BIOREMEDIATION: A REMEDY FOR ENVIRONMENTAL POLLUTION

Abstract

Bioremediation is an emerging biotechnological process that detoxifies the environmental contaminants like pesticides, petroleum hydrocarbon, heavy metal, plastic, etc. to some non-hazardous byproducts. Mainly plants, bacteria and fungi actively take part in this process but now a day's researchers are using earthworm and some genetically modified microorganisms for an effective bioremediation in less time. Microorganisms mainly bacteria consumes these pollutant as their food and use the carbon source and electrons for their growth. Some in-situ strategies like bioattenuation, bioaugmentation, biostimulation and some ex-situ strategies like landfarming, composting, biopiles are implied for convincing remediation of these organic pollutants. Many bacterial species have the hydrocarbon degrading properties and they serves an important role in resolving the global issue "OIL SPILL". Biosurfactants are surface active molecules secreted from microbial cells and they have significant role in agriculture, mining, food formulations and in pharmaceutical industry. "E-waste" is now treated by microorganisms to promote more sustainability and reducing the number of e-waste across the world. Earthworms are now used for bioremediation of some recalcitrant molecules along with phytoremediation, mycoremediation and bacterioremediation. Some genetically modified microorganisms like Pseudomonas diminuta are used for speedy bioremediation of some pollutant pesticides like parathion.

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"Bioremediation" refers to the biotechnological process of detoxifying environmental pollutants from polluted air, water, soil. It's mainly focused to convert parent hazardous molecules to non-hazardous products (such as CO_2 and H_2O) to protect the ecosystem from pollution. This process defends animals and human being from diseases. Mostly bacteria, fungi and plants have the physiological ability to degrade the complex hazardous molecule. Before detoxifying and degrading any specific contaminating chemical by bioremediation technology we must ensure that it is biodegradable and it should not have an ill-effect towardsenvironment. The biodegradation is a complex process which depends on few factors such as the indigenous microbes of the contaminant site, the pollutant's quantity and the environmental factors. Emerging biotechnological approaches are gaining success to detoxify this pollutants without harming our environment. Site characterization is an important task before applying this bioremediation technology. As if environment is not suitable for this process it can create some recalcitrant molecules by degrading the pollutants, which has a harmful effect towards environment and it's life forms.

Two general approaches in bioremediation technology are –

- Modify the environment and
- technological approach.

Bioremediation by modifying the environment by adding nutrients, microbial culture or aeration can give effective results with less expense. But the technological approach to decontaminate a polluted site by separating the soil from the polluted site to another place or incineration needs labor and it's an expensive process. Bioremediation process can potentially degrade some specific pollutant such as heavy metals, plastic, petroleum hydrocarbon, crude oil. Bioremediation can be applied to solve some important environmental hazards like oil spill. Several thousand contaminated sites have been identified where bioremediationtechnology can be applied to detoxify the places.

I. HISTORY OF BIOREMEDIATION

The concept of bioremediation was established by people long ago before they knew the term, as it is a natural process. In the 1940s few scientists discovered that petroleum hydrocarbon could be broken down by various microorganisms. However, they did not understand the exact mechanism of bioremediation and it's wide application in controlling environmental hazard. In 1970s the progression of research field led the scientist in the discovery of few microorganisms which can degrade petroleum hydrocarbon substances like gasoline from contaminated water. First commercial application of bioremediation was made in 1972 for the Sun Oil pipeline spill in Ambler, Pennsylvania. Richard Raymond of Sun Oil continued the bioremediation projects throughout 1970s. In mid 1980s researchers accentuate the bioengineering of the microorganism which are used for bioremediation. But they failed to earn the expected results. In mid 1990s scientists focused on naturalmicroorganisms for improvement of their ability in bioremediation process.

II. MECHANISM OF BIOREMEDIATION

Microorganisms consume the organic pollutants as their food. The transformation of organic pollutant to harmless by products is takes place in microorganisms. The microorganisms use the organic pollutant to grow and reproduce. The carbon source present in the organic compound which is the basic building blocks of new cellular components and some electrons are produced during the transformation. The provided electrons are used as substrates by microorganisms to produce energy. Usually, microorganisms get energy from the organic compound by breaking the chemical bonds from the organic pollutant to an electron acceptor such as oxygen. They produce more cell and cellular constituents by accumulating the source of energy such as carbon, electron. The microorganisms break the chemical bonds and transfer the electrons. This is a high energy yielding reaction which is called oxidation-reduction reaction. As the organic compound loses electron it is said to be oxidized while the chemical by product got electron so it is said to be reduced. Most of the microorganisms break thechemical bonds of organic pollutant with the aid of oxygen so it is called aerobic respiration while some uses other electron acceptor too. The electron donor compound and electron accepting compounds are called primary substrates.

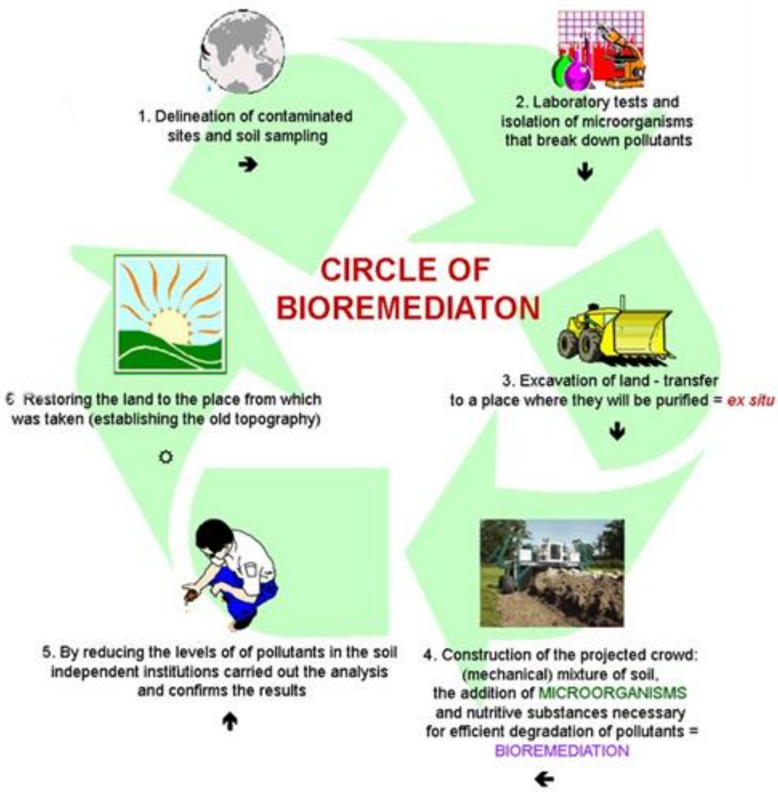


Figure 1: Mechanism of Bioremediation
https://www.onlinebiologynotes.com/bioremediation-concept-types-advantages-and-limitations/

III. FACTORS AFFECTING BIOREMEDIATION

The process of bioremediation depends upon several factors to eliminate pollutants from environment. Some of them are:

1. **Concentration of Contaminants:** The rate of synthesis of degrading enzymes in bacteria is proportional to the concentration of the contaminants. The lower the concentration of contaminants will led to less production of degrading enzymes by bacteria in soil.

2. **Nutrient Availability:** Every microorganisms need some basic nutrients (such as Carbon, Nitrogen, Phosphorus, Potassium) for their physiological activities. The excessive presence of this nutrients in soil directly affect the microbial activity by reducing it synthesizedegrading enzymes.

3. **Temperature & pH:** Microorganisms need optimum pH and temperature to survive and synthesize degrading enzymes. Neutral pH is required for the synthesis of degrading enzymes fordegradation of petroleum hydrocarbon. 30°- 40°c is the optimum temperature for bioremediationprocess in soil.

IV. BIOREMEDIATION STRATEGIES

1. **In-situ Bioremediation**: In-situ Bioremediation refers to the biotechnological process of removal of toxic pollutants in the contaminated site. It is mainly employed in polluted soil and contaminated groundwater through consuming of microorganisms and biotransformation. It is the process involving less area and less exposure to public. In-situ Bioremediation is also and economical process.

2. **Bio-stimulation**: Bio-stimulation process mainly focuses in cultivating the population of helpful bacteria which can degrade the pollutants from environment. This cultivation can be done by adding some water soluble rate limiting mineral nutrient salts like Potassium nitrate (KNO_3), Sodium nitrate ($NaNO_3$), Ammonium nitrate (NH_4NO_3), Di-potassium phosphate (K_2HPO_4). In this process bioremediation can be done by the bacteria which are naturally present in soil.

   Limitations Includes: Due to high concentration of pollutant or low microbial population,bioremediation process could not take place.

3. **Bioaugmentation:** Bioaugmentation process is done by inclusion of exogenous microbial culture to supplement the population of microbes present in that particular environment. This process has shown positive outcomes in wastewater treatment. Bioaugmentation exhibits efficiency in speedy removal of pollutant in wastewater treatment. Example, *Dehalococcus ethenogenes* to remove trichloroethylene (TCE).

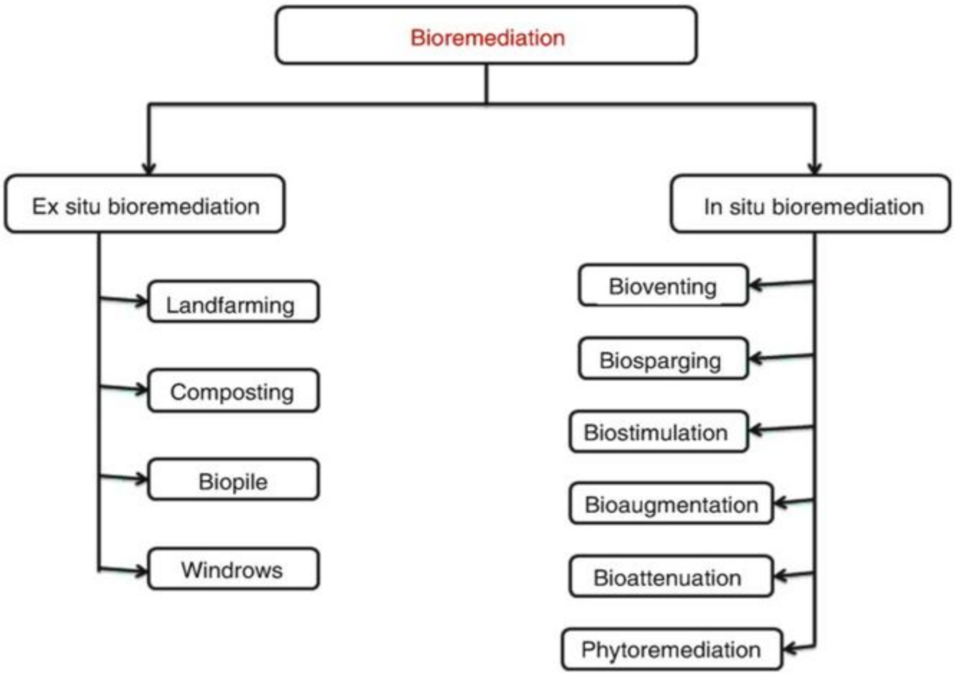
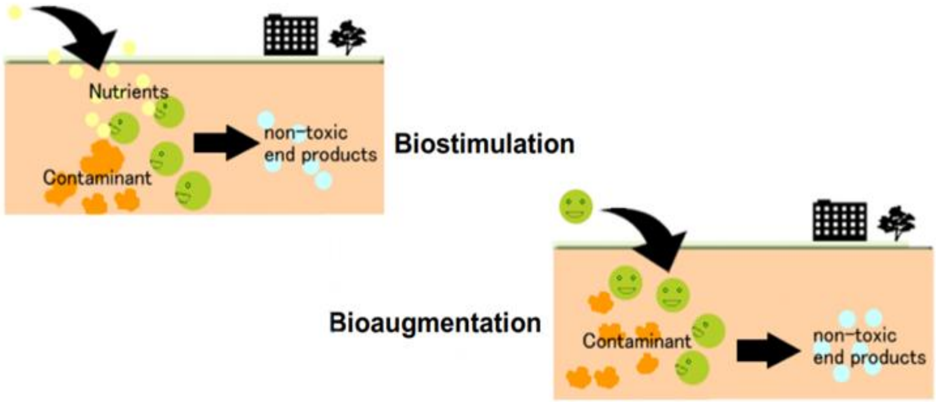


Figure 2: Biostimulation and Bioaugmentation
https://sites.nicholas.duke.edu/superfund/project5_2017-22/

Limitations includes: Due to some inability to adapt in a new environment, exogenous microorganismssometimes could not survive.

4. **Bio-Attenuation:** Bio-attenuation is the process of naturally degrading the pollutants by incorporation of nutrients or bacteria. The present indigenous microorganisms in that environment will determine the metabolic activity. They will take action as natural attenuation.

5. **Bioventing:** Bioventing is the process of inclusion of air and nutrients through specifically constructed wells to treat contaminated soil by stimulating the growth of indigenous microorganisms. It mainly focuses in the biodegradation of pollutants adsorbed into soil in the unsaturated zone. Vacuum pumps are mainly used for low air flow as it will maintain the oxygen concentration and minimizes the volatilization and release of contaminants to the atmosphere. Sometimes, mineral nutrients like Nitrogen and Phosphorus is also required in contaminated soil with petroleum hydrocarbon .As it has been depleted this nutrients due to increased carbon concentration.



6. **Bio-Sparging:** Bio-sparging is the process of injection of compressed air into the contaminated subsurface to increase the oxygen concentration in contaminated ground-water. The increased oxygen concentration will enhance the degradation of pollutants from groundwater. Bio-sparging is comparatively an easy and economical process to treat contaminated groundwater.

7. **Ex-Situ Bioremediation:** Ex-situ Bioremediation refers to the biological process of degrading pollutants from the contaminated area to a remote treatment location. Ex-situ technique usually applied for treating highly contaminated materials. In this technique contaminants usually extracted from the source to a remote place. However during the transport process hazards can be spread or there is a risk of an accidental spill during the transport. Ex-situ technique is rarely applied to treat contaminatedgroundwater.

8. **Landfarming:** Landfarming is the most common method in ex-situ bioremediation. Contaminated soils are removed from the site and placed over a large landfarming area. Around 20-30 cm layers of soils are spread out in a lined cell manner and aeration is done by tilling the mixture. The soil is periodically tiled to stimulate the growth of the indigenous microorganisms. Sometimes additional organic or chemical compounds are also added for their nutrients. Landfarming is the most economical method for bioremediation. Moreover it can provide a speedy result due to less remediation time and easy procedure. Still it's only limitation is it require a large area.

9. **Windrows:** Windrows refers to the process of periodically tilling of contaminated soil to increase theaeration in the soil. It helps the existing microorganisms to grow and enhance the bioremediation rate. The frequent tiling manner is due to spread the pollutants in an even order.

10. **Bio-piles:** Bio-piles refers to the process of converting the highly toxic pollutant to low toxicbyproducts in contaminated soil .Contaminated soils are excavated, piled up and then soils are placed in pad for aeration. The aeration system helps in microbial growth. Bio-piles are designed in such manner that it controls the moisture content, nutrients and optimum temperature and it consists of a drainage system for precipitation exposure.

11. **Composting:** Composting is an ex-situ bioremediation technique mainly employed to treat contaminated soil or lagoon sediment. Soil mixture is excavated after non-hazardous

organic amendments like manure, hay, wood, vegetable wastes are added in the contaminated soil to enhance the microbial growth and soil porosity. The temperature maintained for composting is 55°-65°c.The large microbial population mainly contain mesophilic and thermophilic bacteria that transforms the pollutants to a non-toxic byproducts. It is a feasible process to detoxify and degrading hazardous pollutants from soil.

COMPOSTING	LANDFARMING
Soil mixture is separated and excavated bysieving.	Soil mixture is placrd in a lined cell mannerand pilled .
No mechanics required.	Mechanics are required to separate the contaminants.
Wood, hay, manures are added.	Sometimes crushed limestones are added.
RDX, PAH removal takes place.	PAH, hydrocarbons removal takes place.

V. BIOREACTORS

Bioreactors refers to any reaction vessel or container where contaminants are degraded. Then the contaminants are isolated to collect. Bioreactors can be termed as suspended or attached-growth system. Their activity can be increases by adding activated carbon or nutrients. Activated carbon adsorbs contaminant and releases microorganisms slowly for degrading pollutants. Bioreactors are used for the treatment of contaminated soil, water. In bioreactors the vessels are equipped with a mixing system consists of oxygen and nutrient influent and affluent pumps. It can be operated in cascade manner. Examples-fixed-film bioreactors, plug flow bioreactors, fluidized bed bioreactor, sequencing batch bioreactors, slurrybioreactors, aqueous bioreactors.

1. **Uses of Bioreactors:** They are used in primary treatment of biodegradable organic matter, petroleum hydrocarbon. They are not so effective in pesticide degradation. Recently they are being used in municipal and industrial waste water treatment.

2. **Limitation of Bioreactors:** It cannot work efficiently in degrading high concentration pollutants. Low temperature usually decrease the rate of biodegradation inside the bioreactors. They are expensive than the other bioremediation strategies. Remnants of bioreactors also need waste treatment after disposal from the bioreactors

FEATURES	IN-SITU BIOREMEDIATION	EX-SITU BIOREMEDIATION
Bioremediation place	In situ means bioremediation at contaminant’s original place.	Ex-situ bioremediation means bioremediation outside the originalplace.
Cost	Less expensive	Expensive
Speed of the process	Very slow process	Rapid process

Main focus	It mainly focuses on indigenous microorganisms.	It mainly focuses on Exogenousmicroorganisms and nutrients .
Example	Bioaugmentation,Bio-stimulation, Bio-venting, Bio-sparging.	Composting, Windrows,Bio-piles , Bioreactors.

Forms of Bioremediation: Mainly three forms of bioremediation are seen. They are: A. Phytoremediation ; B. Mycoremediation; C. Bacterioremediation

- **Phytoremediation:** Phytoremediation is the of detoxifying pollutants from the environment by plants.It is a cost- effective technology. It is mainly of five types- Phyto-stabilization, Phyto-degradation, Phyto-extraction , Rhizo-filtration, Phyto-volatilization.
  Hyacinth, Azolla, Duckweed, Cattail, Poplar are some of the example of the plants used in phytoremediation. They have some common features as high tolerance, fast growth, toxic heavy metal accumulation and degradation.

  - **Advantage:** It is a low maintenance economically friendly process.
    Plants can be easily observed.
    It enhance the fertility of the soil.

  - **Disadvantage :** For a long term approach a low growing tree is required.Sometimes plants accumulate the heavy metals in itself but degradation fails to take place which lowers the plant health .
    Phytoremediation is totally dependent upon the surface area of root which is less compared to other bioremediation process.

Types of Phytoremediation	Mechanism	Pollutant	Plants	Microorganis Ms Involved
Phytostabilisation	Stabilizing the pollutant water soil,precipitation.	Inorganics like Cu,Cr,Pb,Zn, As ,Cd	*Hybrid poplar,gras ses Brassica juncea*	*Anthyllis vulneraria , Festuca arvernensis.*
Rhizofiltration	Rhizosphere accumulation through sorption,concentration and precipitation.	Metals like Cd,Cu, Zn, Cr.	*Brassica juncea,Rye, Tobacco.*	*Aruna donax,Eichornia crassipes,Carex penduta.*
Phytovolatilization	Volatilization of pollutants by transpiration.	Chlorinated solvents,inorganics as Se,As.	*Brassica juncea,Alfa alfa,Poplar.*	*Liriodendron tulipifera.*
Phytodegradation	Pollutants effacement.	Phenol,herbicides, Chlorinated solvents,Munitions.	*Stonewart,Black willow.*	*Pueraria sp, Elodea canadensis.*
Phytoextraction	Accumulating inorganics in plant parts.	Inorganics suchas Cu,Cr,Ni,Pb,Cd ,Ag.	*Brassica juncea,Helianthus annus.*	*Rhizophora sp,Rumex crispus,Sedum alfredii.*

- **Mycoremediation**: Mycoremediation is the process of detoxifying and degrading pollutants to valuable byproducts by fungi. This fungi-based remediation is cost effective and environmentally safe. Moreover, fungi can degrade petroleum hydrocarbon, polycyclic aromatic hydrocarbons, pesticides, herbicides, detergents ,industrial wastes. Mycoremediation procedure totally depends upon the enzyme synthesizing ability of fungus which can degrade pollutants Most common fungi used in phyco-remediation are-*Aspergillus niger, Aspergillus flavus, Trichoderma longibrachium*. Two common genus of fungi used in phyco- remediation are-*Agaricus* and *Agrocybe* .

  - **Genus *Agaricus*:** Mushrooms of genus *Agaricus* usually accumulates and detoxify the toxic heavy metals like zinc, cadmium, copper etc. Some of them contains antibacterial content. Eg,-*Agaricus campestris*, *Agaricus bisporus.*
  - **Genus *Agrocybe*:** Mushrooms of this genus transforms the chemical pollutants to beneficial byproducts like laccase an oxidase reductase enzyme which can degrade a wide range of industrial pollutants, lignin. pH 3-5 is the optimum for laccase enzyme activity.

- **Advantage:**
  - It is a natural process which does not harm the environment.
  - It is cost effective process and produce some beneficial byproducts.
  - This process shows result in less time than the other alternative process.

- **Disadvantage:**
  - The fungal structures like mycelium are sensitive to environment.
  - Fungi need optimum pH ,temperature to produce degrading enzyme.
  - However this process is not efficient to degrade some complex chemical products.

- **Bacterioremediation**: Microorganisms like bacteria are major bioremediating population. Bacteria can degrade the harmful contaminant and provide a safe environment for all living beings. Bacteria can easily decompose organic pollutant like herbicide, pesticide, sewage waste, biodegradable industrial waste and evenheavy metals, crude oil and petroleum hydrocarbons. Some bacteria used in bioremediation are: *Pseudomonas aeruginosa, Arthrobacter sp, Streptomyces sp., Bacillus licheniformis, Rhodopseudomonas palustris, Corynebacterium propniquum.*

VI. ROLE OF MICROORGANISMS IN BIOREMEDIATION

Microorganisms are used to transform complex pollutants to harmless by products by cellular metabolism.The by products produced are CO_2 and H_2O. Sometimes fungi produced some beneficial by products such as enzymes as laccase transferase, Aromatic-nitro reductases, Quinone reductases. Mainly bacteria and fungi are employed for this biotechnological process. Nowadays, genetically engineered microorganisms are used in bioremediation . Microorganisms have the adaptive nature which helps them to inhabit in around every location where other living forms cannot survive. Due to this adaptive ability and exceptionally active metabolism they can degrade pollutants from every contaminated place such as hot Sulphur spring, thermal lake, glaciers, volcanic regions and even in the salt lakes. Microbes consume theharmful pollutants as their food and gains energy from it.. Some common bacterial species involved in bioremediation are: *Pseudomonas sp, Mycobacterium sp, Alcaligenes sp*. Some common fungal species are: *Aspergillus niger, Phanerochate chrysporium*. The microorganisms are specific in degrading some pollutants like heavy metals, petroleum hydrocarbon, polycyclic aromatic hydrocarbon.

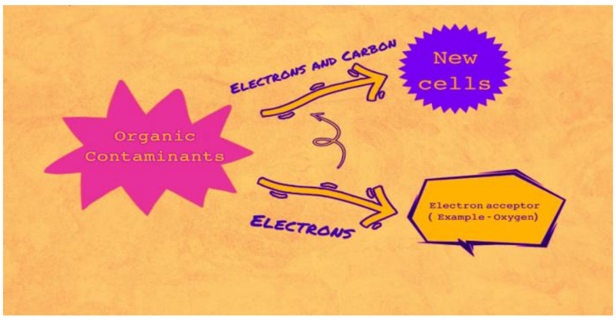


Figure 3: Microbial Metabolism of Organic Contaminants

1. **Bioremediation Efficacy Testing:** To determine the biodegradation strategy of a specific pollutant it is important to keep a record of the required conditions for the bioremediation. This strategy cannot be determined by in-situ bioremediation technology, some laboratory experiments must be done. Laboratory experiments show the efficiency of the particular treatment to degrade a specific pollutant from the contaminated site. Laboratory experiments should take place in a similar environment as the contaminated site to produce effective results. Sometimes samples collected from the polluted site is treated in laboratory to determine the nature of the indigenous microbial population. They help to know the potential of the treatment strategy. Some parameters determined in laboratory for efficient bioremediation are composition of pollutant, nature of indigenous microorganisms, determination and degrading rates, enumeration of microbial population as compared to untreated controls (Microbial Ecology, Atlas and Bartha).The most direct approach in bioremediation to detect the rate of degradation of pollutant from a specific site. This disappearance can cause by biodegradation, leaching, phytoremediation etc. National Environmental Technology Application Corporation (NETAC) has established on 29th April, 1999 by the cooperative agreement between EPA and the University of Pittsburgh Trust to promote biotechnology and bioremediation process. The purpose of NETAC is to the government and private sectors make ease with the biotechnological processes and nation will go forward in solving the environmental problems positively. Then the corporates started to developing these technologies to commercially promote them. Then EPA and NEPAC together convenes a panel of experts who gave strategies to make advancement technology in bioremediation process. The recommended products were tested at EPA Laboratory in Cincinnati, Ohio. Laboratory protocols are essential to determine the efficiency of the laboratory products in increasing biodegradation rate significantly.

2. **Side Effects Testing:** Not only learning the effective bioremediation strategy, we must determine if the bioremediation treatment is producing any harmful toxins and causing environmental damage. Standard tests are performed to determine the toxicity of the specific chemicals. Chronic toxicities and its damage towards cell are also determined by side effect testing methods. The toxicity tests are generally in *Daphnia* bivalves(oyster larvae),fish as rainbow trout. Sometimes the endemic species such as salmon or herring are also used to run this test. The effects of algal growth rates are also determined by side effect testing. This test results help to determine the dose of biofertilizer to be used to enhance biodegradation rate. *Photobacterium phosphoreum* is used in microtox assay. In fuel contaminated soil microtox assay is performed to determine the land treatment possibilities. The mutagenic remnants of soil contaminated with polycyclic aromatic hydrocarbon are assayed for Ames test. In this assay initially the content of mutagenic remnant increases with biodegradation rate but after it progressed a certain level without microsomal enzymes it returned to normal harmless condition. Wyman et al.(1979) found that picric acid(10 µg per plate) produced by the reduction of 2,4,6- trinitrophenol (picric acid) by a strain of Pseudomonas aeruginosa demonstrated mutagenicity (both frameshift and base substation mutation) only after activation with a rat liver homogenate preparation.Picric acid (1µg per plate)induced both base-pair substitution and frameshift mutation without activation of rat liver preparation.(Microbial ecology – Bartha and Atlas)

3. **Oil Spill**: Oil spill refers to the uncontrollable release of petroleum hydrocarbon, crude oil gasoline or other oil products into the environment specially the marine regions. Hazards associated with oil spill is one of the global concern in todays world. It can pollute the

aquatic ecosystem- marine lives like many fishes, invertebrates. Oil spill can affect the soil which is in contact with the polluted water and affects its fertility. It can affect the human beings and other animals through external exposure. This can also affect living forms through consumption or inhalation. Animals and human beings can face eye and skin related problems by affecting with oil spill. According to Marine insight, nearly 706 million gallons of oil pollute the ware every year. Oil spill can smother the small aquatic forms like small fishes, invertebrates. Tarballs are formed in ocean due to oil spill. They are formed by the accumulation of oil in sea areas. By consuming sea food from the shore of the contaminated marine area people facing some serious health hazards. Oil spill prevent the sunrays to enter in the depth of the sea which affect the survival of marine lives. The power plants depending upon marine region faces problem. The loss in the tourism, fishery projects due to oil spill led human beings to great economical loss. Oil spill can be slowly parsed by some microorganisms such as -*Pseudomonas putida, Cladosporium resinae, Candida marina, Pseudomonas aeruginosa, Bacillus submanius. The recent MKN Wakashio spill of Mauritius –about 1000 tones of oil spilled into a wild-life sanctuary for the rarest wild liveforms after the Japaneese ship struck in 2020* .

Figure 4: Degradation Mechanism of Petroleum in Oil Spill

4. **Role of Microorganisms in Oil Spill:** In 1990s Dr. Tatyana Chernikova, research officer in Bangor university, Wales discovered some hydrocarbon degrading properties in bacteria .Many bacterial species can degrade crude oil .Researchers believed that a large population ofmicroorganisms are required to degrade the oil, few microorganisms will not able to degrade crude oil and form CO_2 and H_2O as end product. Presence of high concentration of pollutants can lower the microbial enzyme activity. Assistance of more than few species of microorganisms are required to degrade the petroleum hydrocarbon, crude oil and other forms of oil. Cooperation between microorganisms are required for better bioremediation .Most microorganisms undergo aerobic respiration to degrade the oil ,but running under anaerobic respiration the microorganisms produce some recalcitrant molecules. The most common oil degrading microorganisms are: Flaobacterium, Candida, Chromobacteria, Aeromonas, Cyanobacteria, Aspergillus, Mucor, Bacilli, Fusarium, Moraxella, Nocardia etc.

5. **Best possible tools to handle Oil spill:** Handling oil spill without hampering the whole marineecosystem is not an easy job .But some tools are really helpful to achieve success in this quite challenging task. Some of the tools are:

- **Skimmers**: Spilled oils flow over seawater which can be collected by skimmers before it affect the marine ecosystem. Skimmers can be operated from shore or boat. Skimmer operations largely depends upon the nature of sea. In turbulent condition of sea it cannot be operated. Skimmers can efficiently recover most of the oil spill hence it is a cost effective process.

- **Sorbents**: Sorbents are inert, insoluble molecules which adsorb oil from sea surface. They are mainly of three types: Natural organic sorbent, Natural inorganic sorbent, Synthetic sorbent.

Types of sorbent	**Example**
Natural organic sorbent	Hay, peatmoss, straw.
Natural inorganic sorbent	Clay, vermiculate, sand.
Synthetic sorbent	Polyethylene, Polypropylene.

- **Dispersing Agents:** Dispersing agents are chemical agents which has the ability to breakdown petroleum hydrocarbon, crude oil and other forms of oil into small oil droplets. It usually enhances the rate of oil dispersion in the sea. It can be effectively used in a large area of sea region. The dispersing agents sometimes shows toxic effect towards the marine life forms.

- **Containment Booms:** Containment booms are usually works as floating barrier which prevents the oil to spread over a wide sea region. It is made up of plastic, metal or other materials and reduce the rate of oil exposure in sea. Containment booms usually works efficiently in calm sea but in turbulent condition of sea it usually fails to show the desired results.

- **Burning in-situ:** In this process the spilled oil is ignited over the sea, where oil spill has taken place. It can reduce the oil content effectively. But it can show harmful effect in marine ecosystem and environment. The fumes released by this process can cause major air pollution.But it is effective in fresh spill to reduce the spread of oil in a large area of sea region.

VII. BIODEGRADATION OF ORGANIC POLLUTANTS

Now a days environmental pollution is a subject of global concern. The rapid industrialization, modern techniques of farming using advanced pesticides, herbicides and insecticides are all connected in pollution. Soil acts as reservoirs for the toxic pollutants to disperse and degraded, which are then subject to react with atmosphere and make toxic byproducts. Microorganisms living in soil, are also take part in nutrient cycling and by their metabolic activity the pollutants become degraded and CO_2 and H_2O are formed. Microorganisms degrade this pollutants due to two reasons-Firstly it consumes its required nutrients from the pollutant to generate energy. Secondly, the pollutant may exert toxic pressure on microbial cell and that modified the cell's metabolic activity. Thirdly, sometimes the pollutant resembles like natural substrate for microbial enzymes.

VIII. MICROBIAL METABOLIC ABILITY IN BIODEGRADATION

Microorganisms transforms and detoxify pollutants by secreting specific enzymes, vesicles, cell surface proteins, chelating agents. They target organic pollutants such as hydrocarbons, pesticide, herbicides, insecticides and inorganic pollutants such as heavy metals as their substrates. Microorganisms mainly bacteria and fungi catalyzed reactions to detoxify these pollutants. They produce stable metabolites, harmless by products, biomass. Detoxification mostly occurs due to the unavailability of certain nutrients of microorganisms, then theyuse pollutants to consume nutrients and produce energy. The enzymes involved in metabolisms are of three types: intracellular, extracellular and membrane bound, which present unique challenges in terms of bio-availability. As intracellular and membrane bound enzymes help the microbial cell to bring the substrate into the cell ,whereas the extracellular enzymes works outside of the cell, uptake of pollutants generally not happen in the case of extracellular enzymes.

Fungi produce some beneficial enzymes as laccases and peroxidases that show effects against pollutants.*Pseudomonas chlororaphis* is capable of cleaving tin-carbon bonds in several organotin compounds.(J.D VAN HAMME) Napthalene dioxygenase, for example can catalyze over 76 types of reactions including mono and di-hydroxylations ,desaturations, O- and N-dealkylations and sulfidations against mono and heterocyclic compounds(Ellis et al 2000;Resnick et al 1996).Microorganisms also possess more than one set of genes for the metabolism of specific pollutants, such as pesticides, Polyhydroxy aromatic hydrocarbon(PAH),petroleum, crude oil and heavy metals.

IX. BIODEGRADATION OF PETROLEUM HYDROCARBON COMPOUND

The diverse enzymatic capability of microorganisms led to the evolving method of detoxifying pollutants in the Petroleum industry. The bioremediation of petroleum, crude oil, PAH can be maintained by suitableenvironment establishment. The two methods mostly used in in-situ bioremediation: bioaugmentation and bio stimulation are mainly used to detoxify and degrade the oil forms. Biodegradation of petroleum is a complex process. The Petroleum hydrocarbons consists of four classes:

- Saturate Petroleum hydrocarbon;
- Aromatic Petroleum hydrocarbons
- Resin Petroleum hydrocarbons
- Asphaltenes Petroleum hydrocarbons

As petroleum hydrocarbon binds with soil it is hard to remove them and degrade but microbial catalyzing ability can degrade it. Linear hydrocarbons can degrade more easily than branched or cyclic hydrocarbon. The high molecular weight PAH cannot be easily degrade at all.Some microorganisms capable of Petroleum hydrocarbon, crude oil, PAH degradation are :*Pseudomonas sp* , *Alacaligines sp* , *Acinetobacter sp* , *Arthrobacter sp*, *Flavobacterium sp*, *Baccilus subtilis*, *Corynebacterium sp*, *Brevibacterium sp*,*Micrococcus roseus*, *Sphingomonas* *sp*. It is an eco-friendly process to detoxify thecrude oil, petroleum hydrocarbon, polyhydroxy aromatic compounds (PAH). It is also a cost effective approach in degradation of petroleum. Fungi and yeasts are also used in this method to degrade petroleum hydrocarbons. This process is accomplished rapidly in an aerobic environment. By using peroxidase and oxygenase enzymes bacteria attack organic pollutants and incorporates oxygen. Through Glycolysis, TCA cycle the organic pollutants transforms into harmless by products. From precursor metabolites such as acetyl co-A, succinate, pyruvate the cell biomass is synthesized.

X. BIODEGRADATION OF PESTICIDES

Biodegradation of pesticides generally takes place by the hydrolysis of methyl-carbamate linkage by an enzyme called carbofuran hydrolase. Some bacteria capable of degrading pesticides are: Pseudomonas sp, Pandoraea sp, Klebsiella sp, Phanerochaete chrysporium, Achromobacterium sp, Sphingomonas sp, Flavobacterium sp, Arthrobacter sp. Bioaugmentation, bio-stimulation, phytoremediation are the three approaches taken to detoxifypesticides from environment. The biodegradation of pesticides by bacteria, fungi and algae is the mosteconomical and eco-friendly process.

1. **Organochlorine Pesticides:** Organochlorine pesticides have incessant presence in the environment. Five classes of organochlorine pesticides are derived from are: Dipheny-ethane (DDT, DDE, DDD), Hexachlorobenzene (HCB), Hexachlorocyclohexane (α-HCH, β-HCH, γ-HCH), Cyclodiene (Aldrin, endrin, dieldin) and chlorinated hydrocarbons (Toxaphene, chlordecone). Biotic and abiotic factors determine the fate of the pesticides in the environment. The rate of degradation of pesticides differ from one another. Some pesticides such as Dieldrin, DDT are present in the environment for a fairly long duration and accumulates in the soil. It affect the food chain and balance of the ecosystem got disrupted. Some soil bacteria have the metabolic efficiency to degrade the organochlorine pesticides such as-*Arthrobacter sp, Pseudomonas sp, Bacillus sp ,Micrococcus sp.*

2. **Organophosphate Pesticides:** The organophosphate pesticides are ester derivatives of phosphoric acid. They are not as recalcitrant material as organochlorine pesticides. They are used in a large scale in farming and found in contaminated soil and water from all over the world. Phytoremediation, bioventing, biopiling, biosparging, bioattenuation, landfarming, windrows are some approaches taken to degrade organophosphate pesticides. Some soil habitant bacteria such as *Pseudomonas sp, Arhrobacter sp, Microccus sp, Bacillus sp can degrade the pesticides.* One example of organophosphate pesticide is-Methyl parathion.

3. **Carbamate Pesticides:** Carbamate pesticides are broad spectrum pesticides. They are used inland farming as pest controlling agent extensively. They are soluble in water and thermally unstable. The carbamate pesticides are highly toxic and polar compound. They can be degrade by biodegradation, biotransformation, hydrolysis and oxidation. Some soil bacteria such as Arthrobacter sp, *Phanerochaete chrysporium, Sphingobacterium sp, Flavobacterium sp, Acgromobacterium sp* can degrade this bacteria. Some examples of carbamate pesticides are-aldicarb, carbofuran, oxamyl,methomyl.

- **Biodegradation of Heavy Metals:** Microorganisms can endure metal toxicity but the toxic effect of heavy metals on environment cannot be endured by human beings and other animals. High concentration of heavy metals such as cadmium, arsenic from soil and water is a form of threat towards our lives. The heavy metals and their compounds are crucialfor the welfare and economy of most countries. But most of the heavy metals such as cadmium, vanadium, manganese, arsenic, lead, iron, mercury, zinc, nickel, chromium etc. Are source of disease and discomfort in human life. Microbial bioremediation is the most beneficial strategy to reduce the contamination of heavy metals in environment. Microorganisms specially bacteria have the ability to degrade heavy metals and form some non-toxic byproducts. Bacteria produces iron-chelating substances such as siderophores for mobilizing and up-takingiron from the environment. Most commonly Agrobacterium sp, Bacillus sp, Klebsiella sp, Enterobacter sp, Rhodococcus sp, Pseudomonas sp, Mesorhizobium sp, Microbacterium sp causes heavy metal detoxification. Desulfovibrio desuluricans reacts with sulphate to form H2S and then react with cadmium and Zinc to form insoluble form of metal sulfides.

- **Heavy Metal Tolerance in Microbes**: Most bacteria, fungi, algae, actinomycetes can tolerate a high concentration of metals in soil and water. They have thephysiological ability to tolerate the toxicity of metals.

Microorganisms type	Species	Heavy metal removed	Environment type
Bacteria	*Bacillus cereus* *Pseudomonas veronii* *Pseudomonas putida* *Bacillus subtilis* *Enterobacter cloacae* *Citrobacter sp* *Phormidium valderium* *Ganoderma applantus* *Chlorella vulgaris*	Cr Cd, Zn, Cu Cr Cr Cr Cd Cd, Pb Cu, Hg, Pb Ni	Soil & waterSoil Soil Soil Soil Soil Soil & waterSoil Soil
Algae	*Spirulina Spirogyra* *Cladophora* *Oedogonium*	Cr, Cu, Fe, Mn Pb, Cu Pb, Cu As	Water Water Water Water
Filamentous fungi	*Aspergillus versicolor* *Aspergillus fumigatus* *Rhizopus oryzae* *Gleophyllum sepiarium*	Ni,Cu Pb Cr Cr	Soil Soil Soil Soil
Actinomycetes	*Actinomyces flavoviridis* *Actinomyces levoris*	Hg, Pb Ur Ur	Soil Soil Soil

	Streptomyces viridochromogenes		
Cyanobacteria	*Cyanobacterium sp*	Cd, Ni	Soil
Higher plants	*Thlaspi caerulescens*	Zn, Cd	Soil
Yeast	*Saccharomyces cerevisiae*	Pb, Cu	Soil

- **Mechanism of Heavy Metal Degradation:**

  - Bacteria uptake iron by forming iron-chelating complex or siderophores and other metals byintracellular metal binding proteins.
  - Biochemical pathways then altered to stop the uptake of metals.
  - Enzymes converts toxic metals to harmless compounds .
  - Reduction of metal concentration in bacterial cell and environment.

XI. WHAT IS XENOBIOTIC COMPOUNDS?

A xenobiotic compound is the external compound found to be present in a living cell in which is not expected to be present. Few examples of xenobiotic compounds are: Pesticides, drugs, food additives, flavorings, industrial chemicals, plants components. Without metabolism, xenobiotic component will increase to toxic range so metabolism is required in animal body to keep the balance of toxicity of xenobiotic compounds.

XII. BIOSURFACTANTS

Biosurfactants are surface active molecules derived from diverse group of microorganisms. They are secreted my microbial cell extracellularly or produced by surface of the microbial cell. They are commonly amphipathic molecules containing both hydrophobic and hydrophilic domain which confers them stability by reducing surface and interfacial tension. As they increase solubility by reducing surface tension they have a wide range of application in the industrial processes including foaming, emulsification, solubilization. Biosurfactants have a variety of physical and chemical properties such as low toxicity, stable at extreme Ph & temperature, bio-degradability, foaming ability. They have wide application in cosmetics, oil recovery, agriculture, pharmacy, food and medicine industry. The show antimicrobial and anticancer properties. They have anti-adhesive property so they are used to disrupt the biofilm. They are also used as an anti-aging and wound healing agent. Some methods are applied for screening biosurfactant producing microorganisms by measuringinterfacial and surface tensions. Some of the methods are: Du-Nouy-Ring method, Stalagmometric method, Pendant drop shape technique. This techniques are based on the measurements of some assays ,named hemolytic assay, drop coolapse assay, emulsification assay, oil spreading assay.

1. **Properies of Biosurfactants:**

- **Surface and Interfacial Tension:** Biosurfactants increase solubility and lowers concentration. In low concentration of solvent it's surface and interfacial tension also decreases, which gives them efficiency.

- **Ph and Temperature Tolerance:** Biosurfactants show stability in diverse range of Ph and temperature. The lipopeptide from Bacillus licheniformis is stable at temperature 75°c and Ph around 5 to 12 for up-to 140 hours. They also tolerate salt concentration up-to 10%.

- **Low Toxicity:** Biosurfactants shows antiallergic and low-toxic properties. As most of the population concerned about allergic reactions so biosurfactants are safe to use in cosmetics, pharmaceutical, agricultural industries.

- **Specificity:** Biosurfactants contains diverse functional groups and according to the specific functional group they are specific in their action of degrading specific pollutants from the environment. They are particularly applied in food, pharmaceutical and cosmetics industries due to their specificity.

- **Biodegradability:** Biosurfactants can be easily degraded by microorganisms in soil, water. So they areapplied in bioremediation technology and waste treatment.

- **Bioavailability:** Biosurfactants are produced by most of the microorganisms and can even produced by raw materials and industrial waste.

2. **Classification of Biosurfactants:** Biosurfactants are classified into two categories according to their chemical structure and microbial origin. The two categories of biosurfactants are:

   - **High Molecular Weight Biosurfactants:** Glycolipid, Phospholipid, Lipopeptides.

   - **Low Molecular Weight Biosurfactants:** Polymeric And Particulate Biosurfactants.

3. **Glycolipid Biosurfactants:** Glycolipid is the most studied biosurfactant. They are composed of long chain aliphatic acids or hydroxyl fatty acid. They are linked by ether or ester group. Examples: Sophorolipids are produced by *Candida sp* and Rhamnolipids are produced by *Pseudomonas aeruginosa.*

- **Phospholipid Biosurfactants:** Many microorganisms share similar structure and produce phospholipid surfactants such as Corynomycolic acid. Example: biosurfactant from *Corynebacterium lepus.*

- **Lipopeptides Biosurfactants:** Lipopeptides are also a well known biosurfactant. They are composed of a large number of cyclic lipopeptides containing a lipid connecting to an amino acid chain. Some decapeptide antibiotics like gramicidin and lipopeptide antibiotics like polymyxins show some surface active properties. Example: Surfactin produced by *Bacillus subtilis* is also a cyclic lipopeptide containingseven amino acid ring attached with fatty acid.

- **Polymeric Biosurfactants:** They are high molecular weight biosurfactants. They are mainly composed of a backbone containing three or four repeating sugars with fatty acids linked to the sugars. Example: Emulsan, Liposan, Alasan, Mannoprotein, Biodispersan. Liposan is produced by *Candida lipolytica*. Mannoprotein is synthesized by *Saccharomyces cerevisiae.*

- **Particulate Biosurfactants:** Particulate biosurfactants are also of high molecular weight. They are of two types: Extracellular vesicles and whole microbial cell. Extracellular membrane vesicles synthesizes micro- emulsions by partitioning hydrocarbons. The micro-emulsions are crucial in hydrocarbon uptake by cell of the microorganisms. The whole microbial cell plays the key role in producing biosurfactants.

Biosurfactant Type	Biosurfactant Name	Name of The Microorganisms	Properties
GLYCOLIPID	Rhamnolipis	*Pseudomanas aeruginosa*	Antibacterial, foaming, Anti-aging
	Sorpholipid	*Candida sp*	Antibacterial
	Xylolipid	*Lactococcus lactis*	Antibacterial
	Glucolipid	*Burkholderia cenocepacia*	----------
	Cellobiolipids	*Ustilago maydis Candida*	----------------
	Mannosylerythritolipid	*Candida antarctica*	Antimicrobial Antimicrobial, antifungal
	Flocculosin	*Pseudozyma sp*	------------
	Lipopolysaccharides	*Klebsiella oxytoca*	Antimicrobial
PHOSPHOLIPID	Fatty acid	*Corynnebacterium lupus*	Cleansing agent, Emulsifying agent
	Corynomycolic acid	*Penicillium sp*	----------
	Oleic acid	*Issatchenkia orientalis*	Emulsifying agent
	Neutral lipids	*Nocardia erythropolis*	-----------

LIPOPEPTIDE	Phospholipid	*Acidothiobacillus thiooxidans*	Solubilizer and wetting agent
	Spiculicorpic acid	*Penicillium spiculisporium*	-----------
	Gramicidin	*Brevibacillus brevis*	Anti-biotic
POLYMERIC BIOSURFACTANT	Viscosin	*Pseudomonas flourescens*	------------
		Bacillus subtilis	Anti-aging, Anti-wrinkling agent, Foaming agent, emulsifier
	Fengycin	*Bacillus subtilis*	Antibacterial
	Iturin	*Bacillus subtilis*	Antibacterial & Antifungal
	Lichenysin	*Bacillus licheniformis*	Antibacterial
	Peptide lipid	*Bacillus licheniformis*	---------
	Liposan	*Candida lipolytica*	Emulsifier & stabilizer
	Alasan	*Acinetobacter radioresistens*	Emulsifier
	Emulsan	*Acinetobacter calcoaceticus*	Emulsifier
	Mannoprotien	*Saccharomyces cerevisiae*	Emulsifier
	Biodispersan	*Acinetobacter calcoaceticus*	Emulsifier& stabilizer
	Bioemulsan	*Gordinia sp*	Emulsifier, stabilizer& antioxidant

4. Advantages of Biosurfactants:

- As compared to other chemical surfactants biosurfactants are highly biodegradable.
- Biosurfactants are low-toxic material.
- They are less irritable and aesthetically more acceptable for human skin.
- The most important role of biosurfactants are in bioremediation as they cause no harmful effecttowards our environment.

5. Disadvantages of Biosurfactants:

- They are not produced in a large scale and they are expensive materials.
- In oil spill or other environmental application biosurfactants are required in a bulk quantity sothey may be costly.
- Deficiency in getting pure substances which are required for their important role in cosmetics,pharmaceutical and food industries.

6. Application of Biosurfactants:

- **Anti-adhesive Agent:** Biosurfactants inhibits the adhesion of pathogenic microorganisms to any inanimate surface or infectious causing sites. They help to combat pathogenic microorganisms' colonization in any solid surfaces. So they are used as anti-adhesive agents. Example- Biosurfactants synthesized from *Streptococcus thermophiles, Pseudomonas fluorescens*.

- **In Food Formulations:** Biosurfactants enhance the texture and self -life of starch containing foods.They also improve texture and consistency of fat based food. They control the clumping of the fat globules. Moreover, they also provide stabilization in aerated systems. They also retard staling ofice-cream and bakery products.

- **Oil Storage Tank Cleaning:** Biosurfactants are known for it's property of reduction in surface and interfacial tension. This property helps them to recover, transport of oils through pipelines.

- **Therapeutic and Biomedical Applications:** Biosurfactants can be used as emulsifying, solubilizing and antimicrobial agents. This property helps them in displaying a broad spectrum anti-viral, anti-bacterial and antifungal activity.

- **Biosurfactants in Agriculture:** They are used as biocontrol agents. They combat against crop diseases. They also maintain soil profile Organophosphate pesticides also degraded by biosurfactants.

- **Biosurfactants in Mining:** They are used in detoxifying minerals and toxic pollutants in mining.

Types of antimicrobial activity	**Producing microorganisms**	**Name of Bio-surfactant**
Antibacterial Agent	*Pseudomonas aeruginosa* *Bacillus licheniformis* *Candida antartica*	RhamnolipidsPeptide lipid Mannosylerythritolipid
Anticancer Activity	*Candida antartica* *Pseudomonas aeruginosa* --------------------	Mannosylerythrintol Rhamnolipid Sophorose lipid
Anti-HIV	*Candida bombicola*	Sporolipid
Antifungal	*Bacillus subtilis* ----------------	Iturin Mycosubtilin

7. Role of Biosurfactants in Bioremediation:

Microbial biosurfactants follow two mechanisms to increase the bioremediation rate. They are –

- The increase of the substrate availability for microorganisms
- The interaction of microbial cell with substrate increases the hydrophobicity of microbial cell
- Biosurfactants has the potential role in microbial growth on the surface of pollutants likepetroleum hydrocarbon
- Biosurfactants achieve this goal by increasing the surface area between the oil and water byemulsification
- Organic pollutants like heavy metals are strongly adsorbed into the soil surface, this canincrease the remediation time
- The extension of remediation time can be decreased by the application of biosurfactants
- The mobilization of pollutants from soil takes places at concentration below CMC (Criticalmicelle concentration)
- At the mentioned concentration biosurfactants usually lowers the surface and interfacialtension.
- This led in the increase of contact between soil and petroleum, crude oil
- Solubilization occurs at concentration above CMC (Critical micelle concentration)
- At above CMC (Critical micelle concentration) the hydrophobic tails of microbial surfactants connect together inside micelle, while the head of the hydrophobic moiety directed towards aqueous phase
- They facilitates the microbial cells for up-taking hydrophobic substrates more easily by increasing the hydrophobicity of degrading microorganisms
- This mechanisms will help microorganisms to contact and uptake pollutants like oil drops
- Biosurfactants also play crucial role in bioremediation of heavy metals from soil and

water

- Heavy metals are usually non-biodegradable and they can be accumulated and transformedfrom one toxic chemical form to another less harmful for biodegradable form
- They form complexes with heavy metals
- The complexes can be removed by washing process
- They play significant role in biodegradation of heavy metal, petroleum hydrocarbon, crudeoil or other oil forms.

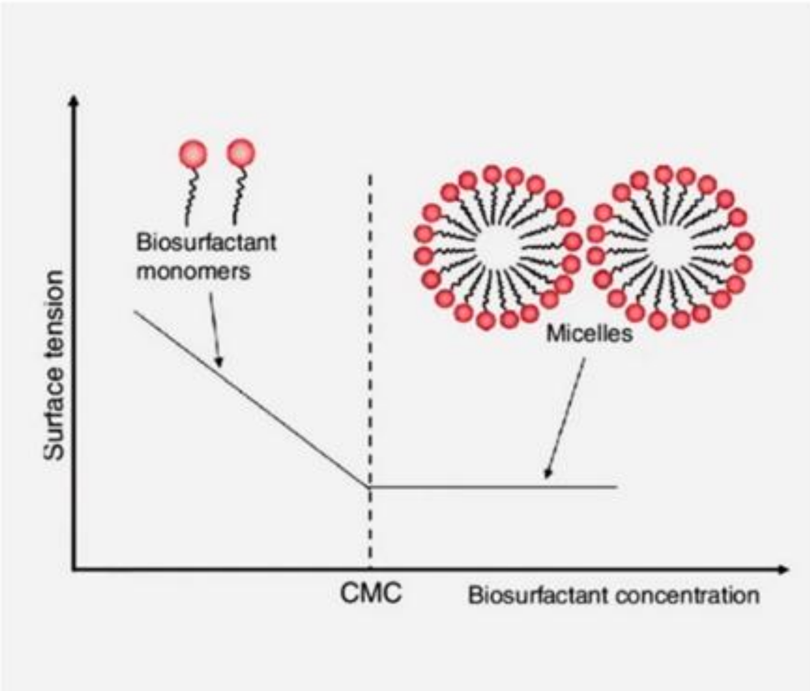


Figure 5: Biosurfactant concentration vs Surface tension graph

XIII. BIODEGRADATION:

Biodegradation refers to the process of the breakdown of organic substances into environmentally acceptable products such as CO_2, H_2O and biomass by naturally available enzymes or microorganisms.

Types of Biodegradation: Biodegradation are of two types based on the principle. They are: 1.Biotransformation; 2. Biomineralization.

1. **Biotransformation:** In this process organic substances are partially degraded, and the remnants is transformed into biomass or other organic compound.

2. **Bio-mineralization:** In biomineralization process, whole organic matters are detoxified andtransformed into non-toxic inorganic compounds like CO_2, H_2O .

XIV. MICROBIAL LEACHING

It refers to the process of collecting metals and minerals from ore- containing rocks using microorganisms. Due to low content of some ore in rocks, all types of metals and minerals extraction by chemical process is not an easy task. Microbial leaching can help in purifying low grade ores which becomes waste in chemical methods. A large quantity of copper can be purified by microbial leaching in low budget. There are also other low-grade ores found throughout the world like Nickel, Lead, Zinc which is collected by this process.

1. The process of microbial leaching is:

- *Thiobacillus ferrooxidans* is the mostly studied microorganism in this process.
- It is a Gram-negative, rod shaped bacterium.
- It acquires carbon from biosynthesis of fixed CO_2 and gains energy from the oxidation of Fe^{2+} to Fe^{3+} or from the oxidation of the elemental Sulphur and reduction of sulphate to Sulphur compounds.
- $4FeSO_4 + 2H_2SO_4 + O_2 \rightarrow 2Fe_2(SO_4)_3 + 2H_2O$
- $2S + 3O_2 + 2H_2O \rightarrow 2H_2SO_4$
- $2FeS_2 + 7O_2 + 2H_2O \rightarrow 2FeSO_4 + 2H_2SO_4$
- Inspection of leaching dumps always shows the presence of mixtures of

Thiobacillus thiooxidans and *Thiobacillus ferrooxidans.*

- The process of leaching can be performed in an order of cascade in pilot-plant reactors.
- In the microbiological laboratory under optimal condition, yields of CO_2 and H_2O with finely ground ores in a tower can be obtained, fermenters can give a better yield but it is less cost effective.

2 Types of Microbial Ore Leaching:

- **Slope-Leaching:** Finely ground ores are kept in a large slope-shaped dump. Then water containing inoculum containing *Thiobacillus sp* culture is continuously sprinkled over the ore pile.

  Then the leach liquid is collected from the bottom and sent for metal extraction. Then the remainingwater is used for possible regeneration of the culture bacteria in an oxidation pool.

- **Heap-Leaching:** In this process, the ore arranged in large heaps. During the heap leaching, water containing Thiobacillus sp culture is sprinkled over the heap. Then the accumulated solution is collected and processed for metal recovery.

- **In-Situ Leaching:** The ore is kept in its natural condition in this leaching process. Water containing *Thiobacillus sp* is pumped through drilled passages within the ore. Then the acidic leach-water seeps through the rock in the lowermost cavity from which it is pumped. Then the water is collectedand sent for metal recovery.

XV. EXAMPLES OF LEACHING

1. **Copper leaching:** Copper leaching is a widely known process. In the production of copper several metal ores like Chalcocite, Chalcopyrite, or covellite are used. Chalcopyrites contains 26% iron, 26% copper,2.5% zinc and 33% Sulphur.5-6% copper processed by microbial leaching process in the world.Copper leaching plants are using to copper throughout the world for many years. The leaching solution contains sulphite and iron as microbial nutrients and the dissolved copper get precipitated from this solution. The leaching solution is then sprinkled over the heap of ore. Then the leach-water accumulates below the heap. The collected copper-rich water then sent for recovery. Mainly copper leaching process has been widely used in Canada, South Africa, United states, Portugal, Spain, Japan, Mexico etc. United state produces up-to 200 tons of copper per day.

   - Chalcopyrite is oxidized as follows:
     $2\ CuFeS_2 + 8\ ^1/_2 H_2O + H_2SO_4 \rightarrow 2CuSO_4 + Fe_2(SO_4)_3 + H_2O$
     Covellite is oxidized as follows: $CuS + 2O_2 \rightarrow CuSO_4$

2. **Uranium leaching:** Uranium leaching is most cost-effective process. But this process is not used widely like the copper leaching process. One ton of uranium is produced from thousand tons of uranium ore. So microbial leaching is an economical process in removing the bulk amount of impurities. This is an indirect process as microbes do not attack on the uranium ore directly, it attacks on the iron oxidant. Ferric sulphate and sulphuric acid can be synthesized by *Thiobacillus ferrooxidans* from pyrite in the uranium ore.The reaction is:

$$2\ FeS_4 + H_2O + 7.5\ O_2 \rightarrow Fe_2\ (SO_4)_3 + H_2SO_4$$

   In this process dissolved uranium is recovered from the leach-water with organic solvents such as tributyl phosphate and uranium is also precipitated. By iron-exchange method uranium can also be obtained. In this process insoluble tetravalent uranium is oxidized with hot H_2SO_4/Fe^{3+} solution to soluble hexavalent uranium sulphate. The reaction is:

$$UO_2 + Fe_2(SO_4)_3 \rightarrow UO_2SO_4 + 2FeSO_4$$

3. **Gold leaching:** Gold leaching is a hydrometallurgical technique. Gold leaching method is called cyanide process as the ore is dissolved in a dilute solution of potassium cyanide or sodium cyanide in the presence of lime and high concentration of dissolved oxygen. This process is discovered by Scottish chemist Robert W. Forrest, John S.Mac Arthur and William forrest in 1887. So, this process is also called Mac Arthur-Forrest process. Gold has wide applications in medicine, electronics, computer and jewelry industry. Cyanide process is mostly performed afterfroth flotation.

   Process:

   - Solubilization of gold/leaching: Generally gold is insoluble and present in small amount in ores. Gold containing ore is first solubilized by adding Sodium cyanide and Pottasium cyanide to separate the gold from ore. The other minerals present in the ore other than goldalso separated by this process.

- Ph Control: After gold became soluble then dilute solution of sodium cyanide is added tomake gold more permeable to move freely. Then lime is added to Sodium cyanide to control the Ph at 10-11 for the reaction to take place.
- Cementation: In this step electrophoresis of gold containing sodium cyanide solution is performed by using Zinc electrode with carbon paste. Gold is extracted by this method anda slurry is formed. The slurry containing cyanide ion is destroyed or recycled.

  Reactions:

  At cathode: $Au(CN)_2 + e^- \rightarrow Au + 2\,CN^-$
  - ♦ Gold is obtained at cathode.

  At anode:1) $Zn + 2OH^- \rightarrow Zn(OH)_2 + 2e^-$
  - ♦ Zinc is oxidized at anode.

  2) $Zn(OH)_2 + 4CN^- \rightarrow Zn(CN)_4^{2-} + 2OH^-$
  - ♦ Slurry is need to be destroyed or recycled as it contains cyanide ion.
- Effect of Cyanide process in environment: Cyanide process is cost effective process for gold, silver extraction from ores. Almost 90% industries used this method. But cyanide ion is highly toxic in nature .It can cause major environmental hazards. Cyanide spill has highly detrimental effect towards the river and it's ecosystem. However, cyanide in aqueous solution rapidly degrade into cyanate or thocyanate form which is less toxic than the cyanide ion.
- Remedy for cyanide process:
- After gold extraction the area should be detoxified by Caro's acid process or any other alternatives. The Caro's acid process converts cyanide into cyanate ion.

  $CN^- + [O] \rightarrow OCN^- \quad OCN^- + 2H_2O \rightarrow HCO_3^- + NH_3$
- The most promising alternative of cyanide process is gold leaching using thiosulfate, mild-chloride solutions, halides, thiourea. The impurity metals present in gold ore are beneficial in this alternatives.The impurities present in gold ore are generally iron, copper which can self oxidized the reaction in presence of Calcium chloride ($CaCl_2$) solution. Gold chloride leaching process is less toxic and more faster than cyanide process. The gold-chloride process is favored by low ph, high chloride concentration and high temperature. Although gold-chloride complexes are less stable than gold- bromide complex.

4. **E-Waste Management:** E-waste or electronic waste are majorly growing problem in today's world. Electronic products are now must have for people of all age group. Using improperly, rapidly replacing the electronic items with the day by day advancement in technology are major cause for producing the 80% of e-waste. People tossed out44.7 million metric tons of e-waste in 2016 and in 2020 the numberincreased in 120 million metric tons. Only 20% of e-wastes are recycled every year and the remainingpercentage of waste take place in landfill or other waste heaps. Although they can be recycled but the most suitable methods are not still used. During recycling of e-waste many hazardous pollutants are released and produces hazardous carcinogens. When humans come in contact with the pollutants it canaffect their brain, heart, liver, kidney and damage skeletal system. Some common methods in recycling e-wastes are hydrometallurgy and pyrometallurgy. In pyrometallurgy the e-waste is heated to more than 1000°c and toxic gases liberated. In hydrometallurgy the e-waste is treated with acids and cyanide but it

produces toxic effluvium. With the advancement in technology e-waste management is going complicated day by day. E-waste can be divided into two groups towards the approach of their management. They are-

- Organic part containing thermos and thermosetting plastic;
- inorganic part containing metallic and non-metallic components.

To promote more sustainability microorganisms are now used in e-waste treatment. Microorganisms can degrade organic as well as inorganic part of e-waste without producing any harmful gases or compounds. Microorganisms like *Sulfobacillus thermosulfidooxidans* and *Pseudomonas balearica* canuptake metals from e-waste. This way the separate metals from e-waste products. *Chromobacterium violaceum* is an e-waste processing bacteria which can degrade the thermosetting plastic compounds like PCB, PBDEs, PCDD etc. Bio-leaching is one of the approaches towards e-waste management by microorganisms. Bio-leaching is more safe and cost-effective method than pyrometallurgy and hydrometallurgy. Scientists are now developing genetically modified microorganisms for an effective e-waste treatment .The strongest microorganisms inhabiting in the hydrothermal vent under the ocean and a soil bacterium is now the centre of the research. If we can detect the gene sequence of the mysterious microorganism of hydrothermal vent and extract the gene fragment and recombine it withthe genome of Arthrobacter sp then it will easily detoxify e-waste and promote endurability. Some other methods in E-waste treatment are: Biosorption; Bioaccumulation; Biotransformation;Biomineralization.

Phyco-remediation is also a sustainable process in e-waste treatment. Some of the plant species used for e-waste treatment are: Black alder (*Alnus glutinosa*), Hybrid poplar (*Populus canadensis)*, Chinese brake (*Pteris vittata*),Yellow burr head (*Limnocharis flava*), Lesser duckweed (*Lemna minor*), Vetiver grass *(Chrysopogon zizanioides).*

5. **Role Of Earthworms In Bioremediation Of Xenobiotic Compounds:** Due to rapid increase in pollution rate researchers are in search of a suitable bioremediation process along with phycoremediation, mycoremediation and bacterial bioremediation. Soils are polluted mostly dueto industrialization, urban waste, excessive application of pesticides, herbicides and fertilizers in soil. The chemical and organic pollutants modifies the soil colloid particles, reduces the bioavailability of nutrients in soil and make the soil imperfect for agricultural purposes. Earthworms are belong to phylum Annelida, class Chaetopoda and are one of the most abundant inverbrates in soil fauna. Theycan degrade xenobiotic compounds in soil so they can also be used bioremediation. Earthworms alone or in association with soil microorganisms can provide immediate and effective remediation. Earthworms are one of the soil organism which have important role in organic matter decomposition and nutrient cycling. Earthworms directly interact with soil substrate that's why they get significantlyaffected by the presence of xenobiotics in soil. The skin of earthworm is highly permeable to water when they uptake water through pores the contaminants also absorbed through the pores. Earthworms ingest a largest proportion of soil as their food .During ingestion of soil their alimentary canal directly exposed towards the absorbed contaminants. Their dermal pores are the major route for heavy metal uptake. Earthworms can accumulate the up taken organic and inorganic

pollutant including toxic heavy metals in their body. They can tolerate the higher metal concentration, specially the Cu, Hg and Zn. Their chloragogenous tissue of post alimentary canal is consist of pedunculated cells which can detoxify the heavy metals. Bioaccumulation of heavy metals among earthworm varies from one species to another species. BSAF or biota to soil accumulation factor is expressed as the bioaccumulation of xenobiotics in earthworms.

BSAF –Concentration of earthworm/Concentration of xenobiotics in soil
A variety of heavy metals are bioaccumulated by different earthworm species. *Lumbricus terrestris* is not capable accumulating higher concentration of Pb but *Apporectoda longa* can accumulate. Earthworms stores cadmium in their gut wall and nephridia in the form of metallomethioneines. Earthworms can accumulate Cd, Zn, Hg efficiently even when the BSAF reaches greater than 1.They can also accumulate pesticides like DDT, Dieldrin, Heptachloroepoxide but PCBs, PAHs are not accumulated in high concentration among earthworms. But there is also a risk of transferring the heavymetals or other xenobiotics from earthworms to animals of higher trophic levels through food chain. Earthworm can ingest soil, so when researchers exposed some species of earthworm in crude oil they isolated two species that can tolerate and degrade crude oil. *Eisenia fetida* and *Apporectodea caliginosa* can degrade crude oil efficiently.

6. **Genetically Modifying Microorganisms to Improve Bioremediation:** Bioremediation using microorganisms is a safe and economic process. But not all microorganisms are capable of degrading toxic pollutant and making non-hazardous byproducts because of their diverse metabolic ability. All microorganisms specially all species of bacteria cannot tolerate high metalconcentrations or crude oil. But heavy metal contamination and oil spills are now a major global issue. Due to the advancement in biotechnology now we can modify or edit the genetic sequence ofmicroorganisms. By manipulating the gene sequence according to our interest we can culture genetically modified microorganisms which can efficiently degrade the xenobiotics. Some advantages of genetically modified microorganisms are-

- More efficiency in bioremediation process.
- They can be monitored easily.
- They can survive in any adverse conditions.

Some genetically microorganisms are: Pseudomonas diminuta can degrade Parathion; Alcaligenes sp can degrade 2,4-Dichlorophenoxyacetic acid; Acinetobacter sp can degrade 4-Chlorobenzene.

- **Advantages of Bioremediation**

  - It is an useful process to degrade a wide variety of contaminants to non-toxicsubstances.
  - It requires less equipments in comparison to other methods.
  - It is a low cost treatment for detoxifying contaminated soil and wastewater.
  - It does not cause pollution or affects in human and other animals' life.
  - This process can be used to degrade any specific pollutants.

- **Disadvantages of Bioremediation**

  - This process is only used to degrade biodegradable compounds.
  - If the temperature, Ph cannot be controlled then it can yield toxic byproducts from theparent toxic substance.
  - Bioremediation takes longer time than any other remediating method.
  - In ex-situ process controlling volatile compounds may be difficult.
  - It is difficult to track the bioremediation rate throughout the process.

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