ENVIRONMENTAL & INDUSTRIAL BIOTECHNOLOGY

UNIT I

Types of Environmental Pollution

Air Pollution

Air pollution involves the introduction of harmful substances into the atmosphere, which can cause adverse effects on human health, the environment, and the climate. Major pollutants include particulate matter (PM), nitrogen oxides (NOx), sulfur dioxide (SO2), carbon monoxide (CO), volatile organic compounds (VOCs), and heavy metals.

Water Pollution

Water pollution is the contamination of water bodies such as rivers, lakes, oceans, and groundwater. Major sources of water pollution include industrial discharge, agricultural runoff, sewage, and chemical spills. Pollutants include heavy metals, organic pollutants, pathogens, nutrients (nitrogen and phosphorus), and plastics.

Control of Air Pollution through Biotechnology

Biofilters

Biofilters are systems that use microorganisms to degrade pollutants in the air. They consist of a support medium (such as compost or wood chips) where microorganisms grow and form a biofilm. Contaminated air passes through the biofilter, and the microorganisms metabolize the pollutants.

Bio scrubbers

Bio scrubbers are air pollution control devices that use a liquid phase (usually water) to capture and remove pollutants. The contaminated air is bubbled through the liquid, where soluble pollutants are absorbed. Microorganisms in the liquid phase then degrade these pollutants.

Bio trickling Filters

Bio trickling filters combine the principles of biofilters and bio scrubbers. In these systems, contaminated air passes through a packed bed, which is continuously irrigated with a liquid. The pollutants are absorbed by the liquid and degraded by the biofilm of microorganisms growing on the packing material.

Water Pollution and Its Management

Measurement of Water Pollution

The measurement of water pollution involves various physical, chemical, and biological assessments:

- Physical Parameters: Temperature, turbidity, color, and suspended solids.
- Chemical Parameters: pH, dissolved oxygen (DO), chemical oxygen demand (COD), biological oxygen demand (BOD), nutrients (nitrates, phosphates), and the presence of heavy metals and organic pollutants.

- Biological Parameters: Coliform bacteria count, presence of pathogens, and bioindicators such as certain algae or invertebrates.

Sources of Water Pollution

- Industrial Discharge: Effluents from factories and manufacturing plants.
- Agricultural Runoff: Pesticides, fertilizers, and animal waste washed into water bodies.
- Sewage: Domestic wastewater containing human waste, detergents, and other household chemicals.
- Chemical Spills: Accidental releases of hazardous substances.

Microbiology of Waste Water Treatment

Aerobic Processes

- Activated Sludge: A process where air or oxygen is pumped into wastewater to support the growth of aerobic bacteria that consume organic pollutants. The sludge formed (biomass) is then settled out.
- Oxidation Ponds (Lagoons): Large, shallow ponds where wastewater is treated through the interaction of sunlight, algae, and microorganisms. Oxygen is provided through photosynthesis and natural aeration.
- Trickling Filters: Consist of a bed of coarse material (rocks, gravel, or plastic media) on which microorganisms grow. Wastewater is trickled over the media, and the biofilm on the media degrades the organic matter.
- Rotating Biological Contactors (RBCs): Consist of a series of closely spaced disks mounted on a rotating shaft. The disks are partially submerged in wastewater, and as they rotate, a biofilm develops on the surface. The microorganisms in the biofilm degrade organic pollutants as the disks rotate through the wastewater.

Anaerobic Processes

- Anaerobic Digesters: Closed containers where organic waste is broken down by anaerobic bacteria, producing biogas (mainly methane and carbon dioxide) and digestate (a nutrient-rich residue).
- Upward Flow Anaerobic Sludge Blanket (UASB) Reactors: Wastewater is introduced at the bottom of the reactor and flows upward through a blanket of granular sludge. The microorganisms in the sludge degrade the organic pollutants anaerobically, producing biogas.

UNIT II

Biodegradation and Bioremediation

Concepts & Principles of Bioremediation

Biodegradation:

- The process by which organic substances are broken down by living organisms, primarily microorganisms.
- Occurs naturally in the environment, driven by bacteria, fungi, and other microorganisms that utilize organic pollutants as a food source, breaking them down into simpler, less toxic compounds.

Bioremediation:

- The use of microorganisms, plants, or microbial or plant enzymes to detoxify and remove pollutants from the environment.
- Aims to restore contaminated sites to their original state or make them safe for use.

Principles of Bioremediation

- **Microbial Metabolism**: Microorganisms metabolize contaminants, using them as energy sources and converting them into harmless products.
- **Environmental Conditions**: Factors such as temperature, pH, oxygen availability, and nutrient levels affect the efficiency of bioremediation.
- **Bioavailability**: The extent to which pollutants are accessible to microorganisms for degradation.
- **Bioremediation Strategies**:
    - **In Situ**: Treatment of contaminated material at the site. Examples include bioventing, biosparging, and phytoremediation.
    - **Ex Situ**: Removal of contaminated material to be treated elsewhere. Examples include biopiles, landfarming, and composting.

Bioremediation of Hydrocarbons

Applications

- **Oil Spill Cleanup**: Use of microorganisms to degrade oil hydrocarbons in marine and terrestrial environments.
- **Contaminated Soil Treatment**: Application of bioremediation techniques to remove hydrocarbons from soil.
- **Groundwater Remediation**: Use of bioremediation to treat hydrocarbon-contaminated groundwater.

Techniques

- **Bio stimulation**: Addition of nutrients or electron acceptors to stimulate the activity of indigenous microorganisms.
- **Bioaugmentation**: Introduction of specific microorganisms known to degrade hydrocarbons to the contaminated site.

Degradation of Pesticides and Other Toxic Chemicals by Microorganisms

Pesticide Degradation

- **Microbial Degradation**: Specific bacteria, fungi, and algae can break down pesticides into non-toxic compounds.
- **Enzymatic Breakdown**: Microbial enzymes such as hydrolases, oxidases, and reductases play a crucial role in degrading pesticides.

Degradation of Other Toxic Chemicals

- **Heavy Metals**: Certain microorganisms can transform or immobilize heavy metals through processes like biosorption and biomineralization.
- **Polychlorinated Biphenyls (PCBs)**: Bioremediation strategies involve aerobic and anaerobic degradation using specific microbial consortia.

Role of Genetically Engineered Microbes

Genetically Engineered Microorganisms (GEMs)

- **Enhanced Degradation**: GEMs are designed to possess specific pathways for the breakdown of pollutants that are difficult to degrade naturally.
- **Bioaugmentation**: Introduction of GEMs into contaminated sites to improve bioremediation efficiency.
- **Examples**:
    - **Pseudomonas putida**: Engineered to degrade various hydrocarbons and aromatic compounds.
    - **Deinococcus radiodurans**: Engineered to detoxify heavy metals and organic solvents.

Concept of Phytoremediation

Phytoremediation

- **Definition**: The use of plants to remove, stabilize, or degrade contaminants from soil and water.
- **Mechanisms**:
    - **Phytoextraction**: Plants absorb contaminants through their roots and accumulate them in their tissues.
    - **Phytodegradation**: Plants break down contaminants through metabolic processes.
    - **Phyto stabilization**: Plants immobilize contaminants in the soil, preventing their spread.

   - **Rhizofiltration**: Plant roots absorb contaminants from water.
   - **Phytovolatilization**: Plants take up contaminants and release them into the atmosphere as less harmful substances.
- **Applications**: Heavy metal removal, treatment of contaminated water, and soil remediation.

Environmental Safety Guidelines

Guidelines for Safe Bioremediation Practices

- **Risk Assessment**: Evaluation of potential risks associated with bioremediation, including the impact on human health and the environment.
- **Regulatory Compliance**: Adherence to local, national, and international regulations governing bioremediation activities.
- **Monitoring and Control**: Regular monitoring of bioremediation processes to ensure effectiveness and prevent unintended consequences.
- **Containment and Mitigation**: Measures to contain and mitigate the spread of contaminants during bioremediation.
- **Public Awareness and Engagement**: Informing and involving the public in bioremediation projects to ensure transparency and address community concerns.

UNIT III

Biofuels are renewable fuels derived from biological sources, such as plants, algae, or organic waste. They are used as alternatives to fossil fuels and are considered environmentally friendly due to their lower carbon footprint.

Types of Biofuels

- **Biogas**: Produced through anaerobic digestion of organic matter.
- **Bioethanol**: Alcohol produced by fermenting sugars from crops such as corn or sugarcane.
- **Biodiesel**: Produced from vegetable oils or animal fats through a chemical process called transesterification.

Biogas

Production Process

Biogas is produced through the anaerobic digestion of organic materials in the absence of oxygen. The process involves the following steps:

1. **Feedstock Preparation**: Organic materials such as agricultural residues, animal manure, sewage sludge, and food waste are collected and prepared.
2. **Digestion**: Microorganisms break down the organic matter in a sealed, oxygen-free environment, producing biogas.
3. **Biogas Composition**: Mainly methane (CH_4) and carbon dioxide (CO_2), with trace amounts of other gases like hydrogen sulfide (H_2S) and ammonia (NH_3).

Microbial Groups Involved

- **Methanogens**: Anaerobic bacteria that produce methane as a metabolic byproduct.
- **Acidogens**: Convert complex organic molecules into simpler organic acids.
- **Acetogens**: Convert organic acids into acetate and hydrogen.
- **Hydrolytic Bacteria**: Break down complex organic molecules into simpler compounds that can be further digested.

Interactions in Biogas Production

- **Syntrophic Relationships**: Cooperative interactions between different microbial groups where each performs a specific step in the breakdown of organic matter.
- **Competition**: Microbes compete for nutrients and substrates, influencing the efficiency of biogas production.
- **Inhibition**: Factors such as pH, temperature, and substrate composition can inhibit microbial activity and biogas production.

Factors Affecting Biogas Production

- **Temperature**: Optimal temperature ranges (typically 35-55°C) for different microbial groups.
- **pH**: Maintaining a neutral pH (around 7) to support microbial activity.
- **Retention Time**: Adequate time for microorganisms to digest organic material and produce biogas.
- **Substrate Composition**: Ratio of carbon to nitrogen (C/N ratio) and the presence of inhibitors or toxic substances in the feedstock.

Biofertilizers

Definition

Biofertilizers are microorganisms (bacteria, fungi, or algae) applied to soil or plants to enhance nutrient availability and soil fertility.

Types of Biofertilizers

- **Nitrogen-Fixing Bacteria**: Convert atmospheric nitrogen into a usable form for plants.
- **Phosphate-Solubilizing Bacteria**: Release phosphate from insoluble compounds, making it available to plants.
- **Mycorrhizal Fungi**: Form symbiotic relationships with plant roots, enhancing nutrient uptake.
- **Azotobacter and Azospirillum**: Nitrogen-fixing bacteria commonly used as biofertilizers.

Benefits

- **Enhanced Nutrient Availability**: Improves nitrogen, phosphorus, and potassium uptake by plants.
- **Soil Health**: Increases organic matter content, improves soil structure, and suppresses soil-borne diseases.
- **Environmental Sustainability**: Reduces reliance on chemical fertilizers and promotes sustainable agricultural practices.

Vermiculture

Definition

Vermiculture is the practice of using earthworms to convert organic waste into nutrient-rich compost.

Process

- **Worm Species**: Red worms (Eisenia foetida or Lumbricus rubellus) are commonly used for vermicomposting.
- **Feedstock**: Organic waste materials such as kitchen scraps, garden waste, and paper are fed to the worms.
- **Digestion**: Worms consume organic matter, digesting it and converting it into castings (vermicompost).

- **Nutrient Enrichment**: Vermicompost is rich in nutrients (nitrogen, phosphorus, potassium) and beneficial microorganisms.

UNIT IV

Definition

Industrially important microbes are microorganisms utilized in various industries for their ability to produce valuable products or perform specific functions. These microbes include bacteria, fungi, yeasts, and algae.

Examples of Industrially Important Microbes

- **Bacteria**:
  - **Escherichia coli**: Used in biotechnology for protein production and genetic engineering.
  - **Bacillus subtilis**: Produces enzymes used in detergent and textile industries.
  - **Streptomyces species**: Source of antibiotics and other pharmaceutical compounds.
- **Fungi**:
  - **Aspergillus Niger**: Produces enzymes for food processing and industrial applications.
  - **Penicillium species**: Source of antibiotics such as penicillin.
- **Yeasts**:
  - **Saccharomyces cerevisiae**: Used in brewing, baking, and bioethanol production.
- **Algae**:
  - **Chlorella and Spirulina**: Used in food supplements, biofuels, and wastewater treatment.

Screening, Selection, and Identification of Industrially Important Microbes

Screening

- **High Throughput Screening (HTS)**: Screening large numbers of microbes quickly to identify those with desired properties (e.g., enzyme production, antibiotic resistance).
- **Primary Screening**: Initial assessment based on observable traits or growth characteristics.
- **Secondary Screening**: Detailed analysis of promising candidates for specific industrial applications.

Selection

- **Criteria**: Selection based on productivity, stability, adaptability to industrial conditions, and regulatory compliance.
- **Genetic Manipulation**: Use of genetic engineering to enhance desirable traits or introduce new functionalities.

Identification

- **Microbial Taxonomy**: Classification based on morphological, biochemical, and genetic characteristics.
- **Molecular Techniques**: DNA sequencing (e.g., 16S rRNA sequencing) for accurate identification.
- **Biochemical Tests**: Assays for specific enzyme activities or metabolic pathways.

Maintenance and Preservation of Industrially Important Microbial Cultures

Preservation Methods

- **Lyophilization (Freeze-Drying)**: Removal of water under vacuum to preserve microbial viability.
- **Cryopreservation**: Storage in liquid nitrogen or ultra-low temperatures to maintain long-term viability.
- **Glycerol Stock**: Preservation in glycerol solution at -80°C for short-term storage.

Culture Collection

- **Microbial Repositories**: Collections like ATCC (American Type Culture Collection) and NCIMB (National Collection of Industrial, Marine and Food Bacteria) maintain diverse microbial strains for research and industrial use.
- **Quality Control**: Regular testing to ensure purity, viability, and genetic stability of stored cultures.

Strain Improvement

Purpose

- **Enhanced Productivity**: Improve yield or quality of desired products (e.g., enzymes, antibiotics, biofuels).
- **Environmental Adaptation**: Modify microbes for better performance in specific industrial conditions (e.g., pH, temperature).

Methods

- **Mutagenesis**: Induce mutations using physical (radiation) or chemical agents to generate genetic diversity.
- **Genetic Engineering**: Introduce specific genes or pathways to enhance metabolic capabilities or product synthesis.
- **Directed Evolution**: Iterative process of selecting and mutating microbes to achieve desired traits over successive generations.

Basic Concepts of Fermentation

Definition

Fermentation is a metabolic process where microorganisms convert sugars into acids, gases, or alcohol in the absence of oxygen. It is widely used in food production, pharmaceuticals, and biofuels.

Fermenter Design and Applications

Design Considerations

- **Sterilization**: Ensure sterile conditions to prevent contamination during fermentation.
- **Aeration and Agitation**: Provide oxygen and mix nutrients to support microbial growth and metabolism.
- **Temperature and pH Control**: Optimize conditions for specific microbial strains and product formation.
- **Monitoring and Control**: Measure parameters like dissolved oxygen, pH, and temperature for process optimization.

Applications

- **Food Industry**: Production of fermented foods such as yogurt, cheese, and sauerkraut.
- **Pharmaceuticals**: Antibiotics (e.g., penicillin), vaccines, and therapeutic proteins.
- **Biofuels**: Production of ethanol from sugars or lignocellulosic biomass.

UNIT V

Microbial technology plays a pivotal role in the commercial production of various products essential in industries ranging from food and beverages to pharmaceuticals. This guide explores key microbial products and their applications in diverse sectors.

Microbial Production of Organic Acids

Lactic Acid

- Production: Fermentation of sugars (e.g., glucose, lactose) by lactic acid bacteria such as *Lactobacillus* species.
- Applications:
  - Food industry (preservatives, pH regulator, flavor enhancer).
  - Pharmaceutical and cosmetic industries (ingredient in formulations).
  - Biodegradable polymer production (polylactic acid, PLA).

Citric Acid

- Production: Fermentation of sugars by *Aspergillus Niger* or *Candida* species.
- Applications:
  - Food and beverage industry (acidulant in soft drinks, flavor enhancer).
  - Pharmaceutical industry (as a pH regulator in medications).
  - Household products (cleaning agents and detergents).

Microbial Production of Amino Acids

Glutamic Acid

- Production: Fermentation by *Corynebacterium glutamicum*.
- Applications:
  - Food industry (flavor enhancer, seasoning).
  - Pharmaceutical industry (excipient in medications).
  - Biotechnology (production of monosodium glutamate, MSG).

Aspartic Acid and Lysine

- Production: Fermentation by various bacteria and fungi.
- Applications:
  - Food and feed additives (nutritional supplements for humans and animals).
  - Pharmaceutical industry (aspartic acid is used in medications and supplements).

- Biotechnology (lysine is an essential amino acid used in animal feed).

Fermentation by Microbes for Food Additives

Dairy Products

- Cheese: Fermentation of milk by lactic acid bacteria (e.g., *Lactococcus lactis*, *Streptococcus thermophilus*) and fungal enzymes (rennet).
- Yogurt: Fermentation of milk by lactic acid bacteria (e.g., *Lactobacillus bulgaricus*, *Streptococcus thermophilus*).

Beverages

- Beer: Fermentation of malted barley by yeast (*Saccharomyces cerevisiae*) to produce alcohol and flavor compounds.
- Wine: Fermentation of grapes by yeast (*Saccharomyces cerevisiae*) to produce alcohol and unique flavors.

Antibiotics

- Streptomycin and Penicillin: Production by *Streptomyces* and *Penicillium* species, respectively, through fermentation.
- Applications:
  - Pharmaceutical industry (antibacterial medications).
  - Agricultural industry (as crop protectants against bacterial diseases).

Industrial Applications and Challenges

Scale-Up

- Fermentation Process Optimization: Control of parameters such as temperature, pH, oxygenation, and nutrient availability to maximize product yield.
- Bioreactor Design: Selection of appropriate fermenter types (batch, fed-batch, continuous) for different microbial cultures and products.
- Downstream Processing: Purification and recovery of microbial products from fermentation broth for commercial use.

Regulatory Compliance

- Quality Control: Adherence to regulatory standards for product safety, purity, and efficacy.
- Environmental Impact: Considerations for waste management and sustainability in microbial