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BHIMAVARAM

DEPARTMENT OF PG MICROBIOLOGY

STUDY MATERIAL

SEMESTER-IV

402-ENVIRONMENTAL MICROBIOLOGY

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IMVIC TEST

- The IMVIC tests are a group of individual test used in microbiology lab testing to identify an organism in the cloliform group.
- A coliform is a gram negitive,aerobic,or facultative anerobic rod, which produces gas from lactose within 48 hours the presence of some coliforms indicate fecal contamination.
- The term “IMVIC” is an acronym for each of these tests.
- "I" is for indole production test;
- "M" is for methyl red test;
- "V" is for Voges-Proskauer test, and "C" is for citrate test. The lower case ("i" is merely for proper pronunciation)

INDOLE PRODUCTION TEST

Aim:

To determine the ability of microbe to degrade the amino acid tryptophan.

Principle:

$$\text{Tryptophan} \xrightarrow{\text{Tryptophanase}} \text{Indole + Pyruvic acid + Ammonia}$$

$$\text{P-dimethylaminobenzaldehyde + Indole} \xrightarrow[\text{Dehydration reaction}]{\text{HCL,alcohol}} \text{Quniodial red-violet compound}$$

Result:

Development of cherry red colour at the interface of the reagent and the broth, within seconds after adding the Kovacs reagent ind indicates the presence of indole and the test is positive. If no colour change is observed then the test is negative and so organisms are not capable of producing tryptophanase.

METHYL RED TEST

Aim:

To differentiate E.coli and E.aerogen and to determine the ability of microbes to oxidize glucose with production and stabilization of high content of acid end product

Principle:

The Methyl Red test is based on the principle that certain bacteria produce stable acids as byproducts of glucose fermentation. When these bacteria are inoculated into a medium containing glucose and a pH indicator such as Methyl Red, they produce acidic end products. If the pH drops below a certain threshold, the Methyl Red indicator will turn red, indicating a positive test result. This test is used to differentiate between bacteria that produce mixed acids and those that do not.

E.coli:

Glucose+ H_2O → Lactic acid, Acetic acid, Formic acid + Methyl Red → CO_2+H, Red colour (positive test) (pH 4)

E. aerogen:

Glucose H_2O → Acetic acid → 2,3butanediol + CO_2+ H_2O + Methyl Red → Yellow colour (negative test) (pH 6)

Result:

A positive methyl red test is indicated by a red colour.

VOGES-PROSKAUER TEST

Aim: To differentiate the E.coli and E.aerogen by the production of 2,3 butanediol and acetoin via glucose fermentation.

Principle:

This test determines the capability of some organisms to produce non- acidic or neutral end products, such as acetyl methyl corbinol (acetoin), from the organic acid that results from glucose metabolism. This test is characterizes E.aerogen. Test identifies bacteria that ferment glucose and leading to 2,3-butanediol accumulation in the medium.

Glucose + ½O_2 → 2 Pyruvate → (CO_2) a-aceto acetate → (CO_2) Acetoin → (CO_2) 2.3-butanediol

40% KOH

Acetion + alpha-napthol → (Absolute alcohol) Diacetyl + Creatine (pink coloured complex)

Result:

A positive voges-proskauer test result is indicated by a pink or red colour.

CITRATE UTILIZATION TEST

Aim: To determine the ability of the microbes to ferment citrate as sole carbon source.

Principle:

- Citrate as a sole carbon source for their energy needs.
- Presence of a citrate permease that facilitates transport of citrate into the bacterium.
- Sodium citrate as the carbon source, NH4+ as a nitrogen source.
- pH indicator-bromothymol blue.
- This test is done on slants since 02 is necessary for citrate utilization.
- When bacteria oxidize citrate, they remove it from the medium and liberate CO2.
- CO2 combines with sodium (supplied by sodium citrate) and water to form sodium carbonate an alkaline product.
- This raises the pH, turns the pH indicator to a blue color, and represents a positive citrate test: absence of a color change is a negative citrate test.
- Citrate-negative cultures will also show no growth in the medium and the medium remains green.

Sodium citrate → (Citrate permease, Citrase) Pyruvic acid + Oxalacetic acid + CO_2

Excess sodium from sodium citrate + CO_2 +H_2O → Na_2 CO, (pH ↓) (green → blue)

Result:

A positive citrate test result is indicated by colour change from green to blue.

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WATER BORN DISEASES

INTRODUCTION:

Waterborne diseases are illnesses caused by drinking contaminated water or by coming into contact with water that has been contaminated with disease-causing microorganisms. These diseases can be caused by various pathogens such as bacteria, viruses, and parasites.
Common waterborne diseases include cholera, typhoid fever, dysentery, and giardiasis. It's crucial to ensure that water sources are properly treated and maintained to prevent the spread of these diseases.

THE IMPORTANCE OF WATER QUALITY:

Water quality is crucial because it directly impacts our health and well-being. Poor water quality can lead to the spread of waterborne diseases, causing illnesses and even fatalities. Clean and safe water is essential for drinking, cooking, bathing, and sanitation. It also plays a significant role in agriculture, industry, and the environment. Monitoring and maintaining water quality help ensure the safety of our water sources and protect public health.

ITS CONTAIN TWO TYPES OF WATER:

- Potable(clean) water
- Contaminated water

Potable(clean) water:

- It is free of all objectional material, including pathogens, tastes, odors, colours, toxins, radioactive material, organisms, oils, gases, etc.

contaminated water:

- Water contaminated with sewage, domestic or industrial waste with chemicals and pathogenic microorganisms.

TYPES OF WATER BORN DISEASES:

- Cholera
- Typhoid
- Amoebic and bacillary dysentery
- Diarrheal diseases

Cholera:

Cholera disease is a serious and potentially life-threatening illness caused by the bacterium Vibrio cholerae. This bacterium is typically found in contaminated water or food, especially in areas with poor sanitation. Cholera can lead to severe diarrhea, vomiting, and dehydration, which can be fatal if not treated promptly. Prevention of cholera involves ensuring access to clean water, proper sanitation, and hygiene practices. Treatment usually includes rehydration therapy and, in severe cases, antibiotics. It's essential to be aware of the symptoms of cholera and seek medical attention if needed.

Previantions:

Preventing cholera disease involves ensuring access to clean water, practicing good sanitation, and maintaining proper hygiene. It's essential to drink safe water, preferably boiled or treated, and avoid consuming untreated water or ice. Proper handwashing before eating and after eating.

Typhoid:

Typhoid is a bacterial infection caused by Salmonella typhi. It is typically spread through contaminated food and water. The symptoms of typhoid fever include a high fever, weakness, stomach pain, headache, and in some cases, a rash. Treatment usually involves antibiotics, and prevention focuses on good sanitation practices, safe drinking water, and proper food hygiene. It's important to seek medical attention if you suspect you have typhoid fever.

Previantions:

practicing good hygiene is crucial in preventing typhoid. This includes washing hands thoroughly with soap and water before eating or preparing food, and after using the bathroom.

Amoebic and bacillary dysentery:

Amoebic dysentery and bacillary dysentery are both types of infectious diarrheal diseases that can cause severe symptoms.

Amoebic dysentery is caused by the parasite Entamoeba histolytica. It is typically contracted by consuming food or water contaminated with the parasite. Symptoms include bloody diarrhea, abdominal pain, and fever. Treatment usually involves specific antibiotics to target the parasite.

Bacillary dysentery, on the other hand, is caused by bacteria such as Shigella species. It spreads through contaminated food or water as well. Symptoms include bloody or mucus-filled diarrhea, abdominal cramps, and fever. Treatment may involve antibiotics targeted at the specific bacteria causing the infection.

Both conditions can be serious and require medical attention. It's important to stay hydrated and seek treatment if you suspect you have either of these types of dysentery.

Previantions:

Hand hygiene water safety, food safety,sanitation,vaccination.

Diarrheal diseases:

Diarrheal diseases, such as cholera, typhoid, amoebic dysentery, and bacillary dysentery, are conditions that lead to frequent and loose bowel movements. These diseases are often caused by

bacteria, viruses, or parasites present in contaminated food or water. Symptoms may include waterystools, abdominal cramps, dehydration, and sometimes fever. Treatment typically involves

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AQUATIC ENVIRONMENT

INTRODUCTION:

Aquatic environments include all types of water bodies like oceans, rivers, lakes, and ponds. These environments are home to a wide variety of plants and animals, each adapted to live in water. Aquatic ecosystems are essential for maintaining biodiversity and play a crucial role in the global water cycle. They also provide valuable resources for humans, such as food, transportation, and recreation.

Aquatic environments face various threats that can harm the delicate balance of these ecosystems. Some common threats include pollution from industrial and agricultural activities, overfishing, habitat destruction, climate change leading to rising sea levels and ocean acidification, invasive species disrupting native ecosystems, and water extraction for human consumption and agriculture. These threats can have severe impacts on aquatic biodiversity, water quality, and the overall health of aquatic ecosystems. Conservation efforts and sustainable practices are essential to protect and preserve these vital environments for future generations.

CHARACTERISTICS OF AQUATIC ENVIRONMENT:

- Plankton
- Phytoplankton
- Zooplankton

Plankton:

Plankton are tiny organisms that drift in aquatic environments, including both freshwater and marine ecosystems. They can be classified into two main groups: phytoplankton, which are plant-like and perform photosynthesis, and zooplankton, which are animal-like and feed on other plankton. Plankton form the base of the aquatic food chain, providing essential nutrients for larger organisms. They play a crucial role in marine and freshwater ecosystems by contributing to nutrient cycling and supporting the overall biodiversity of these environments.

Phytoplankton:

Phytoplankton are like the plant version of plankton in aquatic environments. They are microscopic, single-celled organisms that contain chlorophyll and can photosynthesize, converting sunlight into energy. This process is crucial as it produces oxygen and forms the base of the marine food chain, providing food for zooplankton and other marine organisms. Phytoplankton are essential for regulating the Earth's climate by absorbing carbon dioxide from the atmosphere. They are sensitive to changes in water temperature, nutrient levels, and light availability, making them important indicators of environmental health.

Zooplankton:

Zooplankton are the animal-like plankton that float in aquatic environments. They consume phytoplankton and other small organisms, playing a critical role in the food chain by transferring energy from phytoplankton to larger marine animals. Zooplankton come in various shapes and sizes, ranging from tiny microscopic organisms to larger species like jellyfish larvae. They are an essential part of marine ecosystems, contributing to nutrient cycling and biodiversity.

FRESHWATER ENVIRONMENT:

Freshwater environments, such as rivers, lakes, and wetlands, are crucial habitats that support a wide range of plant and animal species. These environments play a vital role in providing drinking water, irrigation for agriculture, and are essential for various ecosystems. However, freshwater environments face threats like pollution from agricultural runoff, industrial discharges, and urban development, which can degrade water quality and harm aquatic life. Over-extraction of water for human use can also lead to water scarcity and ecosystem disruptions. Conservation efforts, sustainable water management practices, and pollution control measures are essential to protect and preserve freshwater environments for future generations.

Temperature, sunlight, oxygen, and nutrients are factors that determine where organisms live.

• A main factor is salinity.

• Salinity is the amount of dissolved salts the water contains.

LIFE IN LAKE:

- Littoral Zone
- Benthic Zone

Littoral Zone:

- Near the shore and aquatic life is diverse and abundant.
- Littoral Zone is where most plants are found: like reeds and cattails.

Benthic Zone:

- Benthic Zone is the bottom of the pond or lake which is inhabited by decomposers, insect larvae, and clams.

FRESHWATER WETLANDS:

Wetlands are areas where water covers the soil or is present either at or near the surface of the soil for varying periods of time during the year. They include marshes, swamps, bogs, and fens, and are vital ecosystems that support diverse plant and animal species. Wetlands play essential roles in flood control, water purification, shoreline stabilization, and providing habitats for a wide range of organisms.

ENVIRONMENTAL FUNCTIONS:

- They control flooding by absorbing extra water when rivers overflow.
- They provide homes for many species and trap carbon.

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RIVERS:

Rivers are a crucial part of our environment, playing a significant role in the water cycle and supporting various ecosystems. They provide habitats for aquatic plants and animals, act as natural drainage systems, and are a source of freshwater for many communities.

Microbial production of methyl mercury, acid rain water,activated sludge process, trickling filters

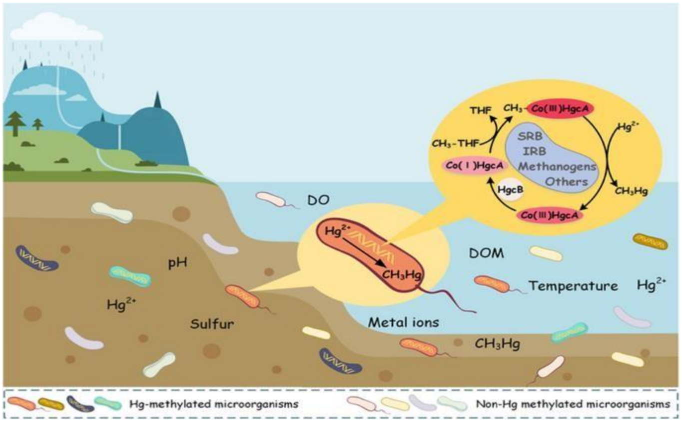
Methyl mercury

Introduction

Methylmercury formation is mainly driven by microbial-mediated process. The mechanism of microbial mercury methylation has become a crucial research topic for understanding methylation in the environment. Pioneering studies of microbial mercury methylation are focusing on functional strain isolation, microbial community composition characterization, and mechanism elucidation in various environments. Therefore, the functional genes of microbial mercury methylation, global isolations of Hg methylation strains, and their methylation potential were systematically analysed, and methylators in typical environments were extensively reviewed. The main drivers (key physicochemical factors and microbiota) of microbial mercury methylation were summarized and discussed. Though significant progress on the mechanism of the Hg microbial methylation has been explored in recent decade, it is still limited in several aspects, including molecular biology techniques for identifying methylators; characterization methods for mercury methylation potential; and complex environmental properties (environmental factors, complex communities, etc.). Accordingly, strategies for studying the Hg microbial methylation mechanism were proposed. These strategies include the following: the development of new molecular biology methods to characterize methylation potential; treating the environment as a micro-ecosystem and studying them from a holistic perspective to clearly understand mercury methylation; a more reasonable and sensitive inhibition test needs to be considered.

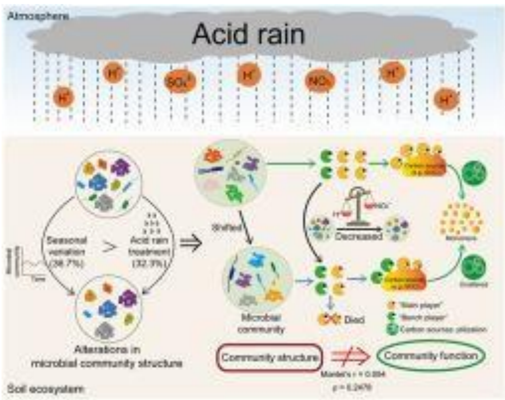
Key points:

- Global Hg microbial methylation is phylogenetically and functionally discussed.
- The main drivers of microbial methylation are compared in various condition.
- Future study of Hg microbial methylation is proposed.



Acid rain water

Acid rain alters soil carbon (C) cycling by influencing the soil microbial community structure and functions. However, the response of soil microbial communities to acid rain with time and underlying mechanisms are still poorly understood. Herein, we conducted a one-year intact soil core experiment to investigate the temporal changes of soil microbial community composition and C sources metabolism under acid rain (pH 5.0, pH 4.0, and pH 3.0) in an agricultural soil of southern China. We found that pH 3.0 acid rain increased the total, bacterial, gram-positive bacterial, and actinomycetal PLFAs at the early stage, but this effect diminished with time. Conversely, the gram-negative bacterial PLFAs contents were reduced under pH 3.0 acid rain at the later stage. Interestingly, pH 5.0 acid rain increased the total, bacterial, gram-positive bacterial, and actinomycetal PLFAs contents at the later stage. In addition, pH 3.0 and pH 5.0 acid rain treatments accordingly altered the soil microbial community structure at the early and later stage. However, acid rain did not change the microbial C sources utilization pattern. The principal response curve analysis revealed that the seasonal variation exerted a greater effect on the overall variance of soil microbial community structure than the acidity of acid rain.



Soil cores collection

On July 2, 2017, we collected 16 intact soil cores using PVC pipes (60 cm diameter, 40 cm depth) from a farmland (113°38′ E, 23°14′ N) located in the Guangzhou, Guangdong, China. The region of this farmland is characterized as a subtropical monsoon climate, with the mean annual air temperature and precipitation of 22 °C and 1976.8 mm, respectively.

Experimental design

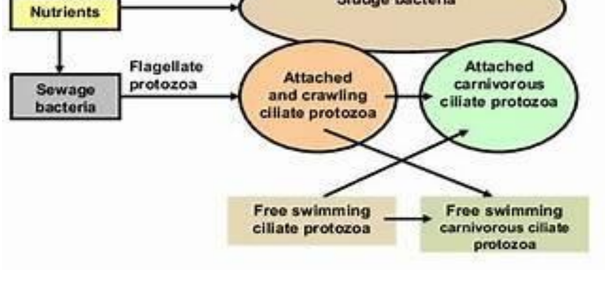
All soil cores were taken to a greenhouse of the ecological farm (113°15′ E, 23°08′ N) of South China Agricultural University in Guangzhou, and these cores were equilibrated under field moisture for three months. After equilibration, the soil cores were used for simulated experiments, in which four acid rain treatments were applied for one year as follows: control (CK, tap water, pH ≈ 7.5), acidity 1 (pH 5.0), acidity 2 (pH 4.0), and acidity 3 (pH 3.0). Of them, pH 5.0 and pH 4.0 were set to simulate the current status of acid rain in South China (Ministry of Ecological Environment of the People's Republic of China, 2017), while the pH 3.0 was used to preview the changes in the soil ecosystems once the acid rain will be getting worse in the future

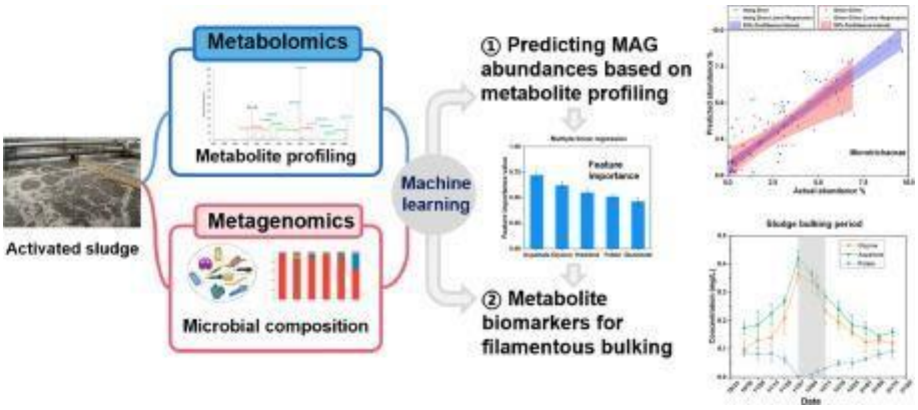
Soil sampling

Soil samples were taken three times (i.e., 90, 270, and 360 d after acid rain treatment) from the soil cores during the experimental period to reflect the temporal dynamics of soil parameters. Briefly, five small soil cores (2.5 cm diameter, 10 cm depth) from each intact soil core were taken with drills, and manually mixed to obtain a composite sample.

Activated sludge

Activated sludge is subjected to transient conditions, resulting in the enhanced production of microbial products (i.e., extracellular polymeric substances, soluble microbial products, and internal storage products). The authors review the status of the microbial products formed by activated sludge in wastewater treatment systems. They outline the fundamental facets of their formation and metabolism, define their key characteristics and important roles, and identify the interrelationships between the microbial products and activated sludge processes as well as discuss their implications. In addition, they appraise present understanding about their conversion kinetics. Finally, the authors also summarize modeling works on the microbial products formation processes and discuss a model incorporating their formation



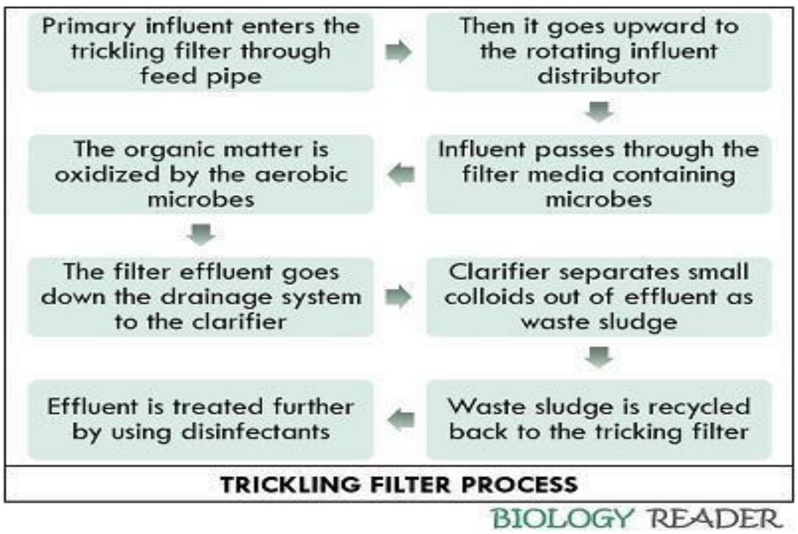


Trickling process

Trickling filter process is one of the types of **aerobic wastewater treatment**. It is a fixed-bed bioreactor that is the part of secondary wastewater treatment, which eliminates the coarse particles, suspended organic and inorganic waste, small colloids etc. out of the primary effluent. A trickling filter is also called **biological filter**, as it makes the use of active microbial mass as a bioweapon to degrade the waste out of primary sewage.

Definition of Trickling Filter Process

Trickling filter process can define as the biological system, which tends to separate or degrade the maximum organic and inorganic waste (**up to 85%**) out of the primary or raw sludge via the slime layer. The designing of a trickling filter unit includes a support structure, pebble or plastic filled media and rotary distributor.



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Introduction of the settled sewage

The raw or primary sewage from the **primary clarifier** tank enters the trickling filter tank at a steady rate. The sewage must be primarily treated via a series of operations like screening, grit removal and primary clarifiers to prevent clogging in the trickling filter system. Primary influent that enter the trickling filter unit is free from coarse objects, suspended solids, rags etc. A **dosing device** (tipping bucket) is a piece of equipment allowing the supernatant to flow upwards to the arms of the perforated rotary distributor.

Sewage flushing

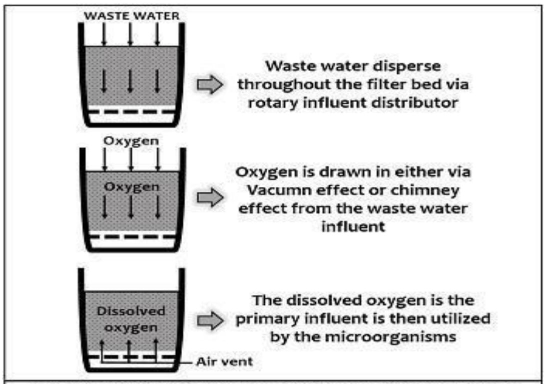
The primary sewage flushes downward via a perforated **rotary distributor**, which evenly distribute the sewage over the filter matrix. The filter bed is packed with constituents like rock, gravel, redwood, synthetic material etc. that acts as a **media** for the attachment of the microorganisms. Therefore, the trickling filter is the best example of an **attached growth system**, in which biomass is directly associated with the media instead of sewage suspension.

Pollutant degradation

The microorganisms attached to the filter bed utilize organic waste as a **food material**. The pollutants like organic and inorganic waste in the sewage suspension go through the **absorption** and **adsorption** into and over the filter bed by the microbial slime layer.

Oxygen utilization by the microorganisms

The treated primary sewage splashes through the arm of a **rotary distributor** provide the oxygen supply in the form of dissolved oxygen. Oxygen is drawn into the filter media via vacuum or chimney effect. The bio-film layer uses the dissolved oxygen trapped within the filter media and **oxidize** the organic compounds by releasing carbon dioxide gas, water and other oxidized end products.



Oxygen utilization by the microorganisms attached to the filter media

SEWAGE TREATMENT , EUTROPHICATION AND ALGAL TOXINS

- Sewage treatment , eutrophication , and algal toxins are interconnected environmental issues.

1. <u>**SEWAGE TREATMENT**</u>:

Sewage treatment is the process of removing contaminants from wastewater, primarily from household sewage

The aim is to produce an effluent that can be safely discharged into the environment or reused.

Treatment typically involves multiple stages:

Stages of Sewage Treatment:

- Preliminary Treatment: Removes large solids and debris through screening and grit removal.
- Primary Treatment: Settles out suspended solids in sedimentation tanks, producing primary sludge.
- Secondary Treatment: Uses biological processes to degrade organic matter. Common methods include activated sludge systems, trickling filters, and biofilters.
- Tertiary Treatment: Further purification to remove nutrients like nitrogen and phosphorus, heavy metals, and pathogens

Techniques include filtration, chemical precipitation, and disinfection (e.g., chlorination, UV treatment).Sludge Treatment: Treats the sludge produced in earlier stages, often involving anaerobic digestion, dewatering, and sometimes composting or incineration.

2. EUTROPHICATION :

- Eutrophication is the process by which water bodies become enriched with nutrients, particularly nitrogen and phosphorus, leading to excessive growth of algae and other aquatic plants.
- This can have detrimental effects on aquatic ecosystems

CAUSES OF EUTROPHICATION:

- **Agricultural Runoff:**
  - Fertilizers rich in nitrogen and phosphorus can run off into water bodies.
- **Urban Runoff:**
  - Contains nutrients from lawns, gardens, and impervious surfaces
- **Sewage Discharge:**
  - Insufficiently treated sewage can release high levels of nutrients.

- **<u>Industrial Discharges:</u>**
  - Certain industries may discharge nutrient-rich wastewater.

<u>Effects of Eutrophication:</u>

- **Algal Blooms**: Rapid growth of algae, which can reduce light penetration and oxygen levels in water.
- **Hypoxia**: Decomposition of algae by bacteria depletes oxygen, creating dead zones where aquatic life cannot survive
- **Loss of Biodiversity**: Low oxygen levels and changes in water chemistry can kill fish and other aquatic organisms.
- **Water Quality Deterioration**: Algal blooms can make water unsafe for drinking and recreational use.

3. **<u>Algal Toxins :</u>**

Algal toxins are toxic compounds produced by certain types of algae, particularly during harmful algal blooms (HABs). These toxins can have severe impacts on human health, aquatic life, and the environment.

Types of Algal Toxins:

- **Microcystins:**Produced by cyanobacteria (blue-green algae), can cause liver damage and are potent liver carcinogens**.**
- **Anatoxins:** Also produced by cyanobacteria, these neurotoxins can cause respiratory paralysis.
- **Saxitoxins:** Neurotoxins associated with paralytic shellfish poisoning.
- **Domoic Acid:** Produced by certain diatoms, can cause amnesic shellfish poisoning.
- **Impact of Algal Toxins**:HumanHealth RisksCan contaminate drinking water and seafood, causing a range of health issues from gastrointestinal illness to neurological damage.

- **Ecological Impact**: Can kill fish, birds, and other wildlife. Decomposing algae further depletes oxygen, worsening hypoxic conditions.
- **Economic Consequences**: Affects fisheries, tourism, and water treatment costs.

Connections Between Sewage Treatment, Eutrophication, and Algal Toxins:

- **Nutrient Loading**: Insufficient sewage treatment can lead to nutrient-rich effluents being discharged into water bodies, contributing to eutrophication**.**
- **Algal Blooms:** Eutrophication promotes the growth of algae, including harmful species that produce toxins.
- **Health and Environmental Risks:** The presence of algal toxins in water supplies poses risks to human health and ecosystems.

Mitigation and Management Strategies:

- **Enhanced Sewage Treatment**: Upgrading treatment plants to tertiary processes that remove nutrients more effectively.
- **Nutrient Management**: Implementing best practices in agriculture to reduce nutrient runoff, such as buffer strips, controlled use of fertilizers, and cover cropping.

Monitoring and Early Warning Systems:

Regularly monitoring water bodies for nutrient levels and algal blooms to provide early warnings and take preventive actions.

Public Awareness and Education: Educating the public about the sources and impacts of nutrient pollution and how they can help reduce it.

Effective sewage treatment and nutrient management are crucial in mitigating the impacts of eutrophication and harmful algal blooms, thereby protecting water quality, ecosystems, and public health.

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MICROBIAL PRODUCTION OF TRIMETHYL ARSINE

- The mechanism for trimethylarsine formation was originallydescribed on the basis of work done with the fungus Scopulariopsis brevicaulis
- which involves a series of steps, in which the reduction of arsenate was followed by oxidative addition of a methyl groupby S-adenosylmethionine as the usual
- range of fungi and bacteria isolated from the environment arecapable of forming trivalent arsines through methylation and hybridization of arsenic to give four compounds, arsine (AsH_3), methylarsine (Me AsH_2), dimethylarsine (Me_2AsH)

and trimethylarsine (Me_3As).

- Only limited knowledge is available on the generation and fateof these arsines in the environment.
- Once released into the atmosphere, there is little accurateinformation relating the stability of arsine and its related species.
- Jakob et al. (2009) measured rates of degradation for the species listed above, in sealed chambers containing the individual species being measured. Based on these studies, estimated atmospheric half-lives for arsine, methylarsine, dimethylarsine, and trimethylarsine were 130 d, 67 d, 17 d, and2 d, respectively.
- Mestrott et al. (2011), determined half-lives of approximately 8 h under daytime conditions (UV light) for all methylated arsines, while the same species were found to be considerablymore stable under night-time (dark) conditions.

- Arsine (AsH_3) showed under both day and night-time conditions, considerably higher stabilities than methylatedarsines.
- How well these two studies simulate actual atmospheric stabilities is difficult to say but they do indicate that the stability of arsine and related species is sufficient to allow atmospheric transport for long distances prior to degradation.
- It has been reported that arsine is oxidized to arsenite or arsenate, while the methylated arsines oxidize under atmospheric conditions rapidly to their pentavalent methylatedarsenic oxides such as methylarsonate (MA), dimethylarsinate (DMA) and trimethylarsine oxide (TMAO) (Jakob et al., 2009).
- Arsenic (As) poses a risk to the human health in excess exposure and microbes play an important role in the toxicity ofAs.
- Arsenic methylation mediated by microbes is a key driver of Astoxicity in the environment and this paper reviews the role of microbial arsenic methylation and volatilization in the biogeochemical cycle of arsenic.
- In specific, little is presently known about the molecular mechanism and gene characterization of arsenic methylation.
- The uptake of methylated arsenic in plants is influenced bymicrobial arsenic methylation in soil
- thus enhancing the volatilization of methylated arsenic is a potential mitigation point for arsenic mobility and toxicity inthe environment.
- On the other hand, the potential risk of methylated arsenic onorganisms is also discussed.

- And the directions for future research, theoretical reference forthe control and remediation of arsenic methylation, are presented.

BOD:Biochemical oxygen demand

-

- BOD – Biochemical Oxygen Demand is applied to determine the aerobic destructibility of organic substances.
- BOD is the biological method used for the measurement of the total amount of dissolved oxygen (DO) used by microbes in the biological process of metabolizing organic molecules present inwater.
- The total amount of oxygen gas present in the water is called dissolved oxygen (DO). The non-compound oxygen present in water may either be a by-product of the photosynthesis of theaquatic plants or the dissolved atmospheric oxygen gas.
- in some water bodies, organic matter is a great source of BOD.
- These organic matters include sewage and other pollutantspresent in the water bodies.
- The greater the BOD, the lower is the dissolved oxygenavailable for aerobic animals such as fishes and other **aquatic** organisms.
- The BOD is accordingly a reliable measure of the organicpollution of water bodies.
- The main reason for treating wastewater prior to its discharge into a water resource is to reduce its BOD level (the demand foroxygen).

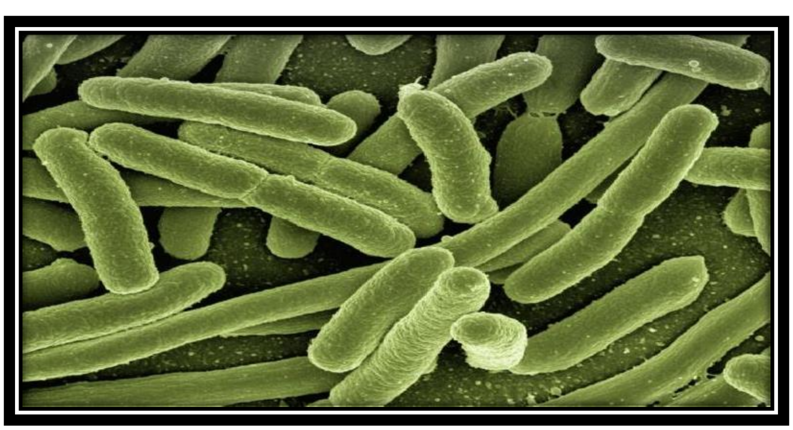
Importance of BOD

- BOD measures the amount of oxygen consumed by microorganisms for the process of decomposition of theorganic matters in the water bodies.
- It indicates the amount of organic pollution present in anaquatic ecosystem.
- BOD is calculated in sewage treatment or wastewater treatment to find the destruction of organic wastes by aerobicmicrobes
- It determines the amount of organic matter present in soils,sewages, sediment, garbage, sludge, etc.
- The biochemical oxygen demand also determines the rate ofrespiration in living beings.
- BOD is also used in the medicinal & pharmaceutical industriesto test the oxygen consumption of cell cultures.

Azotobacter Biofertilizers Production

Introduction

Azotobacter, a genus of soil-dwelling bacteria, has garnered significant attention in agriculture for its remarkable ability to enhance soil fertility and promote plant growth through biological means. As a biofertilizer, Azotobacter plays a pivotal role in sustainable farming practices by reducing the reliance on chemical fertilizers, which can have detrimental effects on soil health and the environment.



Biofertilizer

- Azotobacter represents a significant advancement in sustainable agriculture, harnessing the natural biological processes of nitrogen fixation to enhance soil fertility and crop productivity. These versatile bacteria thrive in diverse soil environments, contributing to soil health by converting atmospheric nitrogen into ammonia, a crucial nutrient for plant growth.

Role of biofertilizers:

1. Better germination
2. Make's nutrients available
3. Growth Promoting Substances are produced
4. Improve quality and quantity of produce
5. More biotic and abiotic stress tolerance
6. Improve soil health
7. Increase the crop yields

Azotobacter

- Azotobacter is known for its ability to fix atmospheric nitrogen into a form that plants can use. This biological nitrogen fixation reduces the dependence on synthetic nitrogen fertilizers, which can be costly and environmentally damaging.
- In addition to nitrogen fixation, Azotobacter species produce various growth-promoting substances such as vitamins, amino acids, and phytohormones (e.g., auxins).
- By converting nitrogen gas (N_2) into ammonia (NH_3) or ammonium ions (NH_4^+), Azotobacter enhances soil fertility and promotes healthier plant growth.

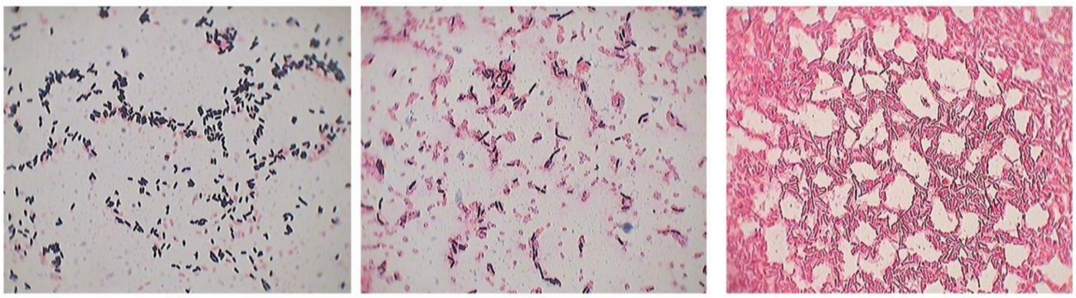
Characteristics of azotobacter

1. **Morphology**: Azotobacter cells are typically rod-shaped (bacilli) and range in size from 1 to 4 micrometers. They are often motile due to the presence of polar flagella, which enable them to move through the soil matrix.

2. **Metabolic Features**: Azotobacter are obligate aerobes, meaning they require oxygen to carry out their metabolic processes. They are capable of fixing atmospheric nitrogen (N_2) into ammonia (NH_3) through the enzyme nitrogenase, which is essential for their role as nitrogen-fixing bacteria.

3. **Growth Conditions**: These bacteria thrive in well-aerated soils with a pH range typically between 6.5 and 7.5. They prefer soils that are rich in organic matter and nutrients, although they can adapt to a variety of soil types and conditions.

4. **Endospore Formation**: Some species of Azotobacter can form cysts or endospores under unfavorable environmental conditions such as drought or nutrient depletion. These endospores allow the bacteria to survive in a dormant state until conditions improve.



(a) (b) (c)

NITROZEN FIXATION

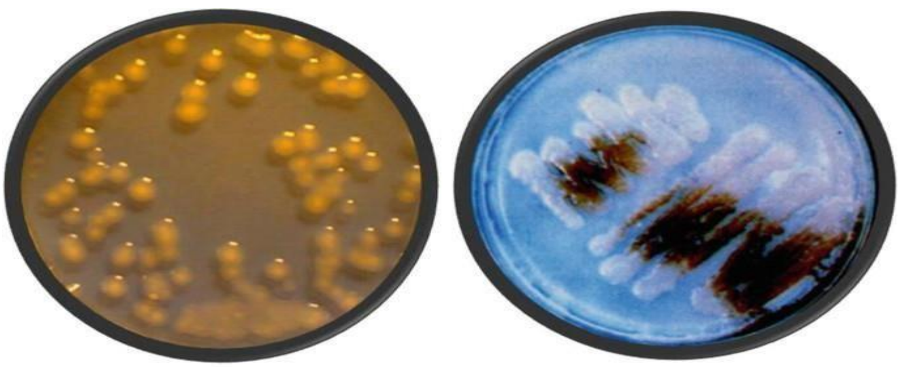
- Nitrogen fixation is a fundamental biological process essential for global nutrient cycling and agricultural productivity.
- Organisms capable of nitrogen fixation, such as Azotobacter bacteria, play a pivotal role in converting atmospheric nitrogen (N_2) into a form usable by plants and other organisms.
- This process is catalyzed by the enzyme nitrogenase, which facilitates the reduction of nitrogen gas to ammonia (NH_3) or ammonium ions (NH_4^+). Nitrogen fixation occurs primarily in anaerobic or microaerobic conditions to protect the nitrogenase enzyme from oxygen, which inhibits its activity.

MODE OF ACTION

- This nitrogen fixation process occurs under anaerobic or microaerobic conditions to protect nitrogenase from oxygen, as it is highly sensitive to its presence.
- Direct mechanism of plant growth improvement.
- Plant growth improvement by biocontrol activity.
- Indirect mechanism.

Isolation of azotobacter

- Collect soil samples from diverse agricultural or natural environments where Azotobacter is likely to be present, such as fields with leguminous crops or organic-rich soils.
- Incubate the inoculated plates at a suitable temperature (typically around 25-30°C) for several days to allow bacterial colonies to develop.
- Maintain isolated Azotobacter cultures in suitable preservation methods such as freezing at -80°C or lyophilization to preserve their viability and characteristics for future research or agricultural applications.



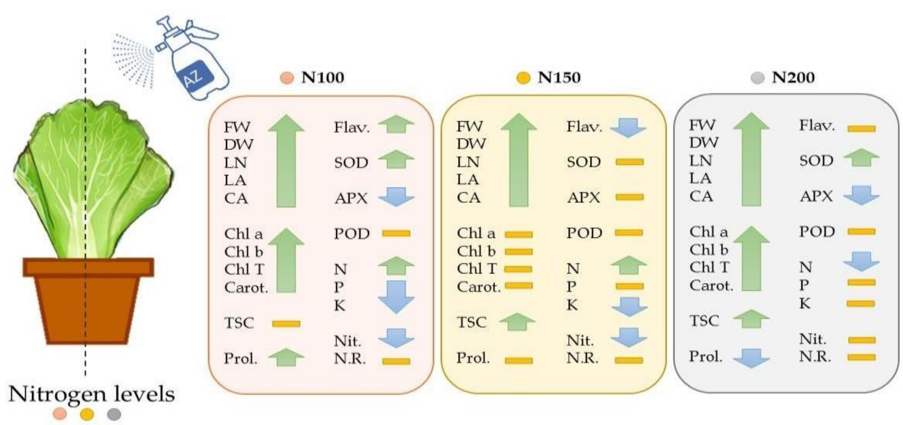
(a) (b)

Applications

APPLICATIONS OF AZOTOBACTERS INOCULANTS IN FIELDS

Azotobacters inoculants can be applied in different ways for different types of plants in various conditions.

- Azotobacter plays a crucial role in enhancing soil fertility by fixing atmospheric nitrogen into ammonia or ammonium ions, which are readily available to plants.
- Azotobacter contributes to soil health by producing extracellular polymeric substances (EPS) that enhance soil structure, water retention, and nutrient availability.
- Azotobacter is particularly valuable in organic farming systems, where synthetic chemical inputs are restricted.
- It supports organic certification requirements by providing natural and sustainable solutions for soil fertility management and crop nutrition.
- This improves soil aeration, microbial activity, and overall soil fertility, supporting healthier root systems and crop growth.



Definition: Blue-Green Algae are a type of photosynthetic bacteria consisting either of single cells or colonies which is also known as the Cyanobacteria. Cyanobacteria contain only one type of chlorophyll, Chlorophyll a, a green pigment. In addition, they also contain pigments such as carotenoids, phycobilin.

These bacteria grow naturally in marine and freshwater systems. They thrive in dams, rivers, reservoirs, lakes and even in hot springs. These bacteria normally look green and sometimes turns blue when scum are dying. Almost all species of these bacteria are buoyant and float on the water surface and forms floating mats.

The accumulation of these algae is termed as ‘blooms’. These blooms discolour the water and produce unpleasant taste and odour. They affect the fish population and reduces water quality. The decomposition of these

Nitrogen fixation by Blue green algae :

Blue green algae are widely spread all over. When they fix carbon from carbon dioxide, some blue green algae fix dinitrogen from the atmosphere. They are called nitrogen-fixing blue green algae and are inclusive of symbiotic and free living forms.

Blue green algae are photosynthetic, some can carry out nitrogen fixation. Its chief photosynthetic pigments are carotenes, chlorophyll a and xanthophylls along with phycobiliproteins, c-phycoerythrin and c- phycocyanin. Some of the blue green algae can fix nitrogen as it contains nitrogenase – an oxygen-sensitive enzyme.

Uses of Blue-Green Algae:

- Blue-green algae contain a small amount of vitamins (including C, E and folate), beta carotene and some minerals. They are a negligible source of nutrients unless you consume huge amounts of algae.
- Blue-green algae are used as a nutrient supplement and also helps inlosing weight.
- It helps in boosting the immune system and controlling cholesterol levels.

- Some species of Blue-green algae naturally fertilise the fields and rice paddies and contributes majorly to the food supply. Anabaena converts inert atmospheric nitrogen into a usable form such a nitrate or ammonia. Anabaena coexists with a fern called Azolla which supplies nitrogen to the plant.
- Certain blue-green algae are processed for various food and medicinal products such as vitamins, drug compounds and growth factors. Spirulina is a popular, high protein food source.

Role of Blue-Green Algae in Paddy Fields:

Cyanobacteria are the major microbes which fix nitrogen in paddy fields. The agricultural importance of cyanobacteria in rice cultivation is because of their nitrogen-fixing ability and other positive effects on soil and plants.

Nitrogen fixation is the process of converting inert atmospheric nitrogen into combined compounds like ammonia, nitrate, nitrite etc.

The cyanobacterium Anabaena forms a nitrogen-fixing symbiosis with Azolla and fixes atmospheric nitrogen in the presence of significant quantities of oxygen.

Problems Caused by Blue-Green Algae:

- Harmful to human health
- Affects the livestock
- Imparts unpleasant odour and taste to water
- Produces toxins which affect the aquatic organisms
- Deplete the oxygen content of water bodies
- Causes the killing of fish
- Incurs high water treatment costs

How to Reduce the Intensity of Blue-Green Algae:

It's not easy to get rid off blue-green algae once they appear in water bodies. However, to some extent it can be reduced with the help of thesefollowing measures:

- By reducing the amount of nitrogen and phosphorus from the waterhelps in reducing the intensity of blue-green algae in the water. Butit may take a long time to effectively remove these compounds from water. The reason for this is that there may be a large amount of these nutrients at the bottom of the water body, and they still serve as the food for the blue-green algae.
- By lowering the oxygen content.
- By reducing the light.
- By lowering the temperature

Bioremediation technology

- **Bioremediation** broadly refers to any process wherein a biological system (typically bacteria, microalgae, fungi in mycoremediation, and plants in phytoremediation), living or dead, is employed for removing environmental pollutants from air, water, soil, flue gasses, industrial effluents etc., in natural or artificial settings.The natural ability of organisms to adsorb, accumulate, and degrade common and emerging pollutants has attracted the use of biological resources in treatment of contaminated environment.[1] In comparison to conventional physicochemical treatment methods bioremediation may offer advantages as it aims to be sustainable, eco-friendly, cheap, and scalable.

- Most bioremediation is inadvertent, involving native organisms. Research on bioremediation is heavily focused on stimulating the process by inoculation of a polluted site with organisms or supplying nutrients to promote their growth. Environmental remediation is an alternative to bioremediation.

- While organic pollutants are susceptible to biodegradation, heavy metals cannot be degraded, but rather oxidized or reduced. Typical bioremediations involves oxidations.Oxidations enhance the water-solubility of organic compounds and their susceptibility to further degradation by further oxidation and hydrolysis.Ultimately biodegradation converts hydrocarbons to carbon dioxide and water.For heavy metals, bioremediation offers few solutions. Metal-containing pollutant can be removed, at least partially, with varying bioremediation techniques. The main challenge to bioremediations is rate: the processes are slow.

- Some examples of bioremediation related technologies are phytoremediation, bioventing,bioattenuation, biosparging, composting (biopiles and windrows), and landfarming. Other remediation techniques include thermal desorption, vitrification, airstripping, bioleaching, rhizofiltration, and soil washing. Biological treatment, bioremediation, is a similar approach used to treat wastes including wastewater, industrial waste and solid waste. The end goal of bioremediation is to remove harmful compounds to improve soil and water quality.

Phytoremediation:

Phytoremediation is a process in which plants are used to sequester toxins and hydrocarbons into plant tissue from contaminated soils. The main mechanisms for phytoremediation stem from complex relationships between roots and rhizobia. Plants secrete sugars, enzymes, and oxygen from roots which provide necessary substrates for rhizobia and associated rhizosphere microbes to stimulate degradation Studies have demonstrated the bioaccumulation abilities of various plants with rhizobial associations, in particular *Chromolaena odorata* were able to remove 80% of petroleum and heavy metal toxins from soils. While more commonly used on terrestrial environments, contaminated marine environments also benefit from plants based bioremediation through the use of various algae and macrophytes.Phytoremediation is most effective when used in conjuction with microbial remediation and Mycoremediation.

Bioventing:

- Bioventing is a process that increases the oxygen or air flow into the unsaturated zone of the soil, this in turn increases the rate of natural *in situ* degradation of the targeted hydrocarbon contaminant. Bioventing, an aerobic bioremediation, is the most common form of oxidative bioremediation process where oxygen is provided as the electron acceptor for oxidation of petroleum, polyaromatic hydrocarbons (PAHs), phenols, and other reduced pollutants. Oxygen is generally the preferred electron acceptor because of the higher energy yield and because oxygen is required for some enzyme systems to initiate the degradation process.Microorganisms can degrade a wide variety of hydrocarbons, including components of gasoline, kerosene, diesel, and jet fuel. Under ideal aerobic conditions, the biodegradation rates of the low- to moderate-weight aliphatic, alicyclic, and aromatic compounds can be very high. As molecular weight of the compound increases, the resistance to biodegradation increases simultaneously.This results in higher contaminated volatile compounds due to their high molecular weight and an increased difficulty to remove from the environment.

Bioattentuation:

- During bioattenuation, biodegradation occurs naturally with the addition of nutrients or bacteria. The indigenous microbes present will determine the metabolic activity and act as a natural attenuation. While there is no anthropogenic involvement in bioattenuation.

Biosparging:

- Biosparging is the process of groundwater remediation as oxygen, and possible nutrients, is injected. When oxygen is injected, indigenous bacteria are stimulated to increase rate of degradation. However, biosparging focuses on saturated contaminated zones, specifically related to ground water remediation.

Biophiles:

- Biopiles, similar to bioventing, are used to remove petroleum pollutants by introducing aerobic hydrocarbons to contaminated soils. However, the soil is excavated and piled with an aeration system. This aeration system enhances microbial activity by introducing oxygen under positive pressure or removes oxygen under negative pressure.

Windrows:

- Windrow systems are similar to compost techniques where soil is periodically turned in order to enhance aeration. This periodic turning also allows contaminants present in the soil to be uniformly distributed which accelerates the process of bioremediation.

Landforming:

- Landfarming, or land treatment, is a method commonly used for sludge spills. This method disperses contaminated soil and aerates the soil by cyclically rotating.This process is an above land application and contaminated soils are required to be shallow in order for microbial activity to be stimulated. However, if the contamination is deeper than 5 feet, then the soil is required to be excavated to above ground. While it is an *ex situ* technique, it can also be considered an *in situ* technique as Landfarming can be performed at the site of contamination.

Pesticides:

- Of the many ways to deal with pesticide contamination, bioremediation promises to be more effective.Many sites around the world are contaminated with agrichemicals.These agrichemicals often resist biodegradation, by design. Harming all manners of organic life with long term health issues such as cancer, rashes, blindness, paralysis, and mental illness. An example is Lindane which was a commonly used insecticide in the 20th century. Long time exposure poses a serious threat to humans and the surrounding ecosystem.

Lindane reduces the potential of beneficial bacteria in the soil such as nitrogen fixation cyanobacteria. As well as causing central nervous system issues in smaller mammals such as seizures, dizziness, and even death. What makes it so harmful to these organisms is how quickly distributed it gets through the brain and fatty tissues. While Lindane has been mostly limited to specific use, it is still produced and used around the world.

- Actinobacteria has been a promising candidate *in situ* technique specifically for removing pesticides. When certain strains of Actinobacteria have been grouped together, their efficiency in degrading pesticides has enhanced. As well as being a reusable technique that strengthens through further use by limiting the migration space of these cells to target specific areas and not fully consume their cleansing abilities. Despite encouraging results, Actinobacteria has only been used in controlled lab settings and will need further development in finding the cost effectiveness and scalability of use.

Advantages:

- Complete remediation of harmful contaminants presents in the environment instead to transferring contaminants from one site to another.
- Cost effective method with minimal requirements of complex tools and equipment
- Environment friendly approach with use of microorganism instead of harmful chemicals
- In majority cases, can be carried out on site reducing transportation cost
- Minimum site destruction and disruption
- Lower liability level
- Low energy consumption
- Being a very effective reliable and easy approach, is therefore accepted publicly and by the regulatory authorities.

Disadvantages:

- Only limited to biodegradable waste and contaminants
- Requires extensive monitoring
- Being a biological process, specificity is a major drawback in terms factors like type of environmental growth conditions, types of microorganisms, type of nutrient requirements and type of contaminants.
- Possibility of production unknown and potentially toxic byproducts
- Comparatively a time consuming process

Biofouling:

- **Biofouling** or **biological fouling** is the accumulation of microorganisms, plants, algae, or small animals where it is not wanted on surfaces such as ship and submarine hulls, devices such as water inlets, pipework, grates, ponds, and rivers that cause degradation to the primary purpose of that item. Such accumulation is referred to as *epibiosis* when the host surface is another organism and the relationship is not parasitic. Since biofouling can occur almost anywhere water is present, biofouling poses risks to a wide variety of objects such as boat hulls and equipment, medical devices and membranes, as well as to entire industries, such as paper manufacturing, food processing, underwater construction, and desalination plants.

- Anti-fouling is the ability of specifically designed materials (such as toxic biocide paints, or non-toxic paints) to remove or prevent biofouling.

- The buildup of biofouling on marine vessels poses a significant problem. In some instances, the hull structure and propulsion systems can be damaged. The accumulation of biofoulers on hulls can increase both the hydrodynamic volume of a vessel and the hydrodynamic friction, leading to increased drag of up to 60%.The drag increase has been seen to decrease speeds by up to 10%, which can require up to a 40% increase in fuel to compensate. With fuel typically comprising up to half of marine transport costs, antifouling methods save the shipping industry a considerable amount of money. Further, increased fuel use due to biofouling contributes to adverse environmental effects and is predicted to increase emissions of carbon dioxide and sulfur dioxide between 38% and 72% by 2020, respectively.

Causes of Biofouling:

- **Settlement of Microorganisms**: Microorganisms like bacteria and diatoms settle on surfaces and create a biofilm.

- **Attachment of Macroorganisms**: Larger organisms such as barnacles, mussels, and seaweed attach themselves to surfaces.

- **Environmental Factors**: Water temperature, salinity, nutrient availability, and light levels can influence the rate and types of organisms that settle on surfaces.

Prevention and Control Of Biofouling:

Antifouling Coatings:

- **Biocidal Coatings**: These release chemicals toxic to fouling organisms. Examples include paints containing copper or organotin compounds.
- **Non-toxic Coatings**: Some coatings prevent organisms from attaching without using biocides, such as silicone-based or foul-release coatings.

Physical Methods:

- **Regular Cleaning**: Removing biofouling manually or mechanically through scraping or pressure washing.
- **Ultrasonic and Electrochemical Systems**: These technologies can deter fouling organisms from settling.

Design Modifications:

- **Surface Roughness**: Smoother surfaces are less likely to accumulate fouling organisms.
- **Material Selection**: Using materials resistant to fouling or easier to clean.

Biological Control:

- Introducing natural predators or competitors of fouling organisms, although this approach is less commonly used due to potential ecological risks.

Regulations and Monitoring:

- International regulations (e.g., IMO's Anti-Fouling Systems Convention) restrict the use of certain biocides to minimize environmental impacts.
- Regular inspection and maintenance to detect and address biofouling early.

402-ENVIRONMENTAL MICROBIOLOGY

prevention of deterioration

- Deterioration refers to the gradual decline or worsening of the condition of materials, structures, or systems over time due to various factors.

Causes of Deterioration:

Environmental Factors:

- **Weathering:** Exposure to elements such as sunlight, rain, wind, and temperature fluctuations.
- **Chemical Exposure:** Contact with pollutants, acids, salts, or other corrosive substances.

Physical Factors:

- **Mechanical Stress:** Pressure, vibrations, or repetitive loading that weakens materials.
- **Abrasion:** Wear and tear from friction or contact with abrasive materials.

Biological Factors:

- **Microbial Growth:** Mold, fungi, or bacteria that degrade organic materials.
- **Insect Infestation:** Damage caused by insects like termites or wood-boring beetles.

Human Factors:

- **Poor Maintenance:** Neglecting regular inspections, repairs, or upkeep.
- **Improper Use:** Misuse, overloading, or inadequate operation practices.

Prevention Of Deterioration:

- Preventing deterioration of materials, whether in infrastructure, buildings, or equipment, involves proactive measures aimed at preserving their integrity and functionality over time.

Proper Design And Control:

- **Material Selection:** Choose materials that are durable and suitable for the intended environment and usage conditions.

- **Design for Drainage:** Ensure proper drainage to prevent water accumulation, which can accelerate deterioration.

Regular Inspection And Maintenance:

- Scheduled Inspections: Regularly inspect structures and equipment for signs of wear, corrosion, cracking, or other forms of damage.
- Prompt Repair: Address any identified issues promptly to prevent them from worsening.

Corrosion Protection:

- **Coatings and Paints:** Apply corrosion-resistant coatings and paints to metal surfaces.
- **Cathodic Protection:** Use techniques such as sacrificial anodes or impressed current systems to protect metal structures from corrosion.

Moisture Control:

- **Waterproofing:** Apply waterproof membranes or coatings to surfaces exposed to moisture.
- **Ventilation:** Ensure adequate ventilation to reduce humidity levels and prevent condensation.

Temperature Control And Environment:

- **Temperature Regulation:** Maintain stable temperatures to minimize thermal expansion and contraction, which can contribute to deterioration.
- **Environmental Monitoring:** Monitor environmental conditions (e.g., humidity, temperature) to identify potential sources of deterioration.

Use Of Protective Clothing:

- **Encapsulation:** Encapsulate materials prone to deterioration (e.g., asbestos, lead-based paint) to prevent their release into the environment.

Proper Use And Handling:

- **Training and Guidelines:** Educate users on proper handling and maintenance practices to minimize accidental damage.

Advanced Materials and Technologies:

- **Nano-coatings:** Use advanced coatings with nanostructures to enhance durability and resistance to degradation.
- **Smart Sensors:** Deploy sensors to monitor structural integrity and detect early signs of deterioration.

Environment Management:

- **Pollution Control:** Implement measures to reduce pollution that can contribute to material deterioration (e.g., acid rain, industrial emissions).

Long – term Planning:

- **Lifecycle Planning:** Develop long-term maintenance plans and budgets to ensure continuous monitoring and upkeep.

By integrating these preventive measures into maintenance routines and project planning, stakeholders can effectively extend the lifespan and functionality of materials, infrastructure, and equipment, reducing long-term costs and environmental impacts associated with deterioration.

Microbial Degration of Oil Spills

Definition:

Microbial degration of oil spills involves oil-degrading bacteria that consume oil hydrocarbons as a souce of energy and carbon.These bacteria break down the oil into less harmful substances,aiding in the natural cleanup of oil- contaminated environments. This bioremediation process is environmentally friendly and can be enhanced by adding nutrients to stimulate bacterial growth.

Oil Spills:

Petroleum contamination of both terrestrial and marine environments results from prospection, extraction, refinement, transport, and storage of oil. Oil spills have been a global issue since the emergence of the oil industry in the early 1900s. The risk of unintentional and intentional spillage has increased as the energy industry and global demand expand. Petroleum is a toxic mixture of organic compounds, trace amounts of heavy metals, and hydrocarbons including many persistent volatile organic compounds (VOCs) and polycyclic aromatic hydrocarbons (PAHs). Discharged into marine environments oil is particularly damaging due to rapid dispersal and the creation of secondary pollutants through photolysis.Petroleum bioaccumulation in terrestrial and marine food chains cause both acute and long term health effects. Exposure to oil damages critical functions within organisms including reproduction, regulation of physiological and chemical processes, and organ function. Large spills alter ecosystem dynamics leading to algae blooms and a mass die-off of marine life. It is estimated that over 1000 sea otters, along with many birds, died from the Exxon Valdez spill. Oil spill clean up efforts commonly employ multiple methods in tandem. Controlled burning and barriers were both used as manual remediation efforts following the Exxon Valdez incident. Chemical solvents and dispersants were briefly used by Exxon in water surrounding the Valdez although discontinued as they required specific conditions and contained carcinogenic compounds. Bioremediation techniques used in the Exxon Valdez spill included nitrogen and phosphorus seeding along coastline increasing available nutrients for indigenous petroleum degrading microorganisms doubling rates of decomposition. Across all remediation techniques less than ten percent of the oil released from Exxon Valdez tanker was recovered.Many genera of plant, microbes, and fungi have demonstrated oil remediating properties including spartina, Haloscarica, Nocardioides, Dietzia, and Microbacterium.

402-ENVIRONMENTAL MICROBIOLOGY

Bioremediation:

Bioremediation refers to the use of specific microorganisms or plants to metabolize and remove harmful substances. These organisms are known for their biochemical and physical affinity to hydrocarbons among other pollutants.Various types of bacteria, archaea, algae, fungi, and some species of plants are all able to break down specific toxic waste products into safer constituents. Bioremediation is classified by the organism responsible for remediation with three major subdivisions: microbial remediation, phytoremediation, and mycoremediation. In most cases, bioremediation works to either increase the numbers of naturally occurring microorganisms or add pollutant-specific microbes to the area. Bioremediation can involve using many varieties of microorganisms as well, either synergistically or independently of each other. The costs and environmental impacts of bioremediation are often negligible when compared to traditional manual or chemical remediation efforts.

Bioremediation of petroleum:

Bioremediation of petroleum contaminated environments is a process in which the biological pathways within microorganisms or plants are used to degrade or sequester toxic hydrocarbons, heavy metals, and other volatile organic compounds found within fossil fuels. Oil spills happen frequently at varying degrees along with all aspects of the petroleum supply chain, presenting a complex array of issues for both environmental and public health. While traditional cleanup methods such as chemical or manual containment and removal often result in rapid results, bioremediation is less labor-intensive, expensive, and averts chemical or mechanical damage.The efficiency and effectiveness of bioremediation efforts are based on maintaining ideal conditions, such as pH, RED-OX potential, temperature, moisture, oxygen abundance, nutrient availability, soil composition, and pollutant structure, for the desired organism or biological pathway to facilitate reactions.[4] Three main types of bioremediation used for petroleum spills include microbial remediation, phytoremediation, and mycoremediation. Bioremediation has been implemented in various notable oil spills including the 1989 Exxon Valdez incident where the application of fertilizer on affected shoreline increased rates of biodegradation.

- Due to their ubiquity across environments, many organisms have evolved to use the hydrocarbons and organic compounds in petroleum as energy while simultaneously denaturing toxins through molecular transfer mechanisms.
- Microbial bioremediation uses aerobic and anaerobic properties of various microbes to respire and ferment compounds transforming toxins into innocuous compounds. These resulting compounds exhibit more neutral pH levels, increased solubility in water, and are less reactive molecularly. Baseline populations of oil-degrading microorganisms typically account for less than 1% of microbiomes associated with marine ecosystems. Remediation techniques which remove reaction limiting factors through the addition of substrate, can boots microbe population towards 10% of the ecosystems microbiome.Dependent on physical and chemical properties, petroleum-degenerative microorganisms take longer to degrade compounds with high-molecular-weight, such as polycyclic aromatic hydrocarbons (PAH's). These microbes require a wide array of enzymes for the breakdown of petroleum, and very specific nutrient compositions to work at an efficient rate.

Microbes work in a step-wise fashion to breakdown and metabolize the components of petroleum.

1. Linear Alkanes
2. Branched Alkanes
3. Small aromatic compounds
4. Cyclic Alkanes

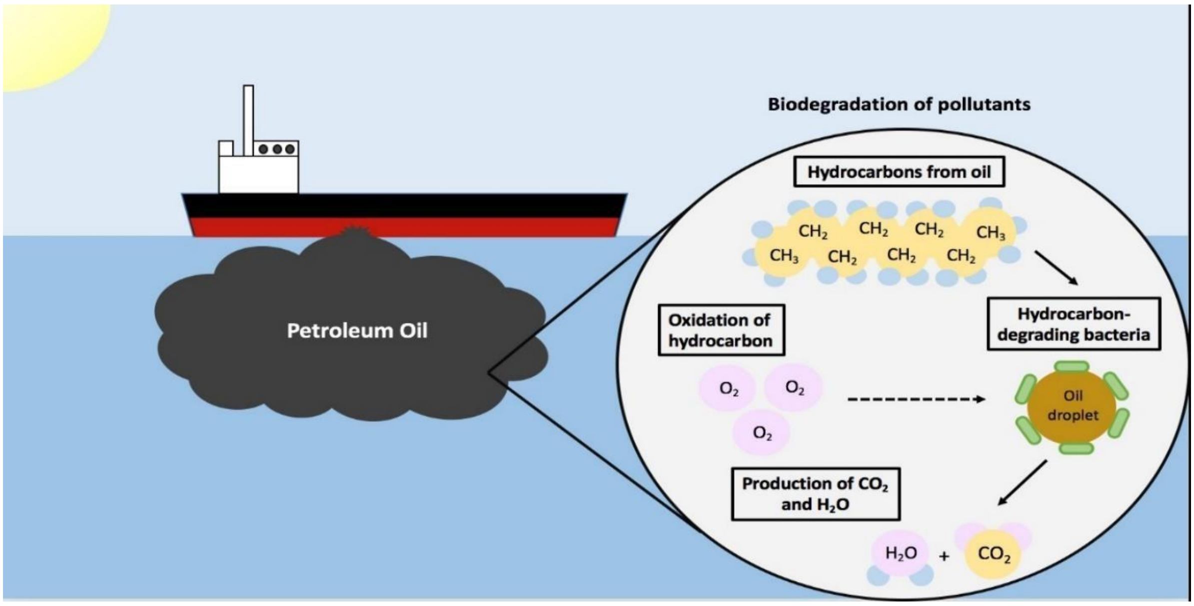
- Treatments that use these breakdown processes most commonly use heat and chemicals to extend the efficacy. Later, more biological systems are used for specific ecosystems that use specific mechanisms.

Phytoremediation:

Phytoremediation is a process in which plants are used to sequester toxins and hydrocarbons into plant tissue from contaminated soils. The main mechanisms for phytoremediation stem from complex relationships between roots and rhizobia. Plants secrete sugars, enzymes, and oxygen from roots which provide necessary substrates for rhizobia and associated rhizosphere microbes to stimulate degradation of organic pollutants.Studies have demonstrated the bioaccumulation abilities of various plants with rhizobial associations, in particular *Chromolaena odorata* were able to remove 80% of petroleum and heavy metal toxins from soils. While more commonly used on terrestrial environments, contaminated marine environments also benefit from plants based bioremediation through the use of various algae and macrophytes.Phytoremediation is most effective when used in conjuction with microbial remediation and Mycoremediation.

Mycoremediation:

Mycoremediation techniques make use of pollutant tolerant fungi which sequester or denature environmental toxins particularly heavy metals. Toxins are sequestered into highly absorbent molecules such chitin and glucan which are found in fungal cell walls. *Saccharomyces cerevisiae* (baker's yeast) can be used to remediate heavy metal contaminated marine ecosystems, with 80% to 90% success in the case of arsenic.Polycyclic aromatic hydrocarbons (PAH) concentrations of soil samples taken from contaminated oil drilling cuttings in Nigeria have been decreased by 7% to 19% using white rot fungi under experimental conditions. Soil contaminated with crude oil displays toxic levels of various heavy metals such as lead, zinc and magnesium. Application of mycoremediation techniques to crude contaminated soils have shown significant reductions of heavy metal concentrations.



Mechanisms involved in bioremediation of toxic compounds

Benefits of Microbial Degration of Oil Spills:

- **Environmental Restoration:** It helps in the natural remediation of contaminatedsites, restoring them to their original condition.
- **Cost Effective**: It is often less expensive than physical or chemical methods of oil spillcleanup.
- **Safety:** It reduces the need for human intervention in hazardous areas, minimizing healthrisks.
- **Eco –** Friendly:This method is biodegradable and does not introduce harmful chemicalsinto the environment.
- **Sustainability:** It leverages natuurally occuring processes, making it a sustainable approach to pollution reduction.

Sanitory Examination Of Water

- A sanitary examination of water refers to the process of assessing the quality and safety of water intended for human consumption or recreational use. This examination involves testing for various physical, chemical, and biological parameters to ensure that the water does not pose health risks to individuals who might come into contact with it.
- Sanitary examinations are crucial for ensuring compliance with water quality standards and regulations set by health authorities. They help in safeguarding public health by identifying potential risks associated with water consumption or recreational activities in water bodies. Results of these examinations inform decisions regarding water treatment, management practices, and public health interventions aimed at minimizing health hazards linked to contaminated water.
- Sanitory examination of water includes the following three tests:

1. MPN Test
2. Membrane Filtration Technique
3. Standard Plate Count

Most Probable Number (MPN) Test :

- Most Probable Number (MPN) is used to estimate the concentration of viable microorganisms in a sample by means of replicating liquid broth growth in ten-fold dilutions. It is commonly used in estimating microbial populations in soils, waters, and agricultural products. MPN test is particularly useful with samples that contain particulate material that interferes with plate count enumeration methods.
- MPN is most commonly applied for quality testing of water i.e to ensure whether the water

is safe or not in terms of bacteria present in it. A group of bacteria commonly referred to as fecal coliforms act as an indicator of fecal contamination of water. The presence of very few fecal coliform bacteria would indicate that water probably contains no disease-causing organisms, while the presence of large numbers of fecal coliform bacteria would indicate a very high probability that the water could contain disease-producing organisms making the water unsafe for consumption.

Principle:

- Water to be tested is diluted serially and inoculated in lactose broth, coliforms if present in water utilizes the lactose present in the medium to produce acid and gas. The presence of acid is indicated by the color change of the medium and the presence of gas is detected as gas bubbles collected in the inverted Durham tube present in the medium. The number of total coliforms is determined by counting the number of tubes giving positive reaction *(i.e both color change and gas production)* and comparing the pattern of positive results *(the number of tubes showing growth at each dilution)* with standard statistical tables.

- MPN test is performed in 3 steps:

1. Presumptive test
2. Confirmatory test
3. Completed test

Presumptive test:

- The presumptive test is a screening test to sample water for the presence of coliform organisms.
- If the presumptive test is negative, no further testing is performed, and the water source is considered microbiologically safe.

Requirements:

- Medium: Lactose broth or MacConkey broth or Lauryl tryptose (lactose) broth
- Glasswares: Test tubes of various capacities (20ml, 10ml, 5ml), Durham tube
- Others: Sterile pipettes.

Preparation of the Medium:

- Prepare medium (either MacConkey broth or lactose broth) in single and double strength concentrations.

For polluted water:

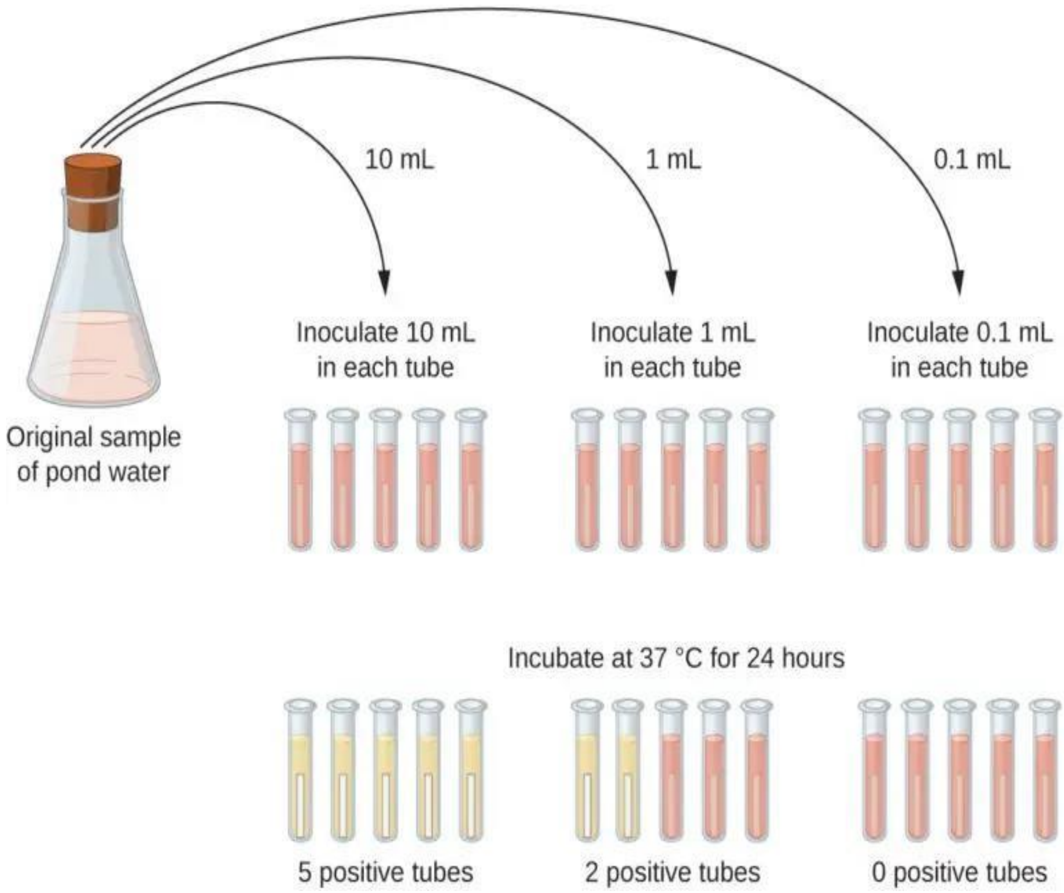
- Dispense the double strength medium in 10 tubes (10mL in each tube) and single strength medium in 5 tubes (10 mL in each tube)and add a Durham tube in an inverted position.

For unpolluted water:

- Dispense the double strength medium in 5 tubes (10mL in each tube) and 50 mL single strength medium in 1 bottle and add a Durham tube in an inverted position.

- Examine the tubes to make sure that the inner vial is full of liquid with no air bubbles.
- Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Procedure of MPN test:



Confirmatory Test:

- Some microorganisms other than coliforms also produce acid and gas from lactose fermentation. In order to confirm the presence of coliform, a confirmatory test is done.
- From each of the fermentation tubes with positive results transfer one loopful of medium to:

1. 3 mL lactose-broth or brilliant green lactose fermentation tube,
2. to an agar slant and
3. 3 mL tryptone water.

- Incubate the inoculated lactose-broth fermentation tubes at 37°C and inspect gas formation after 24 ± 2 hours. If no gas production is seen, further incubate up to a maximum of 48 ±3 hours to check gas production.
- The agar slants should be incubated at 37°C for 24± 2 hours and Gram-stained preparations made from the slants should be examined microscopically.

Completed Test:

- Since some of the positive results from the confirmatory test may be false, it is desirable to do completed tests. For this inoculum from each positive tube of the confirmatory test is streaked on a plate of EMB or Endo agar.

- In this process, a loopful of a sample from each positive BGLB tube is streaked onto selective medium like Eosin Methylene Blue agar or Endo's medium. One plate each is incubated at 37°C and another at 44.5± 0.2°C for 24 hours.

- High temperature incubation (44.5 ±0.2) is for detection of thermotolerant *E.coli.*

- Following incubation, all plates are examined for the presence of typical colonies.

Membrane Filtration Technique:

- Membrane filtration is a technique used in water testing and analysis to concentrate microorganisms from a water sample for detection and enumeration.

Principle:

- The membrane filtration method is based on the principle of passing a known volume of water through a membrane filter with a defined pore size (typically 0.45 micrometers). This filter retains microorganisms present in the water on its surface.

Procedure:

- **Sample Collection:** A representative water sample is collected from the source to be tested. This could be a drinking water source, recreational water body, or wastewater effluent.

- **Filtration:** A known volume (usually 100 mL or 1 L) of the water sample is passed through a sterile membrane filter under controlled vacuum or pressure. The filter captures microorganisms present in the water.
- **Filter Transfer:** After filtration, the membrane filter is carefully transferred onto a nutrient agar plate or other suitable growth medium that supports the growth of microorganisms.
- **Incubation:** The nutrient agar plate with the membrane filter is then incubated at appropriate conditions (usually at a specified temperature for a certain period, such as 24-48 hours). During incubation, any viable microorganisms trapped on the filter begin to grow and form visible colonies.

Applications:

- **Microbial Enumeration:** Membrane filtration is commonly used to quantify the number of total coliforms, fecal coliforms (such as E. coli), and other indicator bacteria in water samples. These indicators help assess the sanitary quality of water and its suitability for drinking or recreational use.
- **Regulatory Compliance:** Many regulatory standards for drinking water and wastewater discharge require testing using membrane filtration to ensure compliance with microbial contamination limits.

Standard Plate Count Technique:

 The standard plate count (SPC) technique, also known as the pour plate method or spread plate method, is a widely used microbiological technique for quantifying the number of viable bacteria or fungal colonies present in a liquid sample.

- **Sample Collection:** Take a sample of the liquid you want to test.
- **Dilution:** Mix the sample with sterile liquid to dilute it several times.
- **Plating:** Take small amounts of each diluted sample and spread them evenly on agar plates (like petri dishes).
- **Incubation:** Leave the plates in a warm place (like an incubator) for bacteria to grow. This usually takes a day or two.
- **Counting:** After incubation, count the visible colonies (groups of bacteria) that have grown on the plates.

- **Calculating:** Based on the number of colonies and the dilution factor, calculate how many bacteria were in the original sample.

Advantages:

- **Quantitative:** Provides a count of viable microorganisms present in the sample.
- **Versatile:** Can be used for a wide range of liquid samples.
- **Standardized:** Widely accepted and used in microbiology laboratories and regulatory agencies.

Microbial Degration of Oil Spills

Definition:

Microbial degration of oil spills involves oil-degrading bacteria that consume oil hydrocarbons as a souce of energy and carbon.These bacteria break down the oil into less harmful substances,aiding in the natural cleanup of oil- contaminated environments. This bioremediation process is environmentally friendly and can be enhanced by adding nutrients to stimulate bacterial growth.

Oil Spills:

Petroleum contamination of both terrestrial and marine environments results from prospection, extraction, refinement, transport, and storage of oil. Oil spills have been a global issue since the emergence of the oil industry in the early 1900s. The risk of unintentional and intentional spillage has increased as the energy industry and global demand expand. Petroleum is a toxic mixture of organic compounds, trace amounts of heavy metals, and hydrocarbons including many persistent volatile organic compounds (VOCs) and polycyclic aromatic hydrocarbons (PAHs). Discharged into marine environments oil is particularly damaging due to rapid dispersal and the creation of secondary pollutants through photolysis.Petroleum bioaccumulation in terrestrial and marine food chains cause both acute and long term health effects. Exposure to oil damages critical functions within organisms including reproduction, regulation of physiological and chemical processes, and organ function. Large spills alter ecosystem dynamics leading to algae blooms and a mass die-off of marine life. It is estimated that over 1000 sea otters, along with many birds, died from the Exxon Valdez spill. Oil spill clean up efforts commonly employ multiple methods in tandem. Controlled burning and barriers were both used as manual remediation efforts following the Exxon Valdez incident. Chemical solvents and dispersants were briefly used by Exxon in water surrounding the Valdez although discontinued as they required specific conditions and contained carcinogenic compounds. Bioremediation techniques used in the

Exxon Valdez spill included nitrogen and phosphorus seeding along coastline increasing available nutrients for indigenous petroleum degrading microorganisms doubling rates of decomposition. Across all remediation techniques less than ten percent of the oil released from Exxon Valdez tanker was recovered.Many genera of plant, microbes, and fungi have demonstrated oil remediating properties including spartina, Haloscarica, Nocardioides, Dietzia, and Microbacterium.

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Bioremediation:

Bioremediation refers to the use of specific microorganisms or plants to metabolize and remove harmful substances. These organisms are known for their biochemical and physical affinity to hydrocarbons among other pollutants.Various types of bacteria, archaea, algae, fungi, and some species of plants are all able to break down specific toxic waste products into safer constituents. Bioremediation is classified by the organism responsible for remediation with three major subdivisions: microbial remediation, phytoremediation, and mycoremediation. In most cases, bioremediation works to either increase the numbers of naturally occurring microorganisms or add pollutant-specific microbes to the area. Bioremediation can involve using many varieties of microorganisms as well, either synergistically or independently of each other. The costs and environmental impacts of bioremediation are often negligible when compared to traditional manual or chemical remediation efforts.

Bioremediation of petroleum:

Bioremediation of petroleum contaminated environments is a process in which the biological pathways within microorganisms or plants are used to degrade or sequester toxic hydrocarbons, heavy metals, and other volatile organic compounds found within fossil fuels. Oil spills happen frequently at varying degrees along with all aspects of the petroleum supply chain, presenting a complex array of issues for both environmental and public health. While traditional cleanup methods such as chemical or manual containment and removal often result in rapid results, bioremediation is less labor-intensive, expensive, and averts chemical or mechanical damage.The efficiency and effectiveness of bioremediation efforts are based on maintaining ideal conditions, such as pH, RED-OX potential, temperature, moisture, oxygen abundance, nutrient availability, soil composition, and pollutant structure, for the desired organism or biological pathway to facilitate reactions.[4] Three main types of bioremediation used for petroleum spills include microbial remediation, phytoremediation, and mycoremediation. Bioremediation has been implemented in various notable oil spills including the 1989 Exxon Valdez incident where the application of fertilizer on affected shoreline increased rates of biodegradation.

- Due to their ubiquity across environments, many organisms have evolved to use the hydrocarbons and organic compounds in petroleum as energy while simultaneously denaturing toxins through molecular transfer mechanisms.
- Microbial bioremediation uses aerobic and anaerobic properties of various microbes to respire and ferment compounds transforming toxins into innocuous compounds. These resulting compounds exhibit more neutral pH levels, increased solubility in water, and are less reactive molecularly. Baseline populations of oil-degrading microorganisms typically account for less than 1% of microbiomes associated with marine ecosystems. Remediation techniques which remove reaction limiting factors through the addition of substrate, can boots microbe population towards 10% of the ecosystems microbiome.Dependent on physical and chemical properties, petroleum-degenerative microorganisms take longer to degrade compounds with high-molecular-weight, such as polycyclic aromatic hydrocarbons (PAH's). These microbes require a wide array of enzymes for the breakdown of petroleum, and very specific nutrient compositions to work at an efficient rate.

Microbes work in a step-wise fashion to breakdown and metabolize the components of petroleum.

1. Linear Alkanes
2. Branched Alkanes
3. Small aromatic compounds
4. Cyclic Alkanes

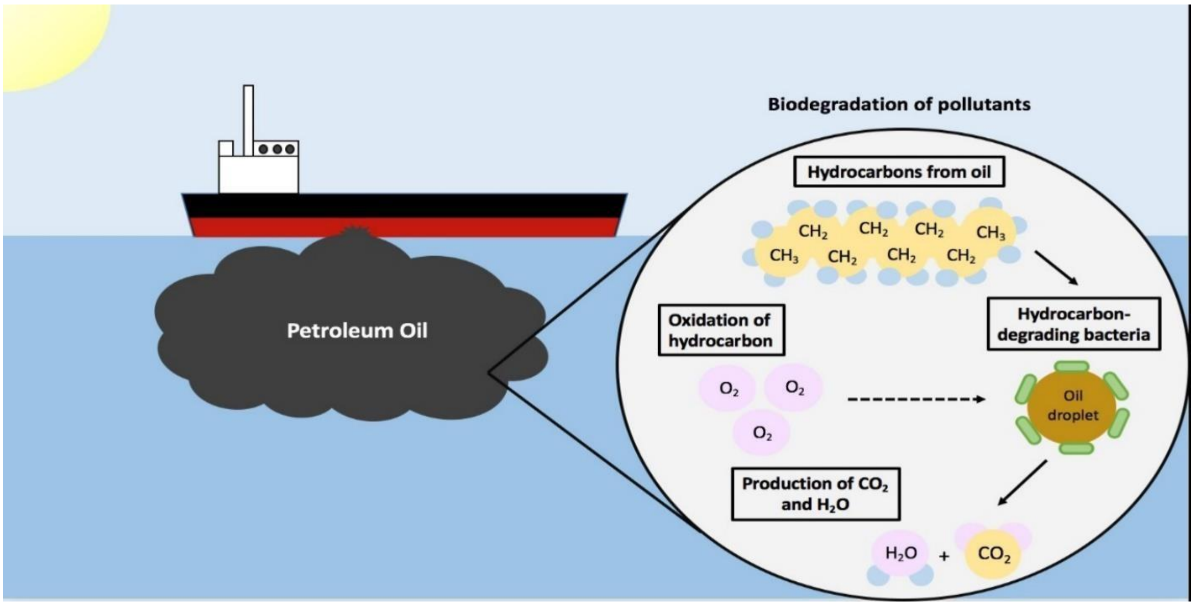
- Treatments that use these breakdown processes most commonly use heat and chemicals to extend the efficacy. Later, more biological systems are used for specific ecosystems that use specific mechanisms.

Phytoremediation:

Phytoremediation is a process in which plants are used to sequester toxins and hydrocarbons into plant tissue from contaminated soils. The main mechanisms for phytoremediation stem from complex relationships between roots and rhizobia. Plants secrete sugars, enzymes, and oxygen from roots which provide necessary substrates for rhizobia and associated rhizosphere microbes to stimulate degradation of organic pollutants.Studies have demonstrated the bioaccumulation abilities of various plants with rhizobial associations, in particular *Chromolaena odorata* were able to remove 80% of petroleum and heavy metal toxins from soils. While more commonly used on terrestrial environments, contaminated marine environments also benefit from plants based bioremediation through the use of various algae and macrophytes.Phytoremediation is most effective when used in conjuction with microbial remediation and Mycoremediation.

Mycoremediation:

Mycoremediation techniques make use of pollutant tolerant fungi which sequester or denature environmental toxins particularly heavy metals. Toxins are sequestered into highly absorbent molecules such chitin and glucan which are found in fungal cell walls. *Saccharomyces cerevisiae* (baker's yeast) can be used to remediate heavy metal contaminated marine ecosystems, with 80% to 90% success in the case of arsenic.Polycyclic aromatic hydrocarbons (PAH) concentrations of soil samples taken from contaminated oil drilling cuttings in Nigeria have been decreased by 7% to 19% using white rot fungi under experimental conditions. Soil contaminated with crude oil displays toxic levels of various heavy metals such as lead, zinc and magnesium. Application of mycoremediation techniques to crude contaminated soils have shown significant reductions of heavy metal concentrations.



Mechanisms involved in bioremediation of toxic compounds

Benefits of Microbial Degration of Oil Spills:

- **Environmental Restoration:** It helps in the natural remediation of contaminatedsites, restoring them to their original condition.
- **Cost Effective**: It is often less expensive than physical or chemical methods of oil spill cleanup.
- **Safety:** It reduces the need for human intervention in hazardous areas, minimizing health risks.
- **Eco –** Friendly:This method is biodegradable and does not introduce harmful chemicals into the environment.
- **Sustainability:** It leverages natuurally occuring processes, making it a sustainable approach to pollution reduction.

Environmental Impact Studies

Definition:

Environmental impact studies are reports that evaluate the possible environmental impacts of a proposed project or development.They are prepared by qualified Ecologists and use a technical, objective and multidisciplinary approach. They also take into account the inter – related socio – economic, cultural and human – health impacts, both beneficial and adverse. Environmental impact studies aims to protect significant natural and habitats during development.

Environmental Impacts:

Environmental impacts are defined as the effects or consequences that anthropic activity can generate in ecosystems in particular and in the environment in general. Virtually any activity has some kind of effect, from the large mining or agricultural companies that modify vast areas of territory to the hikers who are walking quietly on a trail.

Types of Environmental Impacts:

On the one hand, impacts on the environment vary in intensity: depending on the degree of activity, its nature or how they are organized, they can generate a greater or lesser impact. It is not the same to compact the ground with your footsteps than to compact the ground to build a football stadium

On the other hand, we cannot guarantee that the impacts will be negative in all cases.

We distinguish two types of environmental impacts:

- Negative environmental impacts
- Positive environmental impacts

Negative environmental impacts:

- They are considered to have a harmful effect on the environment.
- They are the vast majority.

Positive environmental impacts:

- They have a beneficial effect on the environment.
- For example, some companies committed to the environment are beginning to protect forests as a compensation measure for CO2 generation, which has added value.

What is an environmental impact study:

An environmental impact study (in Spain it is known as EIA, or "Environmental Impact Assessment**")** consists of the evaluation of a project to determine its possible environmental impacts on its surroundings. It is in itself a technical, objective and multidisciplinary study. In many cases this study prior to the project is a legal requirement to carry it out , and it will be the public administration who, based on it, among other requirements, accept it, reject it or decide to modify it.

How to do environmental impact study – simple example:

At the time of do an environmental impact study Big complications tend to arise, especially when the projects that usually require these studies include large works. However, we are going to explain a simple example so that it is understood: a bar near a natural setting.

- **General description of the project:** It includes its location, its objectives, a detailed study of the activities, consumption of materials, occupied land, etc.
- **Exposure of alternatives:** It is required to study several alternatives in terms of materials, processes, etc. It is also required to consider not carrying out the project.
- **Environmental Inventory:** natural spaces, human health, biodiversity, geodiversity, soil and subsoil, air, water, climatic factors, etc.
- **Possible effects and assessment of Impacts:** assessment, assessment and quantification of the foreseeable direct or indirect effects of the programmed activities.
- **Preventive and corrective measures:** closely related to alternatives. For example, to prevent compostable waste from reaching the natural setting, bins can be installed near the site.
- **Environmental monitoring and monitoring program:** his will include, for example, the emptying and maintenance of wastebaskets and the control of noise emissions and water consumption.
- **Summary of the study and conclusions.**

Deterioration of Paper

- The deterioration of paper refers to the process where paper becomes weak and brittle over time.
- This can happen due to environmental factors like light, heat, moisture, and pollutants, or due to the inherent qualities of the paper itself.
- Deterioration of paper is caused by many factors such as acid hydrolysis, oxidative agents, light, air pollution, or the presence of microorganisms.
- The origin of the cellulosic material, as well as pulping and papermaking procedures, additives, and storage conditions play a crucial role.

Common Signs of Paper Deterioration:

- **Yellow or discoloration:** The paper may develop a yellow or brown tint.
- **Brittleness:** The paper becomes fragile and may crumble easily when handled.
- **Fading:** Printed text or images may become lighter and less visible.
- **Strains:** Brown spots, known as foxing, may appear on the paper.
- **Curling or wrapping:** The paper may not lay flat and can have a distorted shape.

These signs indicate that the paper is aging and may need conservation measures to prevent further damage.

Causes Of Paper Deterioration:

Here are some of the common factors that can cause paper deterioration

- Exposure to light or heat can cause certain chemical reactions to break down the paper fibers and fade the inked text.
- Air pollutants,e.g., sulfur dioxide and nitrogen oxides, interact with paper and speed up deterioration. These pollutants contribute to the discoloration and weakening of the fibers.
- If exposed to damp conditions, the paper could develop mold or fungi. These agents don't just damage the fibers but also lead to stains, decay, and foul smells.
- Insects like termites and beetles feed on the fibers of paper.
- Improper handling of the paper can lead to creasing, tears, and sometimes permanent fingerprint stains.

Sewage And Sludge Treatment DO

- Sewage sludge treatment describes the processes used to manage and dispose of sewage sludge produced during sewage treatment. Sludge treatment is focused on reducing sludge weight and volume to reduce transportation and disposal costs, and on reducing potential health risks ofdisposal options.

- Water removal is the primary means of weight and volume reduction,while pathogen destruction is frequently accomplished throughheating during thermophilic digestion, composting, or incineration.

- The choice of a sludge treatment method depends on the volume of sludge generated, and comparison of treatment costs required for available disposaloptions.

- Air-drying and composting may be attractive to rural communities, whilelimited land availability may make aerobic digestion and mechanical dewatering preferable for cities, and economies of scale may

  encourage energy recovery alternatives in metropolitan areas.

Sewage Treatment:

It is a form of waste management. With regards to biological treatment of sewage, the treatment objectives can include various degrees of the following: to transform or remove organic matter, nutrients (nitrogen and phosphorus), pathogenic organisms, and specific trace organic constituents(micro pollutants).

Pollutants in sewage

- BOD(Bio Chemical Oxygen demand)
- COD(Chemical Oxygen demand)

- TSS (Total Suspended Solids)
- **BOD (Biochemical Oxygen demand)**

➢ The BOD is an important measure of water quality .It is measure of the amount of oxygen needed by bacteria and other organisms to oxidize the organic matter present in a water sample over a period of 5 days at 20 degreeC.

COD (Chemical Oxygen Demand)

➢ COD Measures all organic carbon with the exception of some aeromatics (BENZENE,TOLUENE,PHENOL etc.)

➢ which are not completely oxidized in the reaction.

➢ COD is a chemical oxidation reaction .

➢ Ammonia will not be oxidized.

Total Suspended Solids

➢ Total suspended solids(TSS) include all particles suspended in waterwhich will pass through a filter.

➢ As levels of TSS increase, a water body begins to lose its ability to support adiversity of aquatic life.

➢ Suspended solids absorb heat from sunlight, which increases water temperature and subsequently decreases levels of dissolved oxygen(warmerwater holds less oxygen than cooler water).

There are two types of sewage treatment:

1. **Aerobic treatment**
2. **Anaerobic treatment**

1. **Aerobic :**

Sewage treatment in the presence of Oxygen-MBBR, SBR-where aerators/blowers aerators/blowers are installed-generally no smell during treatment.

2. Anaerobic :

Sewage treatment in the absence of Oxygen-UASB- No aerators/blowers arerequired- foul smell during treatment.

Sludge treatment :

- Sludge from municipalities (often called sewage sludge) is a byproduct of wastewater treatment.
- It is normally a mix of organic matter from human waste, food waste particles, microorganisms, trace chemicals and inorganic solids from products and medicine we use, together with water bound to these materials.
- In the United States, sewage sludge is also called wastewatersolids.
- Sludge treatment is an important component of municipal wastewater treatment.
- The primary objectives of sludge treatment are:
- To reduce the harmful microorganisms (such as pathogens) in sludge, lessening the health risks for people and environments thatcome into contact with the material;
- To stabilise some of the organic matter in sludge that would otherwise naturally turn into harmful gases in the atmosphere;
- To decrease its final volume, typically reducing the associatedhandling costs; and
- To collect products and by-products of the treatment process, whichmay be used or sold to offset the sludge treatment costs.

Disposal

The final destination of treated sewage sludge usually is the land. Dewateredsludge can be buried underground in a sanitary landfill.

It also may be spread on agricultural land in order to make use of its value asa soil conditioner and fertilizer. Since sludge may contain toxic industrial chemicals, it is not spread on land where crops are grown for human consumption.

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