BHARATHIDASAN UNIVERSITY

Tiruchirappalli- 620024, Tamil Nadu, India

Programme: M.Sc., Biotechnology(Environment)

Course Title :ENVIRONMENTAL BIOTECHNOLOGY

Course Code : CC08

Unit-II

BIOREMEDIATION

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Bioremediation: Fundamentals, Methods, and Strategies of Application

- Bioremediation is the use of biological organisms, particularly microorganisms, plants, or fungi, to degrade, detoxify, or remove pollutants from the environment. This process exploits the natural abilities of organisms to break down hazardous substances into less harmful compounds, often converting them into harmless end-products like water, carbon dioxide, or biomass. Bioremediation is considered an environmentally friendly, cost-effective, and sustainable method for managing pollution, especially in contaminated soils, water, and air.

- Fundamentals of Bioremediation
- Bioremediation is based on the principles of microbial metabolism and ecological processes. The process utilizes the natural metabolic pathways of microorganisms (bacteria, fungi, algae, etc.) that are capable of degrading or transforming contaminants into less toxic substances. It relies on the following key concepts:
- **Microbial Degradation**: Microorganisms break down organic pollutants (e.g., petroleum hydrocarbons, pesticides, solvents) into simpler, less harmful compounds. Some microorganisms use contaminants as a source of energy and carbon, while others use them as electron donors or acceptors in their metabolic processes.
- **Enzyme Activity**: Microorganisms produce enzymes that catalyze the breakdown of pollutants. These enzymes, such as oxidases, reductases, and hydrolases, help degrade complex molecules like hydrocarbons, heavy metals, and organic pollutants into simpler substances.
- **Nutrient Availability**: For effective bioremediation, microorganisms require essential nutrients (nitrogen, phosphorus, and other minerals) for growth and metabolism. Adequate nutrient supply is crucial to maintaining microbial activity during the bioremediation process.
- **Environmental Conditions**: Factors such as temperature, pH, oxygen levels, and moisture content play a crucial role in the activity of microorganisms involved in bioremediation. Optimizing these factors can significantly enhance the process.

- Methods of Bioremediation
- Bioremediation can be categorized into different methods based on the approach, environment, and organisms used. The two primary approaches are **in situ** (treatment at the contamination site) and **ex situ** (removal and treatment of contaminated material).
- *In Situ Bioremediation*
- In situ bioremediation refers to the treatment of contaminated material directly at the site of pollution without the need for removal or transport. This approach is often used when the contamination is widespread and removal would be impractical or too costly.
- **Natural Attenuation**: This method relies on the natural processes of biodegradation without any human intervention. Microorganisms present in the environment naturally degrade pollutants over time. This approach is suitable for sites where contamination is relatively low and the natural microbial community is capable of handling the pollution.
- **Biostimulation**: Biostimulation involves enhancing the natural microbial population's activity by adding nutrients (e.g., nitrogen, phosphorus) or other substances that encourage the growth and activity of native microbes. By optimizing environmental conditions, biostimulation accelerates the degradation of pollutants.
- **Phytoremediation**: Plants, particularly certain species of hyperaccumulators, can absorb, degrade, or immobilize pollutants from the soil or water. Plants can also release root exudates that promote microbial activity and increase bioremediation effectiveness.

- *Ex Situ Bioremediation*
- Ex situ bioremediation involves removing contaminated material (soil, water, sludge) from the site for treatment in a controlled environment. This approach is used when in situ bioremediation is not feasible or when faster treatment is required.
- **Biopiles**: Contaminated soil is excavated, placed in piles, and treated with the addition of nutrients, oxygen, or moisture to enhance microbial activity. Aeration systems are often used to promote oxygen supply for aerobic microbes, accelerating pollutant degradation.
- **Land Farming**: This technique involves spreading contaminated soil on a prepared surface, allowing microorganisms to degrade the contaminants. The soil is periodically tilled to aerate and provide oxygen to microbes.
- **Bioreactors**: A controlled environment where contaminated water or soil is treated by microorganisms. Bioreactors can be continuously or intermittently fed with nutrients and oxygen to optimize microbial degradation. Bioreactors are commonly used to treat wastewater or liquid contaminants.
- **Composting**: Organic waste, including contaminated soil, is mixed with organic matter such as crop residues and composted. The heat generated during composting can also help degrade contaminants, while microorganisms in the compost break down pollutants.

- Strategies of Bioremediation Application
- Two widely used strategies in bioremediation are **biostimulation** and **bioaugmentation**. Both are aimed at enhancing the natural ability of microorganisms to degrade pollutants but differ in their approach.
- *Biostimulation*
- Biostimulation involves modifying the environment to enhance the activity of indigenous (native) microorganisms in the contaminated site. The goal is to stimulate the growth and activity of naturally occurring microbial populations capable of degrading the contaminants.

- **Mechanism**:
- **Nutrient Addition**: The primary method of biostimulation is the addition of nutrients (e.g., nitrogen, phosphorus, potassium) that are limiting the growth of microorganisms. Nutrients help boost microbial activity and increase the rate of contaminant degradation.
- **Oxygen Supply**: In the case of aerobic bioremediation, oxygen is often added to enhance microbial oxidation of contaminants. This can be done by aerating soil or water.
- **Electron Donors or Acceptors**: For anaerobic conditions, electron donors (e.g., molasses, acetate) or electron acceptors (e.g., sulfate, nitrate) may be added to facilitate microbial redox reactions and promote pollutant degradation.
- **Applications**:
- **Oil Spill Cleanup**: In the case of oil spills, nutrients like nitrogen and phosphorus are added to encourage the growth of hydrocarbon-degrading bacteria.
- **Heavy Metal Reduction**: Biostimulation can be used to enhance microbial reduction of heavy metals like chromium or mercury, converting them into less toxic forms.

- *Bioaugmentation*
- Bioaugmentation is the process of adding specially cultured, pollutant-degrading microorganisms to the contaminated site to enhance bioremediation. The microorganisms added are often selected for their ability to degrade specific contaminants that native microbes may not effectively degrade.
- **Mechanism**:
- **Inoculation of Specific Microorganisms**: The introduced microorganisms may include bacteria, fungi, or algae that possess specific metabolic pathways or enzymes capable of degrading certain pollutants.
- **Genetically Modified Microorganisms (GMMs)**: In some cases, genetically engineered microorganisms with enhanced pollutant-degrading capabilities may be introduced. These microorganisms are designed to degrade specific contaminants more efficiently.
- **Applications**:
- **Oil Spill Cleanup**: Specialized oil-degrading bacteria can be introduced into contaminated marine environments to enhance oil degradation.
- **Pesticide Degradation**: Bioaugmentation can be used to introduce bacteria that can degrade persistent organic pollutants like pesticides or herbicides in agricultural soils.
- **Toxic Waste Treatment**: Bioaugmentation is employed in the treatment of industrial waste sites, where specific microbes are added to degrade toxic chemicals such as solvents, chlorinated hydrocarbons, or polycyclic aromatic hydrocarbons (PAHs).

Feature	Biostimulation	Bioaugmentation
Mechanism	Enhances the activity of native microbes	Adds specific, pollutant-degrading microorganisms
Microorganisms	Native to the environment	Non-native or specially cultured strains
Nutrient Supply	Involves the addition of nutrients and oxygen	Involves the introduction of selected microorganisms
Cost	Generally lower as it utilizes indigenous microbes	Can be more expensive due to microbial cultivation and introduction
Suitability	Effective for moderate contamination levels	Useful for sites with specific, persistent pollutants
Environmental Impact	Minimal, as it relies on native organisms	Can introduce non-native species into the environment
Timeframe	Generally slower, depending on microbial growth	Faster, depending on the introduced microorganisms

Bioremediation of Metals, Radionuclides, and Organic Pollutants

- Bioremediation refers to the use of microorganisms, plants, or enzymes to degrade or transform contaminants into less harmful substances. The bioremediation of metals, radionuclides, and organic pollutants is a promising environmental strategy, particularly in areas of industrial pollution and hazardous waste sites. Different pollutants require different approaches due to their unique chemical properties and biological interactions. This note provides a detailed discussion on the bioremediation of metals (Cr, As, Se, Hg), radionuclides (U, Te), and organic pollutants (PAHs, PCBs, Pesticides, TNT).

- Bioremediation of Metals
- Certain heavy metals like chromium (Cr), arsenic (As), selenium (Se), and mercury (Hg) are toxic to both humans and ecosystems. They can persist in the environment for long periods due to their non-biodegradable nature, making their bioremediation crucial for environmental health.
- *Chromium (Cr)*
- **Chromium in the Environment**: Chromium exists mainly in two oxidation states, Cr(VI) (hexavalent) and Cr(III) (trivalent). Cr(VI) is highly toxic, soluble, and carcinogenic, while Cr(III) is less toxic and insoluble.
- **Bioremediation Mechanisms**:
  - **Reduction**: Microorganisms such as *Shewanella* and *Geobacter* species can reduce Cr(VI) to Cr(III) using various electron donors (e.g., organic carbon sources). This process reduces the toxicity and mobility of chromium.
  - **Bioaccumulation**: Some microorganisms can accumulate chromium, particularly in the form of Cr(III), in their cells. This process can remove chromium from contaminated water and soil.
  - **Precipitation**: Microbial reduction of Cr(VI) to Cr(III) often results in the precipitation of Cr(III) as insoluble compounds like chromite, effectively immobilizing the metal.

- *Arsenic (As)*
- **Arsenic in the Environment**: Arsenic exists mainly in two forms: inorganic arsenic (As(III) and As(V)) and organic arsenic compounds. Inorganic arsenic is highly toxic and carcinogenic, especially in drinking water.
- **Bioremediation Mechanisms**:
  - **Reduction**: Several bacteria, including *Desulfovibrio* and *Pseudomonas*, can reduce arsenate (As(V)) to arsenite (As(III)), which is more toxic but less mobile. In some cases, microorganisms can further oxidize As(III) to As(V) or transform it into less toxic forms.
  - **Methylation**: Certain microorganisms (e.g., *Saccharomyces cerevisiae* and *Pseudomonas spp.*) can methylate inorganic arsenic, producing methylated arsenic species that are less toxic and more easily eliminated from the environment.
  - **Bioaccumulation**: Some plants and microorganisms can accumulate arsenic in their biomass, reducing its concentration in the environment. Hyperaccumulators like *Pteris vittata* (a fern) can absorb arsenic from contaminated soils and water.
- *Selenium (Se)*
- **Selenium in the Environment**: Selenium exists in several oxidation states, including Se(IV) (selenite), Se(VI) (selenate), and elemental selenium (Se^{o}). Se(IV) and Se(VI) are toxic in high concentrations.
- **Bioremediation Mechanisms**:
  - **Reduction**: Microorganisms such as *Bacillus* and *Pseudomonas* can reduce selenate (Se(VI)) and selenite (Se(IV)) to elemental selenium (Se^{0}), which is less toxic and insoluble, allowing it to precipitate out of solution.
  - **Bioaccumulation**: Certain plants and microorganisms can accumulate selenium in their tissues, especially in its elemental form. Some plant species, like *Astragalus bisulcatus*, are hyperaccumulators of selenium.

- *Mercury (Hg)*
- **Mercury in the Environment**: Mercury exists in several forms, including elemental mercury (Hg^0), inorganic mercury (Hg^{2+}), and organic mercury (methylmercury). Methylmercury is highly toxic and biomagnifies in the food chain.
- **Bioremediation Mechanisms**:
  - **Reduction**: Microorganisms such as *Desulfovibrio* and *Geobacter* can reduce mercury salts (Hg^{2+}) to elemental mercury (Hg^0), which is less toxic and can volatilize, escaping into the atmosphere.
  - **Methylation**: Some bacteria, such as *Geobacter sulfurreducens*, can methylate mercury, producing methylmercury, which is more toxic and bioaccumulates in aquatic organisms. However, this process is generally avoided in bioremediation efforts due to the toxicity of methylmercury.
  - **Bioaccumulation**: Plants and microorganisms can also accumulate mercury, particularly in its non-toxic forms like elemental mercury (Hg^0).

- . Bioremediation of Radionuclides
- Radionuclides, such as uranium (U) and tellurium (Te), are radioactive elements that pose serious environmental and health risks. Bioremediation of radionuclides is an emerging field that leverages the abilities of microorganisms to transform or immobilize these pollutants.
- *Uranium (U)*
- **Uranium in the Environment**: Uranium primarily exists in two forms: U(VI) (hexavalent uranium) and U(IV) (tetravalent uranium). U(VI) is highly soluble and mobile, whereas U(IV) is insoluble and tends to precipitate in the environment.
- **Bioremediation Mechanisms**:
  - **Reduction**: Microorganisms like *Shewanella* and *Geobacter* reduce U(VI) to U(IV), leading to the precipitation of uranium as insoluble uraninite (UO_2). This process significantly reduces uranium mobility and toxicity.
  - **Bioaccumulation**: Some microorganisms and plants can bioaccumulate uranium in their biomass, effectively removing it from contaminated water and soil.
- *Tellurium (Te)*
- **Tellurium in the Environment**: Tellurium is a rare element that is toxic to organisms in its oxidized forms (Te(VI) and Te(IV)) but can be reduced to less toxic forms like elemental tellurium (Te^o).
- **Bioremediation Mechanisms**:
  - **Reduction**: Certain bacteria, including *Pseudomonas* and *Bacillus*, can reduce tellurium oxyanions (Te(VI) and Te(IV)) to elemental tellurium (Te^0), which precipitates out of solution and is less toxic.
  - **Bioaccumulation**: Plants and microorganisms can accumulate tellurium in their tissues, potentially providing a strategy for bioremediation.

- Bioremediation of Organic Pollutants
- Organic pollutants, including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides, and explosives (TNT), are persistent and toxic contaminants that pose serious environmental risks. Microorganisms play a critical role in degrading these compounds into less harmful substances.
- *Polycyclic Aromatic Hydrocarbons (PAHs)*
- **PAHs in the Environment**: PAHs are organic compounds composed of fused aromatic rings. They are hydrophobic, persistent, and often carcinogenic, arising from incomplete combustion of organic matter.
- **Bioremediation Mechanisms**:
  - **Degradation**: Bacteria such as *Pseudomonas*, *Mycobacterium*, and *Rhodococcus* are capable of degrading PAHs through enzymatic pathways that break the aromatic rings, leading to simpler and less toxic products.
  - **Bioaugmentation**: In cases where PAH degradation is slow, specialized PAH-degrading strains can be introduced to speed up the bioremediation process.

- *Polychlorinated Biphenyls (PCBs)*
- **PCBs in the Environment**: PCBs are synthetic chemicals composed of carbon, hydrogen, and chlorine. They are persistent pollutants, often found in industrial waste, and are toxic to aquatic life and humans.
- **Bioremediation Mechanisms**:
  - **Dechlorination**: Certain bacteria, including *Dehalococcoides* and *Burkholderia*, can remove chlorine atoms from PCB molecules, reducing their toxicity and making them more biodegradable.
  - **Aerobic and Anaerobic Degradation**: Bacteria can degrade PCBs both in the presence of oxygen (aerobic) and in its absence (anaerobic), using different enzymatic pathways.
- *Pesticides*
- **Pesticides in the Environment**: Pesticides, including organophosphates, organochlorines, and carbamates, are toxic chemicals used to control pests but can cause harm to non-target species and ecosystems.
- **Bioremediation Mechanisms**:
  - **Hydrolysis**: Certain bacteria and fungi can hydrolyze pesticides, breaking them down into less toxic compounds. For example, *Pseudomonas* species can degrade organophosphate pesticides through enzymatic hydrolysis.
  - **Cometabolism**: Some microorganisms degrade pesticides by co-metabolizing them with other substrates, even if they do not use the pesticide as their primary carbon source.

- *TNT (Trinitrotoluene)*
- **TNT in the Environment**: TNT is a toxic explosive chemical that contaminates military sites and other industrial locations. It is highly persistent and carcinogenic.
- **Bioremediation Mechanisms**:
  - **Reductive Degradation**: Certain bacteria, such as *Sphingomonas* species, can reduce TNT by enzymatically transforming the nitro groups, leading to the formation of less toxic compounds.
  - **Co-metabolism**: Some microorganisms can degrade TNT in the presence of other organic compounds through co-metabolism, enhancing the degradation process.

Technological Aspects of Bioremediation (In Situ and Ex Situ)

- Bioremediation is an innovative and environmentally friendly technique that uses microorganisms, plants, or enzymes to degrade, transform, or detoxify contaminants, particularly in polluted soil, water, and air. The application of bioremediation can be broadly classified into two main categories: **in situ** and **ex situ** bioremediation. These approaches differ primarily in how they address the polluted environment — whether treatment occurs at the site of contamination (in situ) or after removal to a controlled environment (ex situ).
- Both in situ and ex situ bioremediation methods are tailored to the type of contaminants, the location of contamination, and the environmental conditions, and each has its own technological aspects that influence its efficiency and effectiveness.

- In Situ Bioremediation
- **In situ bioremediation** involves treating contaminated materials directly at the site of contamination without removing the material from its location. This approach is often used when the contamination is widespread or when it is not feasible or cost-effective to excavate or transport contaminated soil or water.
- *Technological Aspects of In Situ Bioremediation*
- **Natural Attenuation (Natural Bioremediation)**
  - **Concept**: This method relies on the natural processes of microbial degradation that already exist in the environment. No external intervention (e.g., nutrient addition, aeration) is required beyond monitoring.
  - **Technology**: Monitoring the site to assess the natural microbial populations and their ability to degrade contaminants. It's often used for low levels of contamination that are easily degraded by indigenous microorganisms.
  - **Applications**: Suitable for contaminants like low levels of petroleum hydrocarbons, pesticides, or metals that are already subject to natural biodegradation processes.

- **Biostimulation**
  - **Concept**: Biostimulation enhances the activity of indigenous microorganisms by providing additional nutrients or environmental conditions conducive to microbial growth and activity.
  - **Technology**:
    - **Nutrient Addition**: Nutrients such as nitrogen and phosphorus may be added to contaminated sites to stimulate the growth of microorganisms that can break down organic contaminants.
    - **Oxygen or Electron Donor Addition**: In cases where oxygen is limiting, oxygen may be added via aeration systems (e.g., injection of oxygen into groundwater) or through the addition of electron donors like molasses or acetate in anaerobic environments.
  - **Applications**: Used for the treatment of hydrocarbons, chlorinated solvents, and other organic contaminants in groundwater, soils, and sediments.
- **Phytoremediation**
  - **Concept**: The use of plants to remove, degrade, or immobilize contaminants from the soil or water. Certain plants have the ability to accumulate heavy metals, degrade organic contaminants, or even transform pollutants.
  - **Technology**:
    - **Phytoextraction**: Plants take up contaminants from the soil into their tissues. For example, hyperaccumulator plants can absorb metals like lead, arsenic, or cadmium.
    - **Phytodegradation**: Plants break down organic contaminants through processes like enzymatic degradation in their tissues.
    - **Phytostabilization**: Plants immobilize contaminants in the soil or water, preventing their spread.
  - **Applications**: Used for remediation of metal-contaminated soils, petroleum hydrocarbon contamination, and other toxic chemicals in the soil or water.

- **Bioaugmentation**
  - **Concept**: This involves the introduction of specific, pollutant-degrading microorganisms to a contaminated site to enhance the biodegradation process.
  - **Technology**:
    - **Microbial Inoculation**: Cultures of microorganisms that are known to degrade specific contaminants are added to the contaminated environment.
    - **Monitoring**: Regular monitoring of the microbial population and contamination levels is necessary to track the success of bioaugmentation.
  - **Applications**: Commonly used for specific pollutants like petroleum hydrocarbons, heavy metals, or chlorinated solvents, especially when natural microbial populations are insufficient for effective bioremediation.
- **Bioventing**
  - **Concept**: Bioventing is a type of in situ bioremediation that involves enhancing the natural biodegradation of contaminants in soil by supplying oxygen and controlling moisture levels.
  - **Technology**:
    - **Aeration Systems**: An air supply system is installed in the contaminated area to introduce air and oxygen into the subsurface, promoting aerobic microbial activity.
    - **Moisture Control**: The soil moisture is carefully regulated to optimize microbial growth and degradation.
  - **Applications**: Primarily used for the treatment of petroleum hydrocarbons, such as oil spills and diesel fuel contamination in soils.

- Ex Situ Bioremediation
- **Ex situ bioremediation** refers to the removal of contaminated material from its location and treating it in a controlled environment where optimal conditions for microbial growth and pollutant degradation can be maintained.
- *Technological Aspects of Ex Situ Bioremediation*
- **Biopiles**
  - **Concept**: Biopiles involve the excavation of contaminated soil, which is then placed in piles, aerated, and treated with nutrients to promote the microbial breakdown of pollutants.
  - **Technology**:
    - **Aeration**: An aeration system is used to provide oxygen to microorganisms that degrade organic contaminants.
    - **Moisture Control**: Water is added to maintain the correct moisture levels for microbial activity.
    - **Nutrient Addition**: Nutrients such as nitrogen and phosphorus are added to enhance microbial growth and pollutant degradation.
  - **Applications**: Used for the treatment of petroleum hydrocarbons, solvents, and some pesticides in soils.

- **Land Farming**
  - **Concept**: Contaminated soil is spread over a prepared surface, allowing for natural microbial degradation through aeration and nutrient management.
  - **Technology**:
    - **Tilling**: The soil is regularly tilled or turned to enhance oxygen availability for microorganisms.
    - **Nutrient Addition**: Organic or inorganic nutrients are added to enhance microbial degradation.
    - **Moisture Control**: Water is added as needed to ensure proper moisture levels.
  - **Applications**: Suitable for treating organic contaminants such as petroleum products, pesticides, and solvents in soil.
- **Bioreactors**
  - **Concept**: Bioreactors are closed systems where contaminated material (e.g., wastewater, soil) is treated in a controlled environment. The material is exposed to microorganisms that degrade pollutants under optimal conditions.
  - **Technology**:
    - **Batch or Continuous Systems**: Bioreactors can be operated in batch mode (treating a fixed amount of contaminated material) or continuous mode (ongoing treatment of a stream of contaminated material).
    - **Aeration and Mixing**: These systems typically include mechanisms for aeration and mixing to ensure that microorganisms have enough oxygen and that the contaminated material is uniformly exposed to the microbial population.
    - **Nutrient and pH Control**: Nutrient levels and pH are carefully controlled to maximize microbial activity.
  - **Applications**: Widely used for the treatment of industrial wastewater, oil spills, or contaminated soil.

- **Composting**
  - **Concept**: Composting is a biological process that uses organic material to create conditions that favor the growth of microorganisms capable of breaking down contaminants. Contaminated soil or organic waste is mixed with organic matter and allowed to decompose under aerobic conditions.
  - **Technology**:
    - **Aeration**: The mixture is regularly turned to ensure oxygen availability.
    - **Temperature Control**: The composting process generates heat, which can be controlled to ensure optimal microbial activity.
    - **Moisture and Nutrient Management**: Regular moisture and nutrient additions are made to enhance degradation.
  - **Applications**: Primarily used for the remediation of organic contaminants such as hydrocarbons, pesticides, and agricultural wastes.
- **Constructed Wetlands**
  - **Concept**: Constructed wetlands use plants, soil, and microorganisms to treat wastewater or contaminated water by promoting microbial degradation of pollutants.
  - **Technology**:
    - **Vegetation**: Wetland plants, such as cattails and reeds, are planted in the system to provide habitat for microorganisms that break down pollutants.
    - **Substrate and Microbial Activity**: The substrate (soil or gravel) supports microbial activity, facilitating pollutant degradation.
    - **Water Flow Management**: Water is directed through the constructed wetland, where contaminants are removed by plant roots, microbial degradation, and adsorption to soil particles.
  - **Applications**: Used for the treatment of agricultural runoff, industrial wastewater, and stormwater.

Feature	In Situ Bioremediation	Ex Situ Bioremediation
Location of Treatment	Treatment at the site of contamination	Contaminated material is removed and treated elsewhere
Cost	Generally lower, as no excavation or transportation is required	Higher due to excavation, transport, and treatment infrastructure
Speed	Slower, as it relies on natural processes	Faster, as conditions can be optimized for microbial activity
Environmental Impact	Minimal disruption of the surrounding environment	Greater disruption, as soil or water is moved and treated off-site
Applications	Suitable for widespread contamination or groundwater, suitable for large-scale, low-to-moderate contamination	Used for highly contaminated sites or when fast remediation is needed
Examples	Bioventing, phytoremediation, biostimulation	Biopiles, land farming, bioreactors, composting

REFERENCE

- 1. Environmental Biotechnology: Theory and Applications, G. M. Evans and J. C. Furlong (2003), Wiley Publishers.
- 2. Environmental Biotechnology: Principle & Applications, B.Ritmann and P. L. McCarty, (2000), 2nd Ed., McGraw Hill Science.
- 3. Environmental Biotechnology, Scragg
- 4.Biofiltration for Air Pollution Control, J. S. Devinny, M. A. Deshusses and T. S. Webster, (1998), CRC Press.
- 5. Biotechnology – A Multi-Volume Comprehensive Treatise, H. J. Rehm and G. Reed, (2001), Vol. 11, 2nd Ed., VCH Publishers Inc.A., (2005), Pearson Education Limited.


Thank you!