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**S.Y. B.Sc. (Sem. IV) (CBCS)**

**MICROBIOLOGY**

**[401]: APPLIED AND ENVIRONMENTAL MICROBIOLOGY**

**Unit 5**

**ENVIRONMENTAL MICROBIOLOGY**

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## TYPES OF POLLUTION

- Human activities generate tremendous amount of waste materials. These waste materials are ultimately discharged in various components of the environment, where they bring about undesirable changes. This phenomenon is termed as “Environmental pollution”. The word “pollution” is derived from Latin word “polluter”.
- Pollution can be defined as any undesirable change in physical, chemical, biological characteristics of air, water or land (eco system) that may be harmful to human and other life forms, industrial processes, living conditions and cultural assets or cause wastage of our raw materials.

### Pollutants:

- Any substance which causes pollution is called a pollutant. It may be defined as any solid, liquid or gaseous substance present in concentrations which may be injurious to environment. They are residues of substances which we produce, consume or throw away.

### Sources of pollutants:

- Most pollutants are introduced into our environment as sewage, accidental discharge or by-products.
- The compounds such as pesticides used to protect plants and animals also cause pollution. The compounds which are used to purify air and water succeed in polluting them.
- Pollutants come from all types of human activity, agricultural as well as industrial activity.
- Pollution is thus a necessary evil of all developmental activities.

### Types of pollutants:

Depending upon the type of pollutants and upon the time for which they persist in our environments, pollutants are classified in four broad categories.

#### 1) Simple biodegradable pollutants

- This includes simple waste, which are easily and quickly degraded into harmless constituents. E.g., domestic waste, organic matter of plant and animal origin, faecal matter, blood, urine, etc are placed in this group.

#### 2) Complex biodegradable pollutants

- This includes waste which are resistant to degradation by biological entities (activity). Their degradation is very slow due to which they persist in environment for long durations. E.g., Synthetic chemicals, pesticides, polymers, crude petroleum etc are placed in this category.

#### 3) Non degradable pollutants

- This includes waste which is not degraded by biological entities. It may undergo a change of form as chemical combination but are non-destructible in nature. E.g., Heavy metals, toxic trace metals are placed in this group.

#### 4) Pollution caused by physical agents

- This includes the pollution caused by harmful and irritating physical agents such as heat, noise etc. thermal, noise and radioactive pollution are placed in this group. Xenobiotics, Recalcitrant.
- Complex biodegradable wastes. Some compounds have remarkable degree of resistance to natural agencies of decay and decomposition. These harmful substances persist in the environment for long duration of time during which they are taken up in the biosphere, accumulated and biomagnified to concentration toxic to organisms at higher trophic level in food chains. Many of these chemicals are carcinogenic, teratogenic and mutagenic in nature. These chemicals have caused much concern due to their wide spread use and dissemination. These chemicals are grouped into following classes.
  - Chemicals and allied chemicals
  - Detergents
  - Crude petroleum and its derivatives
  - Polymers, plastic and other wastes

### AIR POLLUTION

- Air pollution refers to any physical, chemical or biological change in the air.
- It is the contamination of air by harmful gases, dust and smoke which affects plants, animals and humans drastically.
- Or **Air pollution** refers to the release of harmful contaminants (chemicals, toxic gases, particulates, biological molecules, etc.) into the earth's atmosphere.
- These contaminants are quite detrimental and, in some cases, pose serious health issues. Some causes that contribute to air pollution are:
  - Burning fossil fuels
  - Mining operations
  - Exhaust gases from industries and factories
- The effects of air pollution vary based on the kind of pollutant. But generally, the impact of air pollution ranges from:
  - Increased risk of respiratory illness and cardiovascular problems
  - Increased risk of skin diseases
  - May increase the risk of cancer
  - Global warming
  - Acid rain

- Ozone depletion
- Hazards to wildlife

Among the other types of pollution, air pollution is theorized to have a planet-wide implication. Scientists have even speculated an apocalypse-like scenario where air pollution if left unchecked, can bring about an extreme form of global warming called the runaway greenhouse effect. Though this is purely speculative, it is a phenomenon that has already occurred on Venus.

There is a certain percentage of gases present in the atmosphere. An increase or decrease in the composition of these gases is harmful to survival. This imbalance in the gaseous composition has resulted in an increase in earth's temperature, which is known as global warming.

## TYPES OF AIR POLLUTANTS

➤ There are two types of air pollutants:

### 1) Primary Pollutants

- The pollutants that directly cause air pollution are known as primary pollutants. Sulphur-dioxide emitted from factories is a primary pollutant.

### 2) Secondary Pollutants

- The pollutants formed by the intermingling and reaction of primary pollutants are known as secondary pollutants. Smog, formed by the intermingling of smoke and fog, is a secondary pollutant.

## CAUSES OF AIR POLLUTION

Following are the important causes of air pollution:

- **Burning of Fossil Fuels**

The combustion of fossil fuels emits a large amount of sulphur dioxide. Carbon monoxide released by incomplete combustion of fossil fuels also results in air pollution.

- **Automobiles**

The gases emitted from vehicles such as jeeps, trucks, cars, buses, etc. pollute the environment. These are the major sources of greenhouse gases and also result in diseases among individuals.

- **Agricultural Activities**

Ammonia is one of the most hazardous gases emitted during agricultural activities. The insecticides, pesticides and fertilizers emit harmful chemicals in the atmosphere and contaminate it.

- **Factories and Industries**

Factories and industries are the main source of carbon monoxide, organic compounds, hydrocarbons and chemicals. These are released into the air, degrading its quality.

- **Mining Activities**

In the mining process, the minerals below the earth are extracted using large pieces of equipment. The dust and chemicals released during the process not only pollute the air, but also deteriorate the health of the workers and people living in the nearby areas.

- **Domestic Sources**

The household cleaning products and paints contain toxic chemicals that are released in the air. The smell from the newly painted walls is the smell of the chemicals present in the paints. It not only pollutes the air but also affects breathing.

### **Effects of Air Pollution**

♣ The hazardous effects of air pollution on the environment include:

- **Environmental Issues**

- Environmental issues are the harmful effects of human activities on the environment. These include pollution, over-population, waste disposal, climate change, global warming, greenhouse effect, etc.
- Various environment protection programs are being practised at the individual, organizational and government levels with the aim of establishing a balance between man and environment.
- Some of the current environmental issues that require urgent attention are:

- **Climate Change**

- Climate change is a great concern in today's scenario. This problem has surfaced in the last few decades. Greenhouse gases are the major cause of climate change. Environmental changes have several destructive impacts such as the melting of glaciers, change in seasons, epidemics, etc.

- **Diseases**

- Air pollution has resulted in several respiratory disorders and heart diseases among humans. The cases of lung cancer have increased in the last few decades. Children living near polluted areas are more prone to pneumonia and asthma. Many people die every year due to the direct or indirect effects of air pollution.

- **Global Warming**

- Due to the emission of greenhouse gases, there is an imbalance in the gaseous composition of the air. This has led to an increase in the temperature of the earth. This increase in earth's temperature is known as global warming. This has resulted in the melting of glaciers and an increase in sea levels. Many areas are submerged underwater.

- **Acid Rain**

- The burning of fossil fuels releases harmful gases such as nitrogen oxides and sulphur oxides in the air. The water droplets combine with these pollutants, become acidic and fall as acid rain which damages human, animal and plant life.

- **Ozone Layer Depletion**

- The release of chlorofluorocarbons, halons, and hydro chlorofluorocarbons in the atmosphere is the major cause of depletion of the ozone layer. The depleting ozone layer does not prevent the harmful ultraviolet rays coming from the sun and causes skin diseases and eye problems among individuals.
- **Effect on Animals**
  - The air pollutants suspend on the water bodies and affect the aquatic life. Pollution also compels the animals to leave their habitat and shift to a new place. This renders them stray and has also led to the extinction of a large number of animal species.

## AIR POLLUTION CONTROL

- Following are the measures one should adopt, to control air pollution:
  - **Avoid Using Vehicles**

People should avoid using vehicles for shorter distances. Rather, they should prefer public modes of transport to travel from one place to another. This not only prevents pollution, but also conserves energy.
  - **Energy Conservation**

A large number of fossil fuels are burnt to generate electricity. Therefore, do not forget to switch off the electrical appliances when not in use. Thus, you can save the environment at the individual level. Use of energy-efficient devices such CFLs also controls pollution to a greater level.
  - **Use of Clean Energy Resources**

The use of solar, wind and geothermal energies reduce air pollution at a larger level. Various countries, including India, have implemented the use of these resources as a step towards a cleaner environment.
  - Other air pollution control measures include:
    - By minimizing and reducing the use of fire and fire products.
- 1. Since industrial emissions are one of the major causes of air pollution, the pollutants can be controlled or treated at the source itself to reduce its effects. For example, if the reactions of a certain raw material yield a pollutant, then the raw materials can be substituted with other less polluting materials.
- 2. Fuel substitution is another way of controlling air pollution. In many parts of India, petrol and diesel are being replaced by CNG – Compressed Natural Gas fueled vehicles. These are mostly adopted by vehicles that aren't fully operating with ideal emission engines.
- 3. Although there are many practices in India, which focus on repairing the quality of air, most of them are either forgotten or not being enforced properly. There are still a lot of vehicles on roads which haven't been tested for vehicle emissions.

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4. Another way of controlling air pollution caused by industries is to modify and maintain existing pieces of equipment so that the emission of pollutants is minimized.
5. Sometimes controlling pollutants at the source is not possible. In that case, we can have process control equipment to control the pollution.
6. A very effective way of controlling air pollution is by diluting the air pollutants.
7. The last and the best way of reducing the ill effects of air pollution is tree plantation. Plants and trees reduce a large number of pollutants in the air. Ideally, planting trees in areas of high pollution levels will be extremely effective.

## SOIL POLLUTION

- Soil pollution, also called soil contamination, refers to the degradation of land due to the presence of chemicals or other man-made substances in the soil.
- The xenobiotic substances alter the natural composition of soil and affect it negatively. These can drastically impact life directly or indirectly.
- For instance, any toxic chemicals present in the soil will get absorbed by the plants. Since plants are producers in an environment, it gets passed up through the food chain.
- Compared to the other types of pollution, the effects of soil pollution are a little more obscured, but their implications are very noticeable.
- Some of the common causes of soil pollution are:
  - Improper industrial waste disposal
  - Oil Spills
  - Acid rain which is caused by air pollution
  - Mining activities
  - Intensive farming and agrochemicals (like fertilizers and pesticides)
  - Industrial accidents
- The effects of soil pollution are numerous. Specific wastes, such as radioactive waste become particularly hazardous when they are not well-contained.
- A well-documented example is a nuclear accident in Chernobyl, which has left an area of 2,600 km<sup>2</sup> uninhabitable for several thousand years.
- Other effects of soil pollution include:
  - Loss of soil nutrients, which renders the soil unfit for agriculture
  - Impacts the natural flora and fauna residing in the soil
  - Degrades vegetation due to the increase of salinity of the soil
  - Toxic dust (such as silica dust) can cause respiratory problems or even lung cancer

## WATER POLLUTION

- **Water pollution** is said to occur when toxic pollutants and particulate matter are introduced into water bodies such as lakes, rivers and seas. These contaminants are generally introduced by human activities like improper **sewage treatment** and oil spills. However, even natural processes such as eutrophication can cause water pollution.
- The effects of water pollution are very pronounced in our environment. Furthermore, toxic chemicals can bioaccumulate in living beings, and these chemicals can travel their way up the food chain, ultimately reaching humans.
- Among the other types of pollution, water pollution has a more disastrous consequences on humans. For instance, in 1932, a grave case of water pollution incapacitated the inhabitants of an entire city in Japan with neurological diseases and mental illness for many decades.
- However, the immediate cause was not apparent but was eventually attributed to acute mercury poisoning. Methylmercury was dumped into the surrounding bay and had ultimately bioaccumulated inside the fish. The local population then consumed these fish, and this resulted in the manifestation of ill-effects and neurological diseases.
- Other consequences of water pollution include:
  - Disruption of the ecosystem
  - Threats to marine life
  - Increased risk of water-borne diseases
  - Increases toxic chemicals (such as mercury) in water bodies
  - Eutrophication

Water is one of the most vital natural resources on earth and has been around for a long time. In fact, the same water which we drink has been around in one form or the other since the time of the dinosaurs.

The earth has more than two-thirds of its surface covered with water. This translates to just over 1 octillion liters (1,260,000,000,000,000,000 liters) of water distributed in the oceans, rivers, lakes and streams.

That is a lot of water, however, less than 0.3% is accessible for human consumption. As commercialization and industrialization have progressed, that number continues to dwindle down. Furthermore, inefficient and outdated practices, lack of awareness and a plethora of other circumstances have led to water pollution.

### Sources Of Water Pollution

The key causatives of water pollution in India are:

- Urbanization.
- Deforestation.
- Industrial effluents.



- Social and Religious Practices.
  - Use of Detergents and Fertilizers.
  - Agricultural run-offs- Use of insecticides and pesticides.
- One of the primary **causes of water pollution** is the contamination of water bodies by toxic chemicals.
- As seen in the example mentioned above, the dumped plastic bottles, tins, water cans and other wastes pollute the water bodies.
- These result in water pollution, which harms not just humans, but the whole ecosystem.
- Toxins drained from these pollutants, travel up to the food chain and eventually affect humans.
- In most cases, the outcome is destructive to only local population and species, but it can have an impact on a global scale too.
- Nearly 6 billion kilograms of garbage is dumped every year in the oceans.
- Apart from industrial effluents and untreated sewage, other forms of unwanted materials are dumped into various water bodies.
- These can range from nuclear waste to oil spills – the latter of which can render vast areas uninhabitable.

### Effects Of Water Pollution

- The effect of water pollution depends upon the type of pollutants and its concentration. Also, the location of water bodies is an important factor to determine the levels of pollution.
- Water bodies in the vicinity of urban areas are extremely polluted. This is the result of dumping garbage and toxic chemicals by industrial and commercial establishments.
- Water pollution drastically affects aquatic life. It affects their metabolism, behavior, causes illness and eventual death. Dioxin is a chemical that causes a lot of problems from reproduction to uncontrolled cell growth or cancer. This chemical is bioaccumulated in fish, chicken and meat. Chemicals such as this travel up the food chain before entering the human body.
- The effect of water pollution can have a huge impact on the food chain. It disrupts the food-chain. Cadmium and lead are some toxic substances, these pollutants upon entering the food chain through animals (fish when consumed by animals, humans) can continue to disrupt at higher levels.
- Humans are affected by pollution and can contract diseases such as hepatitis through faecal matter in water sources. Poor drinking water treatment and unfit water can always cause an outbreak of **infectious diseases** such as cholera, etc.

- The ecosystem can be critically affected, modified and destructured because of water pollution.

## Pollution of the Ganges

- Some rivers, lakes, and groundwater are rendered unfit for usage. In India, the River Ganges is the sixth most polluted river in the world. This is unsurprising as hundreds of industries nearby release their effluents into the river. Furthermore, religious activities such as burials and cremations near the shore contribute towards pollution. Apart from the ecological implications, this river poses serious health risk as it can cause diseases like typhoid and cholera.
- Pollution of the Ganges is also driving some of the distinct fauna to extinction. The Ganges River shark is a critically endangered species that belong to the order Carcharhiniforms. The Ganges River dolphin is another **endangered species** of dolphin that is found in the tributaries of the Ganges and Brahmaputra rivers.
- As per a survey, by the end of 2026, around 4 billion people will face a shortage of water. Presently, around 1.2 billion people worldwide do not have access to clean, potable water and proper sanitation. It is also projected that nearly 1000 children die every year in India due to water-related issues. Groundwater is an important source of water, but unfortunately, even that is susceptible to pollution. Hence, water pollution is quite an important social issue that needs to be addressed promptly.

## Control Measures of Water Pollution

- Water pollution, to a larger extent, can be controlled by a variety of methods. Rather than releasing sewage waste into water bodies, it is better to treat them before discharge. Practising this can reduce the initial toxicity and the remaining substances can be degraded and rendered harmless by the water body itself. If the secondary treatment of water has been carried out, then this can be reused in sanitary systems and agricultural fields.
- A very special plant, the Water Hyacinth can absorb dissolved toxic chemicals such as cadmium and other such elements. Establishing these in regions prone to such kinds of pollutants will reduce the adverse effects to a large extent.
- Some chemical methods that help in the control of water pollution are precipitation, the ion exchange process, **reverse osmosis**, and coagulation. As an individual, reusing, reducing, and recycling wherever possible will advance a long way in overcoming the effects of water pollution.

## NOISE POLLUTION

- **Noise pollution** refers to the excessive amount of noise in the surrounding that disrupts the natural balance. Usually, it is man-made, though certain natural calamities like volcanoes can contribute to noise pollution.
- In general, any sound which is over 85 decibels is considered to be detrimental. Also, the duration an individual is exposed plays an impact on their health. For perspective, a normal

conversation is around 60 decibels, and a jet taking off is around 150 decibels. Consequently, noise pollution is more obvious than the other types of pollution.

Noise pollution has several contributors, which include:

- Industry-oriented noises such as heavy machines, mills, factories, etc.
- Transportation noises from vehicles, airplanes, etc.
- Construction noises
- Noise from social events (loudspeakers, firecrackers, etc.)
- Household noises (such as mixers, TV, washing machines, etc.)

Noise pollution has now become very common due to dense urbanization and industrialization. Noise pollution can bring about adverse effects such as:

- Hearing loss
- Tinnitus
- Sleeping disorders
- Hypertension (high BP)
- Communication problems

## MARINE POLLUTION

- Marine pollution can be defined as the introduction of substances to the marine environment directly or indirectly by man resulting in adverse effects such as hazards to human health, obstruction of marine activities and lowering the quality of sea water. While the causes of marine pollution may be similar to that of general water pollution there are some very specific causes that pollute marine waters.
- The most obvious inputs of waste is through pipes directly discharging wastes into the sea. Very often municipal waste and sewage from residences and hotels in coastal towns are directly discharged into the sea.
- Pesticides and fertilizers from agriculture which are washed off the land by carry these materials into rivers and eventually into the sea. from the roads normally enter the sewage system but storm water over industrial chemicals, etc. in huge quantities sometimes to the capacity Ships carry many toxic substances such as oil, liquefied natural gas, pesticides very damaging to the marine environment. Shipping channels in estuaries 350.000 tonnes.
- Ship accidents and accidental spillages at sea therefore can dredged material that may contain heavy metals and other contaminants the entrance to ports of ten require frequent dredging to keep them open. The Offshore oil exploration and extraction also pollute the seawater to a la water is vital for the plants and animals living in it. Wastes, which direct
- **Pollution due to organic wastes:** The amount of oxygen dissolved in the indirectly affect the oxygen concentration, play an important role often dumped out to sea, extent . determining the quality of the water. Normally the greatest volume of a primarily organic in nature and is de graded by bacterial activity . Using to oxygen present in the water these wastes are broken down into the inorganic compounds .

- However as a result of this bacterial activity the gen concentration in the water is reduced . When the oxygen concentration falls below 1.5 mg / lit , the rate of aerobic oxidation is reduced and their play bacteria that can oxidize the organic molecules
- allows below 1.5 mg / lit , the rate of aerobic oxidation is reduced and their par is taken over by the anaerobic bacteria that can oxidize the organic molecules with - out the use of oxygen . This results in end products such as hydro sulphide , ammonia and methane , which are toxic to many organisms .
- This process results in the formation of an anoxic zone which is low in its oxygens content from which most life disappears except for anaerobic bacteria , fans yeasts and some protozoa . This makes the water foul smelling . Control measures : One way of reducing the pollution load on man waters is through the introduction of sewage treatment plants .
- This effluent that is required to be treated . reduce the biological oxygen demand ( BOD ) of the final product before it discharged to the receiving waters . Various stages of treatment such primary , secondary or advanced can be used depending on the quality of the screening and sedimentation to remove pollutants that will settle , and

## THERMAL POLLUTION

- **Sources:** The discharge of warm water into a river is usually called a thermal fer for cooling purposes and then returns the heated water to its source. Power plant heat water to convert it into steam, to drive the turbines that generate electricity. For efficient functioning of the steam turbines, the steam is condensed into water after it leaves the turbines.
- This condensation is done by taking water Eighter than the normal is discharged back into the water body. for a water body to absorb the heat. This heated water, which is at least 15°C: The warmer temperature decreases the solubility of oxygen and creases the metabolism of fish. This changes the ecological balance of the river.
- Within certain limits thermal additions can promote the growth of certain fish and the fish catch may be high in the vicinity of a power plant. However sudden changes in temperature caused by periodic plant shutdowns both planned and unintentional can change result in death of these fish that are acclimatized to living in warmer waters.
- Tropical marine animals are generally unable to withstand a temperature increase of 2 to 30 ° C and most sponges, mollusks and crustaceans are eliminated at temperatures above 37 C. This results in change in the diversity of fauna
- 2 to 30 ° C and most sponges, mollusks and crustaceans are eliminated at temperatures above 37°C. This results in a change in the diversity of fauna as only those species that can live in warmer water survive.
- **Control measures:** The following methods can be employed for control of thermal pollution: (1) Cooling ponds, (2) Spray Ponds & (3) Cooling towers.

- **Cooling Ponds:** Water from condensers is stored in ponds where natural evaporation cools the water which can then be recirculated or discharged in nearby water body.
- **Spray Ponds:** The water from condensers is received in spray ponds. From these fine droplets is dissipated to the atmosphere
- **Dry Cooling Tower:** The heated water flows in a system of pipes. Air is passed over these hot pipes with fans. There is no water loss in this method but installation and operation cost of dry cooling tower is many times higher than wet Cool tower.

## NUCLEAR HAZARDS

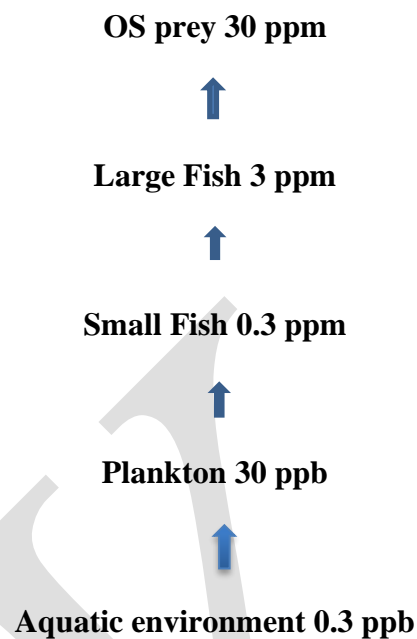
- Radioactive substances are present in nature. They undergo natural radioactive decay in which unstable isotopes spontaneously give out fast moving particles, high energy radiations or both, at a fixed rate until a new stable isotope is formed.
- The isotopes release energy either in the form of gamma rays (high energy electromagnetic or ionization particles i.e., alpha particles and beta particles. The alpha particles are fast moving positively charged particles whereas beta particles are high speed negatively charged electrons. These ionization radiations have variable penetration power.
- Alpha particles can be interrupted by a sheet of paper while beta particles can be blocked by a piece of wood or a few millimeters of aluminum sheet. The gamma rays can pass through paper and wood but can be stopped by a concrete wall,
- **Sources of Radioactivity:** Various sources of radioactivity can be grouped into **Natural Sources:** Sources of natural radioactivity include cosmic rays from outer space, radioactive radon - 222, soil, rocks, air, water and food, and (ii) **Anthropogenic** (man-made) sources. **Anthropogenic sources:** These sources are nuclear power plants, nuclear accidents, X - rays, diagnostic kits, test laboratories etc. where radioactive substances are used.
- **Effects of Radiations:** Ionization radiations can affect living organisms by causing harmful changes in the body cells and also changes at the genetic level.
- Genetic damage is caused by radiations, which induce mutations in the DNA thereby affecting genes and chromosomes.
- The damage is often seen in the offspring and may be transmitted up to several generations.
- Somatic damage includes burns, miscarriages, eye cataract and cancer of bone, thyroid, breast, lungs and skin.
- Many scientists are of the view that due to the body's ability to repair some of the damages, the adverse effects of radiations are observed only beyond a threshold level. However, the other group believes that even a small dose of radiations over a period of time may cause adverse effects.

- They believe that the permissible limits of ionizing radiations should be further reduced.
- Damage caused by different types of radiations depends on the penetration power and the presence of the source inside or outside the body . Alpha particles lack penetration power but they have more energy than beta .
- They will be therefore , dangerous when they enter the body by inhalation or through food . Alpha particles cannot penetrate the skin to reach internal organs whereas beta particles can damage the internal organs .
- Greater threat is posed by radioisotopes with intermediate half - lives as they have long time to find entry inside the human body . Radioisotopes enter the environment during mining of uranium . Tv activity in the earth's crust enters the crops grown there and ultimate in human beings .
- Radionuclides enter the water bodies or the groundwater coming in contact with the contaminated soil or rock cancer of bone marrow.
- Radioactive iodine (113) accumulates in thyroid gland and causes can Similarly, strontium - 90 accumulates in the bones and causes leukemia.
- **Control of Nuclear Pollution** : Siting of nuclear power plants should carefully done after studying long term and short term effects . Proper disposal of wastes from laboratory involving the redioisotopes should be done.

### **Biomagnifications of pesticides:**

- Biodegradability is an essential criterion for any pesticide which used in agricultural field. Different pesticides remain for different time periods in environment , some are degraded quickly and some take very long time for degradation . If the pesticide is not degraded within a short period of time , what happens to it ? If it is persistent and liophilic, on 5.3 them it will undergo " Biomagnifications " .
- Biomagnifications is the increase in concentration of a chemical in biological organisms compared to its concentration in the environment Several chlorinated hydrocarbon , insecticides have been detected in remote Arctic regions which are thousands of miles away from their nearest possible application site . Such persistent pollutants cause detrimental effects fragile ecosystems .
- Such pollutant organochlorines , even though in very much diluted concentrations like ppb ( parts per million ) can cause serious effects when their concentration increases .
- They do so because of biological magnification ; it occurs when an environmental pollutant is both persistent and liophilic . Because of their liophilic character such as compounds accumulate in lipid of prokaryotic and eukaryotic microorganisms and higher eukaryotes . The concentration of such compound may be one to three orders of magnitude higher than those in the surrounding environment . When recalcitrant

pollutant compound passes from different trophic levels in a food chain, its concentration increases up to the magnitude of  $10^4$  to  $10^6$ .



### **Biomagnification of DDT**

- The pollutant enters the microbial cell from surrounding environment and when microorganism is ingested by the members of next higher trophic level in the food chain.
- The pollutant is neither degraded nor excreted.
- Its concentration continuously increases in this manner. Once it reaches in top trophic level organisms such as concentration is increased to a dangerous level. birds of prey, carnivores, predatory fishes or saprophytes, its Such biologically magnified compounds may cause death or serious debilitation of top level animals compounds like DDT causes reproductive failure or death if biomagnified.
- Humans are at less risk because we derive our food from different trophic levels ( as we are omnivorous ) and not from only top level.
- Today the use of DDT is banned in many countries including USA but once an average American was carrying body burden of 4-6 ppm DDT without any occupational exposure.
- This concentration of DDT in human body alarming and explains the trend towards the increasing contamination of higher trophic levels.



## BIODETERIORATION OF PAPER, METAL AND PAINT

### BIODETERIORATION:

- **Biodeterioration** or **Microbial Biodeterioration** is defined as any undesirable change in the properties of a material that decrease the usefulness of that product, caused by the vital activities of microorganisms.
- H.J. Hueck defined Biodeterioration as “**any undesirable change in a material brought about by the vital activities of organisms.**”
- The level of deterioration of products is correlated with the ability of microorganisms to grow over a wide range of organic or inorganic substances.
- The fuels and oils are rich in hydrocarbon and can be easily colonized by the microbes.
- The decay and decomposition of food products brings about undesirable changes which is referred to as food spoilage.
- A slight change in the environment condition may favour the microbial growth, which is essential for the deterioration of the products.
- Fungi are comparatively more important than bacteria as the agents of biodeterioration ability of these organisms accrue huge financial loss to the humans.

#### Types of Biodeterioration

- Physical or mechanical Biodeterioration
- Aesthetic Biodeterioration
- Biochemical Assimilatory Biodeterioration
- Biochemical Dissimilatory Biodeterioration

#### 1) Biodeterioration of paper

- Paper is made in industries by pressing together moist fibres of cellulose pulp.
- The manufacture of paper has 2 main processes:
  - physical and chemical treatment of the cellulosic material for the process of separating and purifying the cellulosic material.
  - fabrication of the fibrous pulp after refinement for redistribution of the fibres in the form of paper.
- Cellulose the main component of paper is highly prone to attack of many bacteria including *Cellulomonas* and *Cellvibrio* Species.
- Enzymes that break down cellulose are cellulase, exo- $\beta$ -1,4-glucanase, endo-  $\beta$ - 1,4-glucanase and  $\beta$ -glucosidase, and hemicellulases.
- When paper is made the cellulose is delignified. Native cellulose is crystalline with some amorphous sites. Delignified cellulose has more amorphous sites and are more susceptible to attack by microorganisms.
- The porous hydrophilic structure of paper also retains water, oxygen and nutrients, providing perfect environments for bacterial growth.



- Susceptibility of paper to the attack of different microorganisms mainly depends on the physical and chemical properties of the paper.
- Components of paper other than cellulose, such as glue or casein also serve as substrate for the growth of microorganisms.
- Most of fungal species found damaging paper belong to *Ascomycota* and *Deuteromycota*.
- The dominant paper deteriorating species were *Chetomium globosum*, *Aspergillus flavus*, *Penicillium sp.*, *Cladosporium cladosporatus*, *Epicoccum perpurance*, *Alternaria alternata*, *Torula sp.*, *Trochoderma sp.*
- Due to deterioration the paper may be stained or discolored due to the growth of microbes and due to the production of microbial metabolites.
- Bacterial and yeast species can form pink slimes. Red or brown slimes are produced by certain 'iron' bacteria that produce ferric hydroxide. Certain fungi also cause blue stain.
- The growth of cellulolytic organisms on paper causes perforation in the material or weakens the fibers and in severe cases, damage the entire paper.
- High heat with low humidity causes dehydration of cellulose fibres and makes paper brittle.

## 2) Biodeterioration of metals

- Microorganisms are known to corrode metals. They make biofilm by colonization/concentration of cells, which release corrosive metabolic products resulting in removal of hydrogen by sulphate reducing bacteria.
- Actually, microbial concentration of the cells appears from oxygen gradient that develops as a microbial colony, in contact with the metal, utilizes the available oxygen.
- These colonies have both oxygen-accessible(border) and oxygen-limiting zone(centre), which are having negative and positive charge particles respectively.
- This leads to metal ion formation by producing insoluble hydroxides. There are *Sphaerotilus*, *Leptothrix*, *Gallionella* and *Siderocapsa* are iron bacteria. Iron corrosion occurs mainly due to a bacterium, *Gallionella*(chemolithotroph) that oxidizes ferrous ions to ferric ions and forms insoluble ferric hydroxide deposits at the site of microbial attack.
- *Thiobacillus* are also responsible for oxidative corrosion, because metabolically they oxidize sulfur compounds to sulphuric acid, the acid around the cell may attack alloys.
- Several groups of microorganisms play an important role in corrosion, including the sulfur bacteria, iron-manganese depositing and slime producing bacteria, fungi, and algae.
- Various organic and inorganic acids are of microbial origin for example *H. resinae* produce sulphuric acid from sulphide. The organism comes in contact of steel/iron resulting in corrosion and breakage/leakage of water pipe lines.
- Fungi produce highly corrosive metabolites including a wide range of organic acids. These acids have been shown to corrode fuel tanks. They survive well at water-fuel interfaces, metabolizing the fuel hydrocarbon as carbon and energy sources. They also are capable of generating corrosive oxidants including Hydrogen peroxide.

### 3) Biodeterioration of paint

- Paints are mainly composed of resins, pigments, filler substances, stabilizers, brighteners, surfactants, solvents and thickeners. These provide both decorative finish and protection to the underlying substrate from the effect of environmental stresses.
- Painted surfaces are not always resistant to microbial disfiguration, but the presence of water can initiate the growth of microorganisms which is usually low.
- The microorganisms derive their carbon and energy from the aliphatic and aromatic compounds for their growth.
- Microbial growth is responsible for deterioration of the paint.
- Cracking and blistering of the coated films takes place due to built up of fungal or algal films either on the painted surface or deeper in the underlying layer of the substrate.
- Chemical preservatives such as cupric dimethylthiocarbonate, copper-8-quinolinolate, Pentachlorophenol, pentachlorophenyl laureate and isothiazolinones are incorporated in the paints to prevent the biodeterioration due to fungi and algae.
- The decolouration of paints is caused by the products of microbial metabolisms resulting into modification of the organic constituents of paints.
- Several molds *Pullularia*, *Phoma*, *Aspergillus*, *Coniophora*, *Chaetomium*, *Cladosporium*, *Alternaria* and bacteria *Pseudomonas*, *Flavobacterium* cause extensive damage to the painted surface,
- Defacing of the painted wooden surface is commonly caused by molds such as *Alternaria alternata*, *Aspergillus flavus*, *Fusarium oxysporum*, *Paecilomyces*, *Trichoderma*, *Cladosporium spp.* And by algae like *Oscillatoria*, *Pleurococcus*, *Scytonema* and *Trentetoh*.

### Biobleaching

- Microbial leaching (also referred to as biobleaching or biomining) is the extraction of specific metals from their ores through the use of microorganisms, especially from the ores with low metal content Sulphur-oxidizing *Thiobacilli* are commercially employed in bio-leaching operations for the recovery of copper and uranium.
- Protocol of metal recovery from low-grade ores by biobleaching using the activity of *Thiobacillio ferroxidans* (*Acidithiobacillus ferroxidans*). *ferroxidans* is a **chemolithotrophic** bacterium that derives energy through the oxidation of either a reduced sulphur compound or ferrous iron.
- It exerts its action directly by oxidizing the metal sulphide and/ or indirectly by oxidizing the ferrous iron content of the ore to ferric iron, the ferric iron in turn chemically oxidizes the metal to be recovered by leaching.

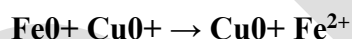
### Copper Leaching

- Biobleaching copper ores is very economical because copper sulphate, formed during oxidation of copper sulphide ores, is very water soluble.

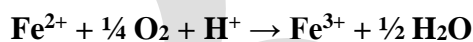
- The leaching is operated as simple heap leaching process or combination of both heap leaching and in situ leaching process. Low-grade ore or waste rock is dumped in a large pile and a dilute sulphuric acid solution (pH 2) is percolated down through the pile.
- The liquid coming out of the bottom of the pile, rich in dissolved metals, is collected and transported to precipitation plant, metal is reprecipitated and purified. Liquid is pumped back to the top of the pile and cycle is repeated. For removal of copper, the ores commonly used are chalcocite ( $\text{Cu}_2\text{S}$ ), chalcopyrite ( $\text{CuFeS}_2$ ) or covellite ( $\text{CuS}$ ).
- The most important reaction in copper leaching operations involves chemical oxidation of the copper ore with ferric ions formed by the bacterial oxidation of ferrous ions. Ferric iron is an excellent electron acceptor for sulphide minerals and the spontaneous reaction of  $\text{CuS}$  with ferric iron results in solubilization of copper and the formation of ferrous iron as shown in the reaction below.



- The soluble copper from the leaching solution is recovered at the precipitation plant. Scrap iron  $\text{Fe}^0$ , is generally added to recover copper from the leach liquid by the reaction shown below:



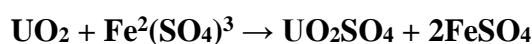
- The liquid containing  $\text{Fe}^{2+}$  ions is pumped to the oxidation pond where *Acidithiobacillus ferrooxidans* grows and oxidizes it to  $\text{Fe}^{3+}$



- Sulphuric acid is added to maintain the low pH and the liquid is allowed to pump to the top of the pile and the ferric iron being used to oxidize more sulphide mineral. Because of the high temperature associated with some leaching operations, thermophilic sulphur oxidizing bacteria such as *Sulfolobus* obligate thermophilic acid tolerant archaea, is used in bio-leaching metal sulphides.

## Uranium Leaching

- Uranium that is used as a fuel in nuclear power generation is recovered by microbial bioleaching from low-grade uranium deposits and from low-grade nuclear wastes by using the bacterium *Thiobacillus ferrooxidans*. In the pyrite containing uranium ores, the  $\text{Fe}^{3+}$  may be produced and regenerated at lower cost by using *T. ferrooxidans*. The bacterium oxidizes the ferrous iron in pyrite ( $\text{FeS}$ ), which often accompanies uranium ores, to ferric iron which in turn acts as an oxidant to convert  $\text{UO}_2$ , (tetravalent uranium, insoluble oxide) chemically to leachable  $\text{UO}_2\text{SO}_4$  (hexavalent uranium, soluble sulphate). The reaction involved is as follows:



## Gold Leaching

- Gold is found in nature intermixed with and enclosed by arsenopyrite. *T. ferroxidans* solubilizes the arsenopyrite minerals, releasing the trapped gold (Au) as shown in the reaction below:



- After the leaching process by *T. ferroxidans*, the gold is then complexed with cyanide by traditional gold mining methods. Gold leaching takes place in small bioreactor tanks where arsenic is removed as a ferric precipitate and cyanide by its microbial oxidation to carbon dioxide and urea in later stages of the gold recovery process. Because both the reactor and the heap leaching process generate heat that can inhibit the activity of *T. ferroxidans*, *Sulfolobus* (thermophilic Archaea) is being substituted for, or is used in combination with *T. ferroxidans*.

## Bio enhanced oil recovery

- Biobleaching of oil shales has potential, to enhance the recovery of hydrocarbons. Many oil shales contain large number of carbonates and pyrites and the removal of these minerals increases the porosity of the shale, enhancing recovery of the oil.
- Acid dissolves the carbonates and can be produced by *Thiobacillus* species growing on the sulphur and iron in the pyrite. Such microbial leaching appears to have the potential for making recovery of hydrocarbons from oil shales economically feasible.
- The tertiary recovery of petroleum, that is the use of biological and chemical means to enhance oil recovery, and the enhanced recovery of hydrocarbons from oil shales are important since readily recoverable oil supplies have diminished. Tertiary recovery of oil employs solvents, surfactants, and polymers to dislodge oil from geological formations.
- The use of tertiary recovery methods has the potential of recovering 60 – 120 billion barrels of oil in united states reserves alone that otherwise could not be recovered.
- Xanthan gums produced by bacteria, such as *Xanthomonas campestris*, are promising compounds for the tertiary recovery of oil.
- These polymers have high viscosity and flow characteristics that allow them to pass through small pores in the rock layers containing oil deposits. When added during water flooding operations, that is when water is pumped into petroleum reservoirs to force out the oil, xanthan gums help push the oil toward the production wells.
- These polymers are produced by conventional fermentation processes in which *X. campestris* is grown and the xanthan gums are recovered.

## **MICROBIAL TECHNOLOGY FOR SUSTAINABLE ENVIRONMENT**

### **Bio Fuels**

- Biofuels are the liquid or gaseous fuels derived from the living things, or from the fermenting biomass and the waste they produce. Microbial fuel produced can contribute to meeting world energy requirements.
- Emphasis is on the production of liquid biofuels such as ethanol and methanol via fermentation of agricultural products such as sugarcane, wheat, wood, corn and agricultural wastes.
- The current emphasis is on the production of liquid biofuel from fast growing grasses grown especially for liquid biofuel production. In many countries' gasoline is routinely supplemented with ethanol produced by microbial fermentation.
- Many waste - treatment plants and landfills capture methane produced by methanogenic archaea and use the methane to generate electricity. Hydrogen production is being developed as potential automatic fuel that would alleviate global warming due to carbon dioxide production. Three important biofuels are ethanol produced by *Zymomonas* and *Thermotoga*, methane by *Methanobacterium* and hydrogen by photosynthetic microorganisms.
- Biomass is the total cellular dry weight or organic material produced by an organism usually from CO<sub>2</sub>, and sunlight. Biomass includes wood, crops, herbaceous plants, residues from agriculture and forest products, animal waste etc. The use of biomass may be by direct burning to generate (as dung cakes or wood used for cooking), thermochemical conversion by pyrolysis (destructive distillation of wood to yield coal and gasification (thermal degradation of carbonaceous material to yield high amount of gases) and by biological conversions involving conversions of organic material into more useful energy forms. i.e., gaseous or liquid fuels by using microbes. Biological conversion includes anaerobic digestion to yield methane (CH<sub>4</sub>) and fermentation to produce ethanol.
- The biofuels have several desirable features, such as:
  - they are ecofriendly being derived from biomass, which is renewable.
  - they have low cost and are easily available saving lot of foreign exchange for the developing countries the substrate used for biofuels production is often a waste which not only generates a more valuable product from low-cost substrate but also help in cleaning up the environment.

### **Ethanol**

- Fermentation of carbohydrates to ethanol by yeasts is well - known in the production of many alcoholic beverages such as wine and beer. Gasohol --the mixture of gasoline - ethanol (9:1) is an efficient fuel lowering the release of atmospheric hydrocarbon

pollutants and can be used without any engineering modification of the automobile engine.

- The various alcohol producing bacteria are: *Clostridium acetobutylicum* , *Leuconostoc mesenteroides* , *Zymomonas mobilis* , *Sarcina ventriculi* , and *Klebsiella pneumoniae* . The fungi include *Aspergillus oryzae*, *Saccharomyces cerevisiae* and *Neurospora crassa*.
- Ethanol can be produced from a variety of feedstocks such as sugar cane, bagasse, miscanthus, sugar beet, sorghum, grain sorghum, switchgrass, barley, hemp, kenaf, potatoes, sweet potatoes cassava, sunflower, fruit, molasses, corn, stover, grain, wheat, straw, cotton, other biomass, as well as many types of cellulose waste and harvestings.
- Commercial bioethanol production is primarily from sugar cane, maize (corn ) and sugar beets. Five countries have developed bioethanol fuel programs Brazil, Colombia, China, Sweden and the United States.
- Ethanol is normally produced from simple carbohydrates by yeasts. However, the yeasts used in commercial products are unable to attack complex polymers , and it is usually necessary initially to degrade plant polymers with enzymes produced by the plant itself or by microbes other than yeast.
- The coculture of *Aspergillus niger* , an amylolytic mold with *Saccharomyces cerevisiae* convert potato starch wastes to ethanol is known . Similarly , if cellulosic materials are used for ethanol production it will be necessary initially to catalyze the breakdown to this polymer to simple carbohydrates before yeasts or genetically engineered microorganisms ( GEMs ) with the accessory enzymes can be utilized for the production of ethanol, the basic steps for large scale production of ethanol are : microbial fermentation of sugars , distillation , dehydration and denaturing . Prior to fermentation , some crops require saccharification or hydrolysis into carbohydrates.
- Saccharification of cellulose is called cellulolysis. Other preproduction steps can be necessary for certain like corn which requires refinement into starch and liquification.
- Fermentation of ethanol is carried out in a large fermentor . The inoculum of microorganisms maintained in fermentor at the optimum growth conditions such as temperature , pH oxygen and concentration of carbohydrates , the substrates . Before starting the fermentation , pure inoculum ( Starter inoculum ) of *Saccharomyces cerevisiae* is prepared by inoculating the well - defined and sterilised medium .
- At the same time , fermentation medium is formulated , sterilized and transferred to the sterile fermentor. Liquid media in fermentor is inoculated with small amount of inoculum of yeast. Growth conditions of liquid broth is maintained to provide optimum conditions such as temperature , pH and oxygen for the production of ethanol.
- After incubation at optimum growth conditions for different periods , the culture fluid is filtered when growth of the microorganism is over . From the supernatant , products are recovered and purified.



- Recovery of ethanol from the fermentation broth is by distillation which exploits the difference in boiling points of ethanol ( 87 ) and water ( 100C ) As a result when water ethanol mixture having 95 % ethanol is heated , vapour has a greater concentration of ethanol than the liquid phase .
- This dilute ethanol water mixture can be repeatedly distilled to obtain more concentrated ethanol . The principle of sequential distillation is used in a cylindrical distillation column which is divided into a series of chamber by perforated plates The ethanol - water mixture is boiled using steam and vapour rises into column Essentially , each chamber of the column functions as a distillation unit so that the proportion of alcohol goes on sequentially increasing as it rises to the upper chamber of the column A properly designed column would yield 95 % ethanol from its top most chamber.

## **Biodiesel**

- Biodiesel is a renewable fuel that is made from methyl or ethyl ester of fatty acids ( made from pure or used vegetable oils edible and nonedible , animal fats and algae ) or from hydrocarbons produced by some plant and algae .
- Biodiesel is biodegradable and nontoxic , and typically produces about 60 % less net carbon dioxide emissions than petroleum - based diesel , as it is itself produced from atmospheric carbon dioxide via photosynthesis in plants .
- Biodiesel is a light to dark yellow liquid . It is practically immiscible with water , has a high boiling point and low vapor pressure . Typical methyl ester biodiesel has a flash point of - 150 C ( 300 ° F ) . making it rather nonflammable Biodiesel has a density of - 0.88 g / cm<sup>3</sup> , less than that of water .
- Biodiesel uncontaminated with starting material can be regarded as nontoxic . It has a viscosity similar to petrodiesel ( diesel produced from petroleum ) . It can be used as an additive in formulations of diesel to increase the lubricity of pure ultralow sulphur diesel ( ULSD ) fuel which is advantageous because it has virtually no sulphur content .
- Biodiesel can be used in pure form or may be blended with petroleum diesel at any concentration in most modern diesel engines . Pure , nonblended biodiesel can be poured straight into the tank of any diese vehicle . Biodiesel can also be used as a heating fuel in domestic and commercial boilers .
- A variety of oils such as soybean and rapeseed can be used to produce biodiesel . Soybean oil alone accounts for about ninety percent of all fuel stocks used for producing biodiesel . Other crops such as mustard , flax , sunflower , canola , palm oil , hemp , and algae are capable of producing biodiesel .the waste vegetable oil (wVO) can also be used as a substrate for its production. Animal fats including tallow, lard , yellow grease , chicken fat , and the by - products of the production of Omega - 3 fatty acids form fish oil are other example of substrates. Cultivation of *jatropha* plant of the family Euphorbiaceae is being emphasized for the production of biodiesel .

## Biogas

- Biogas is the gas produced by anaerobic microorganisms, primarily methane. It is also known as swamp gas, marsh gas, landfill gas or digester gas produced through methanogenesis (biological production of methane) by a group of strictly anaerobic Archaea called the methanogens.
- It is an extremely important fuel source used in the generation of mechanical, electrical and heat (energy) (eg. *Methanotracterium*, *Methanococcus* and *Methanosarcina*).
- It can be used as a fuel source for homes and industry and can also be converted by microbial action or chemical means to methanol, which can be used as a fuel in internal combustion engines.
- However, methanogenesis can be an ecological problem as methane absorbs infrared radiation and thus is a greenhouse gas and its overproduction in nature may significantly promote future global warming.
- In the biogas digestors, the biodegradable feed stock is converted into two useful products: gas and digestate. The digestate comprises manure and the remnants of the anaerobic microorganisms.
- One of the most promising ways of methane production is through the bioconversion of waste materials where methane is a normal byproduct. The production of methane is carried out by a methane mixed microbial community. Of these, some microbes break the organic molecules and, processes used to generate methane as a fuel from polymeric waste materials.
- complex polymers and fatty acids into hydrogen, carbon dioxide and alcohols. Methanogens utilize these fermentation products for methane production.
- Methanogenesis is commonly carried out by microbial populations involved in synergistic and mutualistic relationships. The evolution of methane from anaerobic digestors and other bioconversion processes occurs simultaneously with the evolution of carbon dioxide.
- The ratios of two gases, ie, methane and carbon dioxide depend on the chemical composition of the substrate and the environmental conditions under which the bioconversion is carried out. Biogas is generally composed of 50-75% methane, 25-50% CO<sub>2</sub>, 0-10% nitrogen, 0-1% hydrogen, 0-3% H<sub>2</sub>S and 2% oxygen.
- In India, cowdung is widely used to produce biogas, locally called gobar gas. Two types of biogas plants used are floating gas holder type biogas plant and fixed dome type biogas plant. Biogas plant is mainly based on the design of the digester, an airtight tank in which anaerobic decomposition is carried out.
- It is well-shaped tank inside the ground, made up of concrete bricks and cement.



There lies a cylindrical container above the digester to collect the gas, which is made up of having a valve, from which gas is taken out and used for cooking and other purposes.

- The slurry so prepared is fed in the digester through the inlet pipe and left for fermentation and the spent slurry is collected from the outlet pipe.
- Several microorganisms are involved in anaerobic digestion and biogas production. The breaking down of the complex organic materials is achieved through hydrolysis, acidification and methanogenesis. The biological production of methane occurs through a series of reactions involving novel coenzymes as methanofuran, methanopterin, Coenzyme M<sub>b</sub> (Coenzyme F<sub>130</sub> and F<sub>420</sub>). Methanogens mainly use carbon dioxide and acetate for methane production.
- During organic compounds such as alcohols can be oxidized to yield electrons for CO<sub>2</sub> reduction. The major steps involved in CO<sub>2</sub> reduction by H<sub>2</sub> followed in sequence are reduction of CO<sub>2</sub> to formyl, reduction of formyl to methylene and then to methyl - reduction of methyl group to methane.
- When acetate is the substrate for methanogenesis, it is first activated to Acetyl CoA. The latter acts with carbon monoxide dehydrogenase of the Acetyl CoA pathway. Then the methyl group of acetate is transferred to the Corrinoid enzyme to yield methyl Corrinoid, and from there it goes through the CoM mediated terminal step of methanogenesis. Simultaneously CO group is oxidized to CO<sub>2</sub>.
- Biogas production from cowdung is affected by various factors. Maximum gas production results when the pH is between 6 and 7, temperature 35°C, C / N ratio between 20 and 30, dung / water ratio 1:1, optimum loading rate of raw materials and normal retention time. Addition of Zymogenium algae in the digester increases biogas production.

## Hydrogen

- Hydrogen can be produced by various microorganisms and could become a major fuel source in the coming days.
- Hydrogen produced as an end product from the fermentation of carbohydrates by bacteria has less energy (approximately 33%) compared to methane (85%) from organic matter. Phototrophic organisms have the capacity of biophotolysis, that is the production of hydrogen from water using solar energy significantly in higher amounts in contrast to mixed acid bacterial fermentation.
- The phototrophic organisms belong to photosynthetic bacteria. The bacterium *Desulfovibrio gigas* has a hydrogenase that functions as a catalyst for hydrogen production and consumption. *Anabaena variabilis*, a cyanobacterium, converts fructose to hydrogen and CO<sub>2</sub> in a light-dependent reaction when incubated in the presence of argon, an inert gas.

- The concentration of fructose increases the production of hydrogen . Phototrophic bacteria , such as *Rhodospirillum rhodosseudomonas* can be used for production of hydrogen from waste materials .

## BIOPLASTIC

- **Bioplastics** are plastic materials produced from renewable biomass sources, such as vegetable fats and oils, corn starch, straw, woodchips, sawdust, recycled food waste, etc. Bioplastic can be made from agricultural by-products and also from used plastics (i.e. plastic bottles and other containers) by using microorganisms. Bioplastics are usually derived from sugar derivatives, including starch, cellulose, and lactic acid. Common plastics, such as fossil-fuel plastics (also called petrobased polymers) are derived from petroleum or natural gas.
- As of 2014, bioplastics represented approximately 0.2% of the global polymer market (300 million tons). Although bioplastics are not commercially significant, research continues on this topic.
- In addition to being decoupled from the petrochemical industry, bioplastics are attractive because they are biodegradable. Not all bioplastics are biodegradable nor biodegrade more readily than commodity fossil-fuel derived plastics.
- **IUPAC definition**
  - Bioplastic is misleading because it suggests that any polymer derived from the biomass is *environmentally friendly*.
  - The use of the term "bioplastic" is discouraged. Use the expression "biobased polymer".
  - A biobased polymer similar to a petrobased one does not imply any superiority with respect to the environment unless the comparison of respective *life cycle assessments* is favourable.

## TYPES OF BIO PLASTIC

### Starch-based plastics

- Thermoplastic starch represents the most widely used bioplastic, constituting about 50 percent of the bioplastics market. Simple starch bioplastic film can be made at home by gelatinizing starch and solution casting. Pure starch is able to absorb humidity, and is thus a suitable material for the production of drug capsules by the pharmaceutical sector. However, pure starch-based bioplastic is brittle. Plasticizer such as glycerol, glycol, and sorbitol and can also be added so that the starch can also be processed thermo-plastically. The characteristics of the resulting bioplastic (also called "thermoplastic starch") can be tailored to specific needs by adjusting the amounts of these additives. Conventional polymer processing techniques can be used to process starch into bioplastic, such as extrusion, injection molding, compression molding and solution casting.[17] The properties of starch bioplastic is largely influenced by amylose/amylopectin ratio.

Generally, high-amylose starch results in superior mechanical properties. However, high-amylose starch has less processibility because of its higher gelatinization temperature and higher melt viscosity.

- Starch-based bioplastics are often blended with biodegradable polyesters to produce starch/polylactic acid, starch/polycaprolactone or starch/Ecoflex (polybutylene adipate-co-terephthalate produced by BASF) blends. These blends are used for industrial applications and are also compostable. Other producers, such as Roquette, have developed other starch/polyolefin blends. These blends are not biodegradable, but have a lower carbon footprint than petroleum-based plastics used for the same applications.
- Starch is cheap, abundant, and renewable.
- Starch-based films (mostly used for packaging purposes) are made mainly from starch blended with thermoplastic polyesters to form biodegradable and compostable products. These films are seen specifically in consumer goods packaging of magazine wrappings and bubble films. In food packaging, these films are seen as bakery or fruit and vegetable bags. Composting bags with this films are used in selective collecting of organic waste. Further, starch-based films can be used as a paper.
- Starch-based nanocomposites have been widely studied, showing improved mechanical properties, thermal stability, moisture resistance, and gas barrier properties.

### **Cellulose-based plastics**

- A packaging blister made from cellulose acetate, a bioplastic
- Cellulose bioplastics are mainly the cellulose esters, (including cellulose acetate and nitrocellulose) and their derivatives, including celluloid.
- Cellulose can become thermoplastic when extensively modified. An example of this is cellulose acetate, which is expensive and therefore rarely used for packaging. However, cellulosic fibers added to starches can improve mechanical properties, permeability to gas, and water resistance due to being less hydrophilic than starch.
- A group at Shanghai University was able to construct a novel green plastic based on cellulose through a method called hot pressing.

### **Protein-based plastics**

- Bioplastics can be made from proteins from different sources. For example, wheat gluten and casein show promising properties as a raw material for different biodegradable polymers.
- Additionally, soy protein is being considered as another source of bioplastic. Soy proteins have been used in plastic production for over one hundred years. For example, body panels of an original Ford automobile were made of soy-based plastic.

- There are difficulties with using soy protein-based plastics due to their water sensitivity and relatively high cost. Therefore, producing blends of soy protein with some already-available biodegradable polyesters improves the water sensitivity and cost.

### **Some aliphatic polyesters**

- The aliphatic biopolyesters are mainly polyhydroxyalkanoates (PHAs) like the poly-3-hydroxybutyrate (PHB), polyhydroxyvalerate (PHV) and polyhydroxyhexanoate (PHH).

### **Polylactic acid (PLA)**

- Mulch film made of polylactic acid (PLA)-blend bio-flex
- Polylactic acid (PLA) is a transparent plastic produced from maize or dextrose. Superficially, it is similar to conventional petrochemical-based mass plastics like PS. It has the distinct advantage of degrading to nontoxic products. Unfortunately it exhibits inferior impact strength, thermal robustness, and barrier properties (blocking air transport across the membrane). PLA and PLA blends generally come in the form of granulates with various properties, and are used in the plastic processing industry for the production of films, fibers, plastic containers, cups and bottles. PLA is also the most common type of plastic filament used for home fused deposition modeling.

### **Poly-3-hydroxybutyrate**

- The biopolymer poly-3-hydroxybutyrate (PHB) is a polyester produced by certain bacteria processing glucose, corn starch or wastewater. Its characteristics are similar to those of the petroplastic polypropylene. PHB production is increasing. The South American sugar industry, for example, has decided to expand PHB production to an industrial scale. PHB is distinguished primarily by its physical characteristics. It can be processed into a transparent film with a melting point higher than 130 degrees Celsius, and is biodegradable without residue.

### **Polyhydroxyalkanoates**

- Polyhydroxyalkanoates are linear polyesters produced in nature by bacterial fermentation of sugar or lipids. They are produced by the bacteria to store carbon and energy. In industrial production, the polyester is extracted and purified from the bacteria by optimizing the conditions for the fermentation of sugar. More than 150 different monomers can be combined within this family to give materials with extremely different properties. PHA is more ductile and less elastic than other plastics, and it is also biodegradable. These plastics are being widely used in the medical industry.

### **Polyamide 11**

- PA 11 is a biopolymer derived from natural oil. It is also known under the tradename Rilsan B, commercialized by Arkema. PA 11 belongs to the technical polymers family and is not biodegradable. Its properties are similar to those of PA 12, although emissions

of greenhouse gases and consumption of nonrenewable resources are reduced during its production. Its thermal resistance is also superior to that of PA 12. It is used in high-performance applications like automotive fuel lines, pneumatic airbrake tubing, electrical cable antitermite sheathing, flexible oil and gas pipes, control fluid umbilicals, sports shoes, electronic device components, and catheters.

- A similar plastic is Polyamide 410 (PA 410), derived 70% from castor oil, under the trade name EcoPaXX, commercialized by DSM. PA 410 is a high-performance polyamide that combines the benefits of a high melting point (approx. 250 °C), low moisture absorption and excellent resistance to various chemical substances.

### **Bio-derived polyethylene**

- *Main article: Renewable Polyethylene*
- The basic building block (monomer) of polyethylene is ethylene. Ethylene is chemically similar to, and can be derived from ethanol, which can be produced by fermentation of agricultural feedstocks such as sugar cane or corn. Bio-derived polyethylene is chemically and physically identical to traditional polyethylene – it does not biodegrade but can be recycled. The Brazilian chemicals group Braskem claims that using its method of producing polyethylene from sugar cane ethanol captures (removes from the environment) 2.15 tonnes of CO<sub>2</sub> per tonne of Green Polyethylene produced.

### **Genetically modified feedstock**

- With GM corn being a common feedstock, it is unsurprising that some bioplastics are made from this.
- Under the bioplastics manufacturing technologies there is the "plant factory" model, which uses genetically modified crops or genetically modified bacteria to optimise efficiency.

### **Polyhydroxyurethanes**

- The condensation of polyamines and cyclic carbonates produces polyhydroxyurethanes. Unlike traditional cross-linked polyurethanes, cross-linked polyhydroxyurethanes are in principle amenable to recycling and reprocessing through dynamic transcarbamoylation reactions.

### **Lipid derived polymers**

- A number bioplastic classes have been synthesized from plant and animal derived fats and oils. Polyurethanes, polyesters, epoxy resins and a number of other types of polymers have been developed with comparable properties to crude oil based materials. The recent development of olefin metathesis has opened a wide variety of feedstocks to economical conversion into biomonomers and polymers. With the growing production of traditional vegetable oils as well as low cost microalgae derived oils, there is huge potential for growth in this area.

### Proposed applications

- Flower wrapping made of PLA-blend bio-flex
- Bioplastics are used for disposable items, such as packaging, crockery, cutlery, pots, bowls, and straws. Few commercial applications exist for bioplastics. Cost and performance remain problematic. Typical is the example of Italy, where biodegradable plastic bags and shoppers are compulsory since 2011 with the introduction of a specific law. Beyond structural materials, electroactive bioplastics are being developed that promise to carry electric current.
- Biopolymers are available as coatings for paper rather than the more common petrochemical coatings.
- Bioplastics called **drop-in bioplastics** are chemically identical to their fossil-fuel counterpart but made from renewable resources. Examples include bio-PE, bio-PET, bio-propylene, bio-PP, and biobased nylons. Drop-in bioplastics are easy to implement technically, as existing infrastructure can be used. A dedicated bio-based pathway allows to produce products that can not be obtained through traditional chemical reactions and can create products which have unique and superior properties, compared to fossil-based alternatives.

## BIO FERTILIZERS

### INTRODUCTION

- A biofertilizer is a substance which contains living microorganisms, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply of availability of primary nutrients to the host plant, Bio-fertilizers add nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth-promoting substances.
- Biofertilizers are natural fertilizers that are microbial inoculants of bacteria, algae and fungi (separately or in combination) which may help biological nitrogen fixation for the benefit of plants. They help build up the soil micro-flora and thereby the soil health.
- Biofertilizer also include organic fertilizers (manure, etc.) Use of bio-fertilizer is recommended for improving the soil fertility in organic farming



## TYPES OF BIOFERTILIZERS



### Bacterial, Fungal, Algal, Aquatic fern, Earthworms

#### Bacteria:

- Symbiotic nitrogen fixers.
- *Rhizobium*, *Azospirillum* spp
- Free living nitrogen fixers.
- *Azotobacter*, *Klebsiella* etc.,

#### Algal biofertilizers:

- BGA in association with *Azolla*
- *Anabena*, *Nostoc*, *Oscillatoria*

#### Phosphate solubilising bacteria:

- *Pseudomonas*, *Bacillus megaterium*

#### Fungal biofertilizer

- VAM

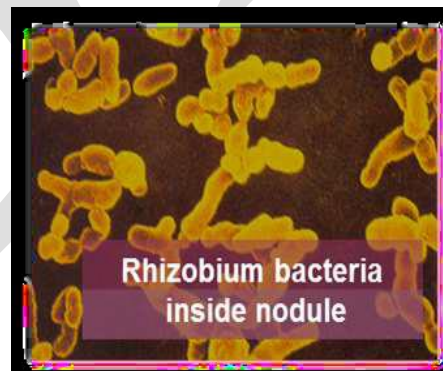
#### Earthworms

### Bacterial biofertilizers

- The live cells of bacteria used as a biofertilizers These microbes contains unique gene called as Nif-Gene which make them capable of fixing nitrogen The nitrogen fixing bacteria work under two conditions, Symbiotically Free living bacteria (non-symbiotic).
- The symbiotic bacteria make an association with crop plants through forming nodules in their roots. The free living bacteria do not form any association but live freely and fix atmospheric nitrogen. **Symbiotic nitrogen fixers.** Most important symbiotic Nitrogen fixing bacteria is *Rhizobium* and *Azospirillum*.

#### *Rhizobium*:

- *Rhizobium* lives in the root hairs of the legumes by forming nodules
- Plant root supply essential minerals and newly synthesized substance to the bacteria
- The name *Rhizobium* was established by Frank in 1889.
- This genus has seven distinct species based on "Cross Inoculation Group Concept".
- More than twenty cross-inoculations groups have been established.
- A new classification has been established for *Rhizobium*.
- That is 'slow growing rhizobia' known as *Bradyrhizobium* and the other group is 'fast growing rhizobia' called *Rhizobium*.
- *Rhizobium* can fix **50-300** kg/ha

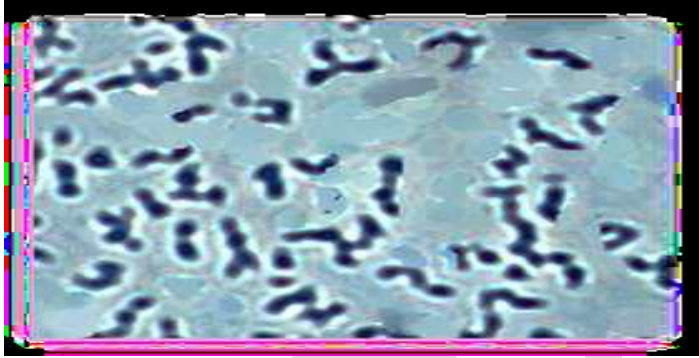


#### *Azospirillum*:

- It mainly present in cereal plants.
- inhabits both root cells as well as surrounding of roots forming symbiotic relation and increasing nitrogen fixing potential of the cereal plant.
- Azospirillum is recognized as a dominant soil microbe



- nitrogen in the range of 20- 40 kg/ha in the rhizosphere in non-leguminous plants such as cereals, millets, Oilseeds, cotton etc.
- Considerable quantity of nitrogen fertilizer up to 25-30 % can be saved by the use of *Azospirillum* inoculant.
- These species have been commercially exploited for the use as nitrogen supplying Bio-Fertilizers.



### Free living bacteria

- Large number of free living or non -symbiotic bacteria (does not form nodules but makes association by living in the rhizosphere) present in soil.
- Commonly used free living bacteria are *Azotobacter* *Klebsiella* it will not associated with plant.
- *Azotobacter* is a biofertilizer which provides the required amount of nitrogen to the plant from the soil.

### *Azotobacter*

- *Azotobacter* is a heterotrophic free living nitrogen fixing bacteria present in alkaline and neutral soils.
- *Azotobacter* is the most commonly occurring species in arable soils of India.
- Apart from its ability to fix atmospheric nitrogen in soils, it can also synthesize growth promoting substances such as auxins and gibberellins and also to some extent the
- vitamins.
- Many strains of *Azotobacter* also exhibit fungicidal properties against certain species of fungus.
- Response of *Azotobacter* has been seen in rice, maize, cotton, sugarcane, pearl millet, vegetable and some plantation crops.

- It improves seed germination and plant growth. *Azotobacter* is heaviest breathing organism and requires a large amount of organic carbon for its growth.

### Mass production

- isolated bacterial cultures were subculture in to nutrient broth
- The cultures were grown under shaking condition at  $30\pm 2^{\circ}\text{C}$
- The culture incubated until it reaches maximum cell population of  $10^{10}$  to  $10^{11}$
- Under optimum condition this population level could be attained within 4-5 days for *Rhizobium* 5-7 days for *Azospirillum* and 6-7 days for *Azotobacter*.
- The culture obtained in the flask is called **Starter culture**
- For large scale production , inoculum from starter culture is transferred in to large flasks / fermentor and grown until required level of cell count is reached prepare appropriate media for specific to bacterial Inoculated with specific bacterial strain for aseptic condition Incubated at  $30\pm 2^{\circ}\text{C}$  for 5-7 days in rotary shaker Observe growth of the culture and estimate the population ( starter culture) The above the media is prepared in large quantities in fermentor Sterilized and cooled well Media in a fermentor is inoculated with the log phase of culture grown in large flask (usually 1-2 % of inoculum is sufficient) cells are grown in fermentor by providing aeration & continuous
- Stirring Broth is checked for the population of inoculated organisms Cells are harvested with the population load of  $10^9$  cells/ml

### Carrier material

- the use of ideal carrier material is necessary for the production of good quality of biofertilizer Peat soil, lignite, vermiculture, charcoal, press mud, farmyard manure and soil mixture are used as a carrier materials Neutralized peat soil/lignite are found to be better carrier materials Ideal carrier material should be
- **Cheaper in cost**
- **Locally available**
- **High organic matter content**
- **No toxic chemical**
- **Water holding capacity of more than 50%**
- **Easy to process**

**Preparation of inoculants packet**

- Neutralized and sterilized carrier material is spread in a clean, dry, sterile metallic or plastic
- Bacterial culture drawn from the fermentor is added to the sterilized carrier and mixed well by manual or mechanical mixer
- Inoculants are packed in a polythene bags sealed with electric sealer

**Specification of the polythene bags**

- Polythene bags should be of low density grade
- Thickness of bag should be around 50-75 micron
- Packet should be marked with the
  - **Name of the manufacture**
  - **Name of the product**
  - **Strain number**
  - **The crops to which recommended**
  - **Method of inoculation**
  - **Date of manufacture**
  - **Batch number**
  - **Date of expiry**
  - **Price**
  - **Full address**
  - **storage instruction**

**Vesicular Arbuscular Mycorrhiza (VAM)**

- The term mycorrhiza was taken from Greek language meaning '**fungus root**'. term was coined by Frank in 1885 The mycorrhiza is a mutualistic association between fungal mycelia and plant roots. VAM is an endotrophic (live inside) mycorrhiza formed by aseptated phycomycetous fungi. VAM help in **nutrient transfer** mainly of phosphorus, zinc and sulfur.



- Mycorrhizae is the symbiotic association between plant roots and soil fungus of the 7 types of mycorrhizae, VAM plays a great role in inducing plant growth. VAM are symbiotic entophytic soil fungi, which colonize the roots of approximately 80% plants.
- The VAM hyphae also help in retaining moisture around the root zone of plants
- It increases the resistance to root borne or soil borne pathogens and Nematodes.
- They also **mobilize different nutrients** like Cu(copper), K(potassium), Al(aluminum), Mn(manganese), Fe (iron) and Mg (magnesium) from the soil to the plant roots. They possess vesicles (sac like structure) for storage of nutrients and arbuscular for funneling them into root system.
- **Morphology**
  - External hyphae
  - Arbuscles
  - Vesicles



### **Mechanism of Action**

- The VAM forms an association with plant roots. It **penetrates in the root cortex** and spreads around the roots of the plant. As the name indicates, they possess sac like

structure called **vesicles** which stores phosphorus as phospholipids. The other structure called arbuscule helps bringing the distant nutrients to the vesicles and root.

### Mechanism of Action

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- Soil in the pot along with roots of host plant is macerated Dried till it attains 5% moisture
- Dried soil inoculants used for field application

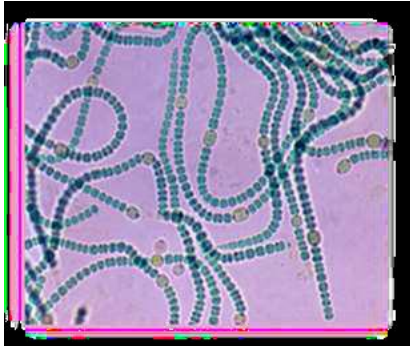
### Uses of VAM

- Enhances the feeding areas of the plant root is as the
- hyphae spreads around the roots.
- Mobilizes the nutrients from distance to root.
- Stores the nutrients (sp. phosphorus).
- Removes the toxic chemicals (example : phenolics) which otherwise hinder nutrient availability.
- Provide protection against other fungi and nematodes
- It increase growth rate in plants (*citrus, maize, wheat, etc.*)
- It reduces sensitivity of crop towards high level of salts and heavy metals

### Algae as a biofertilizer

- Another group of free living nitrogen fixers are cyanobacteria. Commonly called as **Blue green algae**.
- More than 100 species of BGA can fix nitrogen. Nitrogen fixation takes place in specialized cells called '**Heterocyst**' BGA very common in rice field. Unlike *Azotobacter* BGA are not inhibited by the presence of chemical fertilizers. No chemical fertilizers added, inoculation of the algae can result in 10-14% increase in crop yields.
- They are easy to produce Usually they are mass produced in cement tanks filled with
- fresh water. Not require any processing Quite and cheap Cost of 10kg may be Rs.30-40 only Beneficial in certain crops like vegetables, cotton, sugarcane. Eg. of some algal biofertilizers are
- *Anabena*

- □ *Nostoc*
- □ *Oscillatoria*



### **Azolla as a bio fertilizer**

- Azolla is a tiny fresh water fern common in ponds, ditches and rice fields. It has been used as a biofertilizer for a rice in all major rice growing countries including **India, Thailand, Korea, Philippines, Brazil and West Africa**. The nitrogen fixing work is
- accomplished by the symbiotic relationship between the fern and BGA,
- ***Anabena azollae***.
- In addition to nitrogen the decomposed Azolla also provides K, P, Zn and Fe to the
- Crop



- *Azolla* biomass gets doubled within 5-7 days by vegetative methods. fix 40-80 kg nitrogen / ha / year. good manure for flooded rice. Increase of crop yield up to 15-20% has been observed while fertilizing the rice with *Azolla* Hybrids are growing faster Tolerant to heat and cold Fix 4-5% more nitrogen

### **Bio - fertilizers application methods**

- There are three ways of using these N-fixing/P.S.M. bacteria.
- Seed treatment
- Root dipping



- Soil applications
- Seed Treatment
- Seed treatment is a most common method adopted for all types of inoculant. The seed treatment is effective and economic.
- Seed treatment with *Rhizobium*, *Azotobacter*, *Azospirillum* along with *P.S.M.*
- seed treatment can be done with any of two or more bacteria. no side effect. important things has the seeds must be coated first with *Rhizobium* or *Azotobacter* or *Azospirillum* when each seeds get a layer of above bacteria then the P.S.M. inoculant has to be treated on outer layer of the seeds. This method will provide maximum number of population
- of each bacteria required for better results. Mixing the any of two bacteria and the treatment of seed will not provide maximum number of bacteria of individuals.

### Root dipping

- Application of *Azospirillum* with the paddy/vegetable plants this method is needed. The required quantity of *Azospirillum* has to be mixed with 5-10 ltr of water at one corner of the field and all the plants have to kept for minimum ½ an hour before sowing .

### Soil application

- P.S.M. has to be used as a soil application use 2 kgs of P.S.M. per acre. Mix P.S.M. with 400 to 600 kgs of Cowdung along with ½ bag of rock phosphate if available. The mixture of P.S.M., Cowdung and rock phosphate have to be kept under any tree shade or ceiling for over night and maintain 50% moisture. Use the mixture as a soil application in rows or during leveling of soil.

### Precautions

- Store biofertilizer packets in cool and dry place away from direct sunlight and heat.
- Use right combination of biofertilizers *Rhizobium* is crop specific, so use in specified crop
- Do not mix with chemicals Use the packet before expiry, only on the specified crop, by
- the recommended method.

### Advantage of biofertilizers

- Renewable source of nutrients
- Sustain soil health
- Supplement chemical fertilizers.

- Replace 25-30% chemical fertilizers
- Increase the grain yields by 10-40%.
- Decompose plant residues, and stabilize C:N ratio of soil
- Improve texture, structure and water holding capacity of soil
- No adverse effect on plant growth and soil fertility.
- Stimulates plant growth by secreting growth hormones.
- Secrete fungistatic and antibiotic like substances
- Solubilize and mobilize nutrients
- Eco-friendly, non-pollutants and cost effective method

### **Disadvantages**

- Biofertilizers require special care for long-term storage because they are alive.
- must be used before their expiry date.
- If other microorganisms contaminate the carrier medium or if growers use the wrong strain, they are not as effective.
- Biofertilizers lose their effectiveness if the soil is too hot or dry.