizal fungi. They form a fungal sheath that encompasses the roots of the plant; however, the hyphae of the arbutoid mycorrhiza penetrate the cortical cells of plant roots, differentiating it from ectomycorrhizal fungi.

Arbutoid mycorrhizas are, like those of Ericoid and Monotropoid mycorrhizas, found in the plant order Ericales. Like ericoid mycorrhizas, the family Ericaceae is represented, with arbutoid mycorrhizas being formed in the genera Arctostaphylos and Arbutus. Arbutoid associations are also found in the Pyrolaceae family of the Order Ericales

Orchidoid mycorrhizae

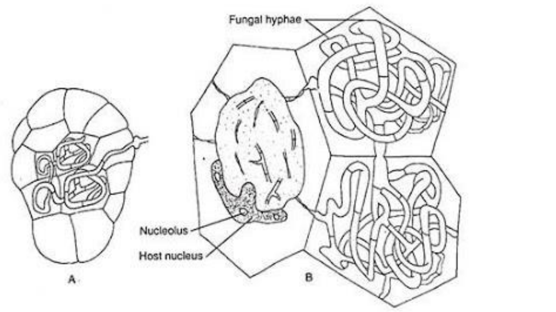


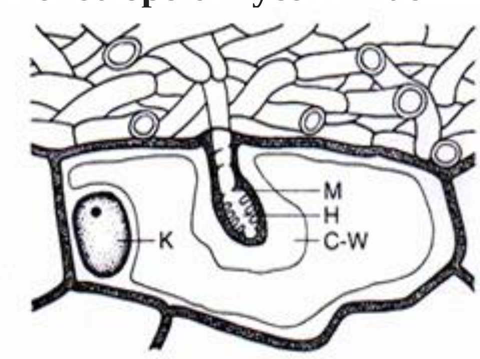
Fig. 4. 106 : Orchidoid mycorrhizae : A. Swollen embryo with initial infection, B. Cortical cells of *Dactylorchis* root showing coiled active and lysed hypae

Orchids produce millions of tiny seeds per capsule, weighing about 0.3-14µg. The embryo of seeds contains 10-100 cells and there is virtually no storage of food. The embryo is encircled in a thin-walled net-like testa that helps in their dispersal.Thus, majority of seeds are unable to germi-nate without exogenous supply of carbohydrates. Therefore, mycorrhizal association is obligatory for the seeds to germinate. The fungus provides C-nutrition to the seeds.

Initially the fungus enters the embryo and colonises, being restric-ted to the cortical cells and provides the nutrition (Fig. 4.106). For non-green orchids, this is obli-gatory throughout their lives. Apparently, it is a case of parasitism by orchids on the mycorrhizal fungi.

Fungi like *Rhizoctonia* (Basidiomycotina), are recognised by hyphal characteristics. Corticium, Ceratobasidium of Aphylloporales are associated in this type of mycorrhiza.

Monotropoid mycorrhizae



Monotropa hypopitys is a non-green saprophytic herb. It has short fleshy roots that are invested with a hyphal sheath and often forming Hartig net in the corti-cal zone. Due to absence of chlorophyll, they are unable to synthesise and supply carbohydrate to the fungus. *Boletus* is a mycorrhizal fungus associated with roots of both pine and Monotropa.

Significance of mycorrhizae association.

- Absorption of water,
- Dissolving essential minerals present in the organic debris and handing over the same to the plant. This allows the plant to grow in areas deficient in inorganic minerals,
- Absorbing inorganic salts present in the soil from over a large area. The forest trees like pines and birches are known to absorb 2-3 times more of potassium, nitrogen and phosphorus in the presence of mycorrhiza than in its absence,
- The fungus produces various growth promoting substances,
- It secretes antimicrobial substances which protect the young roots from attack of pathogens.

Role of Mycorrhizae in Agriculture and Foresty:
Role in Agriculture:

- The mycorrhizal association helps in the formation of dichotomous bran-ching and profuse root growth, thus enhances plant growth.
- Ectotrophic mycorrhiza helps in uptake of mineral ions and also acts as reservoir.
- They also help in absorption of nutri-ents.
- In nutrient deficient soil, the mycelial association helps in the absorption of N, Ca, P, Zn, Fe, Na and others.
- Mycorrhizal association is obligatory for the germination of orchid seeds.

- Mycorrhizal growth in orchids (Rhizoctonia repens with Orchis militaris tuber tissues) causes the synthesis of phytoalexins — orchinol and hirsinol. Both the compounds act as a barrier to pro-tect infection by other pathogens.
- Inoculation of VAM as biofertiliser provides a distinct possibility for the uptake of P in phosphorus-deficient soil.

Role in Foresty

- Mycorrhiza plays an important role to establish forest in unfavourable loca-tion, barren land, waste lands etc.
- Trees with facultative endomycorrhiza act as first invader in waste lands as pio-neer in plant succession.
- The application of mycorrhizal fungi in forest bed enhances the formation of mycorrhizal association that prevents the entry of fungal root

pathogens. This method is very much effective in the root of Pinus clausa against Phytophthora cinnamoni infection.

- Mycorrhiza mixed nitrogenous com-pounds such as nitrate; ammonia etc. is available to the plants. Thus it helps in plant growth, especially in acid soil.

SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
Accredited "A" Grade by NAAC | 12B Status by UGC | Approved by AICTE
www.sathyabama.ac.in

SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – V–Soil & Agricultural Microbiology– SMB2201

BIOFERTILIZER

Biofertilizers or microbial inoculants are carrier based ready to use live bacterial or fungal formulations,which on application to plants, soil or composting pits, help in mobilization of various nutrients by their biological activity.

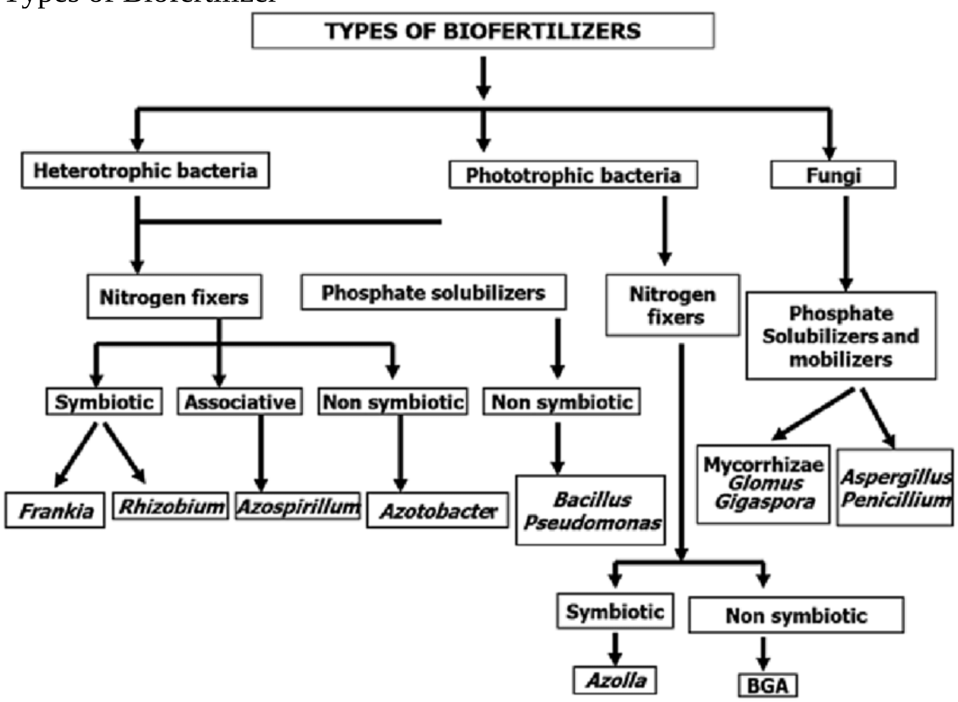
Biofertilizer are classified based on

1) For nitrogen -Rhizobium for legumes crops -Azotobacter/ Azospirilium for non legume crops -Acetobacter for sugarcane only. -Blue –Green Algae (BGA) and Azolla for low land paddy.

2) For phosphorus -Phosphate solubilizing Bacteria (PSB) for all crops to be applied with Rhizobium, Azotobacter, Azospirilium and Acetobacter

3) For enriched compost -Phosphate solubilizing Bacteria (PSB) and Azatobacter culture -Cellulolytic fungal culture

Types of Biofertilizer



Rhizobium

- Inoculum-vary in number, effectiveness and nitrogen fixation
- Inoculation improve nodulation, nitrogen fixation,
- crop growth and yield
- Inoculation increase yield is well documented

Essentials for inoculation in agriculture

- Population density of species specific rhizobia is low
- The same or symbiotically related legume is not grown in immediate past
- Wastelands have to be reclaimed
- Legumes follows a non-leguminous crop in a rotation
- Soil is poor in mineral N,
- Soils are acidic, alkaline and saline

Factors influence the rhizobium-legume symbiosis

1. Physico-chemical parameters
2. Soil temperature, moisture and reaction
3. Temperature tolerant strain of Rhizobia(up to 40o C)
4. Moisture stress affect the rhizobium survival
5. High salt concentration is detrimental, tolerance limit is 0.5 to 5% Na Cl
6. Fast growers are more tolerant (up to 0.34 M), legumes are more sensitive as compared to Rhizobium.
7. Acidity-Legumes are more sensitive as compared to Rhizobium.
8. May limit persistence in soil and reduce nodulation
9. High soil nitrogen levels, decrease nodulation
10. Other nutrients like P, K, Mo, Zn, Fe, Mg, S, Co, Ca, Cd, Mn, Cu
11. Organic matter-Favorable effect on nodulation and N_2 fixation.
12. Indigenous heterotrophic microbes and predators reduces the activity of Rhizobia
13. Bdellovibrio, an intracellular bacterial parasite.

For increasing the crop yield certain parameters to be studied such as the quality of the inoculants,effective inoculants delivery system and formulation of the policy to exploit symbiotic nitrogen fixation successfully

AZOTOBACTER

These are the freeliving bacteria which grow well on a nitrogen free medium.These bacteria utilize
atmospheric nitrogen gas for their cell protein synthesis. Old population bacteria are encapsulated forms and increase resistance to heat, desiccation and adverse condition.

Factors affecting the growth of Azotobacter

Azotobacter sp are sensitive to acidic pH, high salts and temperature above 35^{o}C. There are six species of Azotobacter, A. chroococcumis are commonly found in Indian soils. Nitrogen fixation. The species of Azotobacterare known to fix from 10-30 mg of N/g of sugar in pure culture. This is a poor competitor for nutrients in soil.

Functions of Azotobacter

It fixes atmospheric nitrogen in the rhizosphere.The most important function is the production of indol acetic acid and gibberellins. Also produce thiamin, riboflavin, nicotine óIt improves seed germination and control plant diseases

Uses as biofertilizer

- It is beneficial to cereals, vegetables and certain fruit crops.
- It play an importance role in vegetable crops where farmer add sufficient quantities of manures as it is highly respiratory micro-organism and require about 1000 kg of organic carbon to fix 30 kg of N/ha.
- Its inoculation increase the crop yield 15-20% over uninoculated treatment

AZOSPIRRILLUM

- This is important biofertilizer for maize, paddy and wheat crops.
- Increase the nitrogen content of soil after the crop harvest.
- It substitute the nitrogen up to 25-30 percentand increase the crop yield by 20-25 percent over uninoculated control**.**

BLUE GREEN ALGAE AND AZOLLA

- Increase the availability of nitrogen and phosphorus and certain micronutrients.
- Moderate the soil pH conditions.
- Azolla being green manure can substitute 40 -50 kg N per hectare.
- Azolla is source of nutrients to poultry, fish and water animals**.**

PHOSPHATE SOLUBILIZER

Phosphate solubilizing bacteria (PSB) are beneficial bacteria capable of solubilizing inorganic phosphorus from insoluble compounds. They have the capability of dissolving soil phosphorus which have been adsorbed and can mineralize organic P to become inorganic P, hence increasing the avalibility of P in the soil

- Phosphate solubilizing microbes are
- Bacteria-*Bacillus* spp
- Fungi- *Penicillium, Aspergillus*
- Efficiency of bacteria is more than fungal strains.
- Substitute 15-20 percent dose of phosphorus
- Mechanism is by phosphatase production

PHOSPHATE MOBILIZER

- Gigaspora, Acullosporaare the important sp.
- Phosphorus is being mobilized.
- Increase the availability of P, Mg, Ca and Zn.
- VAM do not produce phosphatase

FORMULATION TRAITS OF BIOFERTILIZER

1. The product must be carrier based or liquid formulation, capable of holding very high population of specific microorganisms for size able period of time**.**
2. Incase of carrier based formulations the product should have30-50% of moisture throughout the shelf life period to sustain microbial population.
3. For carrier based formulations the microbial population should be in the range of107 to 109 cells/g of moist product.
4. In case of liquid formulations the cell load should be in the range of 1x108
5. To 1x1010 during the entire period of shelflife.
6. It should be free from other contaminating microorganisms.
7. The microbial strain present in the product should be able to produce adequate nodulation in case of Rhizobium, be able to fix atleast 10-15mg of N/g ofcarbon source used in case of free living N_2 fixers and be capable of solubilizing significant quanity of fixed soil P
8. It should have sufficient shelf life minimum -6 months for carrier based and 12 months for liquid

PRODUCTION TECHNOLOGY

It involves 3 steps:

a) Isolation and identification of appropriate strains of targeted microorganisms.

b) Up-scaling of microbial biomass.

c) Impregnation of carrier with fully grown microbial broth or immobilization of grown cells to obtain liquid formulations

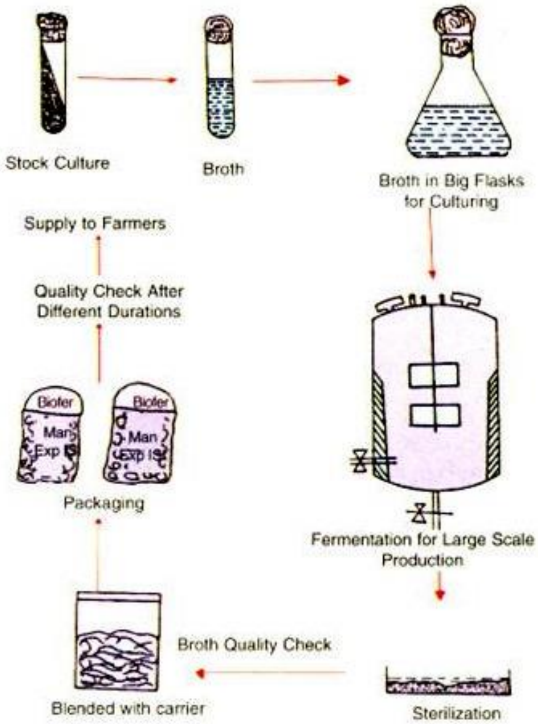


Fig. 34.1 : Commercial production of biofertilizer.

Isolation and identification of strains:

The success of any biofertilizer in the field primarily dependsuponthestrainofthe micro-organisms used in theproduct. The strain,besides possessing specific attributes (such as host specificity, nodulation potential and N_2fixation potential) should also have the ability tocolonize the soil and rhizosphere, be able to successfully compete with the native soil microorganisms and should have enough capacity to survive in the soil for long time in association with other soil microorganisms.

Up-scaling of biomass

To deliver a very high population of microorganismsin biofertilizers, it is very much essential to cultivate these microorganisms under appropriate conditions to achieve very high population Per unit of growing medium. Usually a final cell count of >109 cells per ml of broth should be achieved. This is being done in laboratories under controlled conditions in small glass containers (for small scale production) or large scale fermenters (for large scale production units).

Preparation of carrier based formulations:

Once the optimum growth of microbial cells is achieved in the multiplication process,it has to be mixed with the suitable carrier material, which can provide ideal home for these micro-organisms for about 6 months to 12 months time. The first step in this process involves the selection of suitable carrier materials.
The carrier materials are as follows:
Peat, charcoal, lignite, charcoal-soil mixture, press mud, vermiculite and **coco peat** have been identified as good carrier materials.

1. Ideal carrier should be:
2. Cheap in cost
3. Locally available
4. High organic content
5. No toxic chemical to inoculant
6. Water holding capacity of more than 50%
7. Easy process

For preparation of finished goods the pure bacterial liquid containing very high population is required. Microorganisms is mixed with the carrier material to obtain moist powdered formulation, which is packed in polythene bags and supplied to the farmers. Depending upon the facilities, mixing of bacterial liquid with carrier.
Material is being done either manually or under complete sterile conditions. Packets prepared by manual mixing method have shorter shelf life (2-3 months), while packets prepared under complete sterile conditions have longer shelf life (6-12months).

Preparation of liquid formulations:

In this process, in some cases the fermentation is taken up with specialized formulations with cell immobilizers added at different stages of growth. Finally prepared brothwith immobilized cell shaving A cell count of $>1x10^{10}$ is harvested and packed in bottles. In somecases,the vegetative cells after being cultivated to desired level are converted into cystsorspores. These spores are further treated to keep the minimal dormant position. Final preparation is packed in bottles. If sterile conditions can be arranged then instead of bottles plastic pouches can also be prepared.

APPLICATION

There are three major ways in applying the biofertilizer to the field namely

- Seed treatment
- Seedling root dip treatment
- Soil treatment

Seed treatment- Suspend200g each of Nfixing and PSB in 300-400ml of water and mix thoroughly. Pour this slurry on10to12kg of seed and mix by hands, till all the seeds are uniformly coated. Dry the treated seeds in shade and sow immediately. For acidic and alkaline soils it is always it is advisable to use1 kg of **slacked lime or gypsum** powder respectively for coating the wet biofertilizer treated seeds.

Seedling root dip treatment

Suspend 1 to 2 kg each of nitrogen fixing (Azotobacter/Azospirillum) and PSB into just Sufficient quantity of water (5-10lit depending upon the quantity of seedlings required to be planted in one acre). Dip the roots of seedlings in this suspension for 20-30 min before transplanting. In case of paddy make a sufficient sizebed (2mtx1.5mtx0.15mt)in the field, fill it with 5cm of water and suspend 2kg each ofAzospirillum and PSB, mix thoroughly. Then dip the roots of seedlings in this bed for 8-12 hours(overnight) and then transplant.

Soil treatment

For soil treatment depending upon the total number of plants per acre 2-4 kg of Azotobacter/Azospirillum and2-4kg of PSB are requiredfor oneacre. Mix two types of biofertilizer
in 2-4liters of water separately and sprinkle this suspension on two separate heaps of 50-100 kg of
compost. Mix the two heaps separately and leave for incubation overnight. After12 hours,mix the two heaps together. For acidic soils mix 25 kg limes with this mixture.

VERMICOMPOST

Vermicomposting

Vermicomposting is a type of composting in which certain species of earthworms are used to enhance the process of organic waste conversion and produce a better end-product. It is a mesophilic process utilizing microorganisms and earthworms. Vermicomposting is an ecofriendly, low cost effective and a effective way to recycle agricultural and kitchen waste. The application of vermicompost not only adds plant nutrients (macro and micro) and growth regulators but also increases soil water retention, microbial population, nutrient content and carbon content of the soil. Vermicompost refers to an organic manure produced by earthworms.

It is mixture of worm castings, organic material including humus, live earth worms and their cocoons and other organisms. Vermicomposting is a appropriate technique for the disposal of non-toxic solid and liquid organic wastes. It helps in cost effective and efficient recycling of animal wastes agricultural residual and industrial wastes using low energy.

Earthworms

Earthworms feed on any organic waste, consume two to five times their body weight and after using 5-10 per cent of the feed stock for their growth, excrete the mucus coated undigested matter as wormcasts worm cast consists of organic matter that has undergone physical and chemical breakdown through the activity of the muscular gizzard which grinds the material to a particle size of 1-2 micron. The nutrients presents in wormicast are readily soluble in water for the uptake of plants. Worm casts are rich source of macro and micro nutrients, vitamins, enzymes, antibiotics, growth harmones and immobilized microfloras.

Earthworms are cosmopolitan in distribution. They are important invertebrate macrofauna. Macroinvertebrates, in general, play a definite and prominent role in regulating soil process; among them, earthworms play a vital role in maintaining soil fertility and efficient nutrient cycling.

Earthworms also act as biological indicators of the soil type and its properties.They also serve to detect toxicity of industrial chemicals and act as indicators of soil fertility because the presence of earthworms support healthy populations of bacteria, actinomycetes, fungi, protozoans, insects, spiders, millipedes and a host of other organisms that are essential for sustaining healthy soil.

Earthworms species for vermicompost

1. *Lampito mauriti* -Sandy loam soil
2. *Octochaetona serrata*- Clay soil
3. *Lumbricus terrestris* -Deep boarings
4. *Allolobophora caliginosa* -shallow boarings
5. *Eudrilus eugeniae*
6. *Eisenia foetida*
7. *Perionyx excavatus*
8. *Pheretima elongate*

The *Eisenia foetida* an epigeic species of earthworms is used for vermicomposting. The general characteristics of *Eisenia foetida* are as under:

Habit: Epigeic
Size: 8-10 cm
Diameter: 0.8-1.0 mm
Duration of life: 70 days
Temperature range: 15-40 0C
Approximate weight of single earthworm: 1.0-3.0 g
Moisture tolerance range: 30-70%

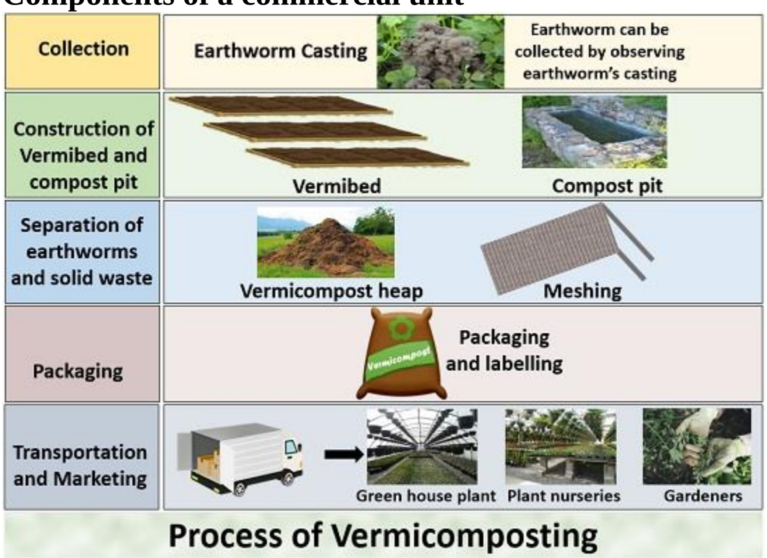
Habit and Habitats: Living on surface and organic matter rich areas.

Process of vermicomposting

A wide range of organic residues, such as straw, husk, leaves, stalks, weeds etc can be converted into vermicompost. Other potential feedstock for vermicompost production are livestock wastes, poultry litter, dairy wastes, food processing wastes, organic fraction of MSW, bagasse, digestate from biogas plants etc. Earthworms consume organic wastes and reduce the volume by 40–60 percent. Each earthworm weighs about 0.5 to 0.6 gram, eats waste equivalent to its body weight and produces cast equivalent to about 50 percent of the waste it consumes in a day. The moisture content of castings ranges between 32 and 66 percent and the pH is around 7. The level of nutrients in compost depends upon the source of the raw material and the species of earthworm.

There are nearly 3600 types of earthworms which are divided into burrowing and non-burrowing types. **Red earthworm species, like *Eisenia foetid*a**, and are most efficient in compost making. The non-burrowing earthworms eat 10 percent soil and 90 percent organic waste materials; these convert the organic waste into vermicompost faster than the burrowing earthworms. They can tolerate temperatures ranging from 0 to 40°C but the regeneration capacity is more at 25 to 30°C and 40–45 percent moisture level in the pile. The burrowing types of earthworms come onto the soil surface only at night. These make holes in the soil up to a depth of 3.5 m and produce 5.6 kg casts by ingesting 90 percent soil and 10 percent organic waste.

Components of a commercial unit



Location

Suburbs of cities and villages around urban centres can be ideal locations for practice of vermicomposting on a large scale, from the view point of availability of raw material and marketing of the produce. As use of the compost is said to have ameliorative effect on product from fruit, flower and vegetable crops, vermicomposting units may be located in areas with concentration of fruit and vegetable growers and floriculture units.

Sheds

For a vermi-composting unit, whether small or big, this is an essential item and is required for having the vermi beds. They could be of thatched roof supported by bamboo rafters and purlins, wooden trusses and stone pillars. If the size is so chosen as to prevent wetting of beds due to rain on a windy day, they could be open sheds. While designing the sheds adequate room has to be left around the beds for easy movement of the labour attending to the filling and harvesting the beds.

Vermi-beds

Normally the beds are 75 cm - 90 cm thick depending on the provision of filter for drainage of excess water. The entire bed area could be above the ground. Care should be taken to make the bed with uniform height over the entire width to the extent possible to avoid low production owing to low bed volumes. The bed width should not be more that 1.5 m to allow easy access to the centre of the bed.

Land

About 0.5-1 acre of land will be needed to set up a vermiculture production cum extension centre. The centre will have at least 8-10 sheds each of about 180-200 sq.ft. It should also have a bore well, and pump set or watering arrangement and other equipments as described in the scheme economics. The land can be taken on lease of at least 10-15 years. Even sub marginal land also will serve the purpose.

Seed Stock

This is an important item requiring considerable investment. Though the worms multiply fast to give the required numbers over a period of 6 months to a year, it may not be wise to wait till such a time having invested on the infrastructure heavily. Thus, worms @ 350 worms per m3 of bed space should be adequate to start with and to build up the required population in about two cycles or three without unduly affecting the estimated production.

Fencing and Roads/Paths

The site area needs development for construction of structures and development of roads and pathways for easy movement of hand-drawn trolleys/wheel barrows for conveying the raw material and the finished products to and from the vermi-sheds. The entire area has to be fenced to prevent trespass by animals and other unwanted elements. These could be estimated based on the length of the periphery of the farm and the length and type of roads/paths required. The costs on fencing and formation of roads should be kept low as these investments are essential for a production unit, yet would not lead to increase in production.

Water Supply System

As the beds have always to be kept moist with about 50% moisture content, there is need to plan for a water source, lifting mechanism and a system of conveying and applying the water to the vermi-beds. Drippers with round the clock flow arrangement would be quite handy for continuous supply and saving on water. Such a water supply/application system requiring considerable initial investment, however, reduces the operational costs on hand watering and prove economical in the long run. The cost of these items depend on the capacity of the unit and the type of water supply chosen.

Machinery

Farm machinery and implements are required for cutting (shredding) the raw material in small pieces, conveying shredded raw material to the vermi-sheds, loading, unloading, collection of compost, loosening of beds for aeration, shifting of the compost before packing and for air drying of the compost, automatic packing and stitching for efficient running of the unit. Costs of providing necessary implements and the machinery have to be included in the project cost. ;;’

Transport

For any vermi-composting unit transport arrangement is a must. When the source of raw material is away from the production unit, an off-site transport becomes major item of investment. A large sized unit with about 1000 tonnes per annum capacity may require a 3-tonne capacity mini-truck. With small units particularly with the availability of raw material near the site, expending on transport facility may become infructuous. On-site transport facilities like manually drawn trolleys to convey raw material and finished products between the storage point and the vermi-compost sheds could also be included in the project cost.

Furniture

A reasonable amount could also be considered for furnishing the office-cum-stores including the storage racks and other office equipment. These enhance the efficiency of operations.

Operational Costs

In order to operate the unit, expenditure on some items have to be incurred on a recurring basis. These items include salaries of the staff, wages to the labourers, cost of raw material, fuel cost on transport of raw materials and finished goods, packing material cost, repairs and maintenance, power, insurance, etc. The number of office personnel and labourers have to be decided breaking each activity into a number of sub-activities and for each sub-activity estimating the work involved and the capacity of the labour to finish the work in a given time. The number of persons should be so chosen to keep them engaged throughout by providing enough persons at various work points like stores, vermi-beds and equipping them with adequate number of implements to avoid undue waiting.

Applications of Vermicompost

The worm castings contain higher percentage of both macro and micronutrients than the garden compost. Apart from other nutrients, a fine worm cast is rich in NPK which are in readily available form and are released within a month of application. Vermicompost enhances plant growth, suppresses disease in plants, increases porosity and microbial activity in soil, and improves water retention and aeration.

Vermicompost also benefits the environment by reducing the need for chemical fertilizers and decreasing the amount of waste going to landfills. Vermicompost production is trending up worldwide and it is finding increasing use especially in Western countries, Asia-Pacific and Southeast Asia.

A relatively new product from vermicomposting is vermicompost tea which is a liquid produced by extracting organic matter, microorganisms, and nutrients from vermicompost. Unlike vermicompost and compost, this tea may be applied directly to plant foliage, reportedly to enhance disease suppression. Vermicompost tea also may be applied to the soil as a supplement between compost applications to increase biological activity.

Uses

- The wastes are pulverized as they pass through the worm,
- Surface area of the material increases which in turn helps as base for nutrients
- Supply nutrients and growth enhancing hormones to plants
- Improves the soil structure leading to increase in water and nutrient holding capacities of soil. Chemical fertilizer in moderate doses can go along with vermicomposting.

Wasteland reclamation using microbes

Wasteland reclamation of wasteland means re-claiming it or to use it for productive purpose. Wasteland reclamation is the process of turning barren, sterile wasteland into something that is fertile and suitable for habitation and cultivation.

The green revolution is the result of intensive agriculture with extensive use of chemical fertilizers. In general, the production of chemical fertilizers has led to depletion of fossil fuel environmental pollution and occupational hazards. In particular, the application of chemical fertilizers in agricultural fields reduces the fertility of the soil.

Due the removal process of desired mineral materials, soil textures have been destroyed, various nutrient cycles have been disturbed, and microbial communities have been altered, affecting vegetation and leading to the destruction of wide areas of land in many countries. Therefore, soil restoration of abandoned mining lands became a very important part of sustainable development strategies.

Contiuous agricultural activities, remove the top soil layer transforming these lands less fertile and more susceptible for soil hazards. Growing cover crops is one of the best practices to improve the organic matter content of the soil and hence soil health and quality. Vegetation layers—besides adding plant material to the soil contributing to its organic matter replenishment—can also fix N in the soil, moderate soil temperature protecting hence soil organisms and can provide habitat for beneficial insects and other organisms. The fertility of soil can be enhanced by Stimulate existing soil microbes, improve the nutrient and water efficiencies, introduce stress tolerance plants, add biofertilizer to increase the nutrient, with agricultural crop rotation and high fertilization doses depending on the location.