MICROBIAL BIOTECHNOLOGY

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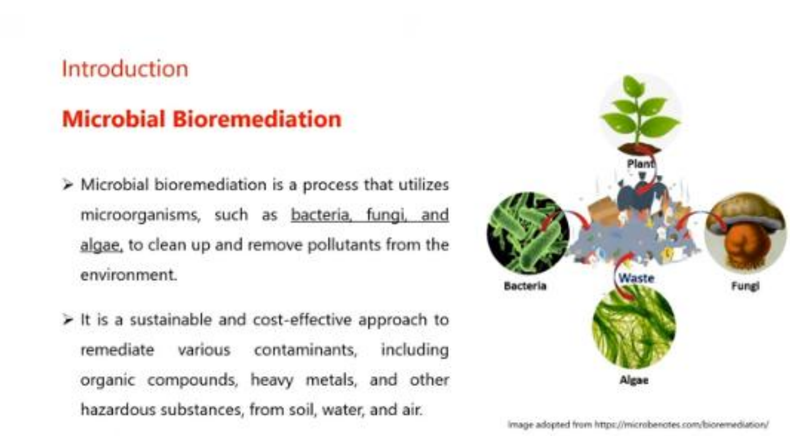
Lecture-28
Lec 28: Microbial Bioremediation

Hello friends, welcome to my course on microbial biotechnology. We are in module number eight, environmental biotechnology, where today we will discuss microbial bioremediation. This lecture is broadly divided into two sections. In section one, we will have a brief introduction to bioremediation. Then we will discuss the major groups of pollutants and identify the sources of environmental pollutants.

We will also cover the impact of environmental pollutants, why bioremediation is necessary, the advantages of microbial bioremediation, and the potential risks. Additionally, we will discuss the two main types of bioremediation: in situ and ex situ. Microbial bioremediation is a process that utilizes microorganisms such as bacteria, fungi, and algae to clean up and remove pollutants from the environment. We also sometimes use plants, but that falls under a different subject called phytoremediation. Microbial bioremediation is a sustainable and cost-effective approach to remediate various contaminants, including organic compounds, heavy metals, and other hazardous substances from soil, water, and air.

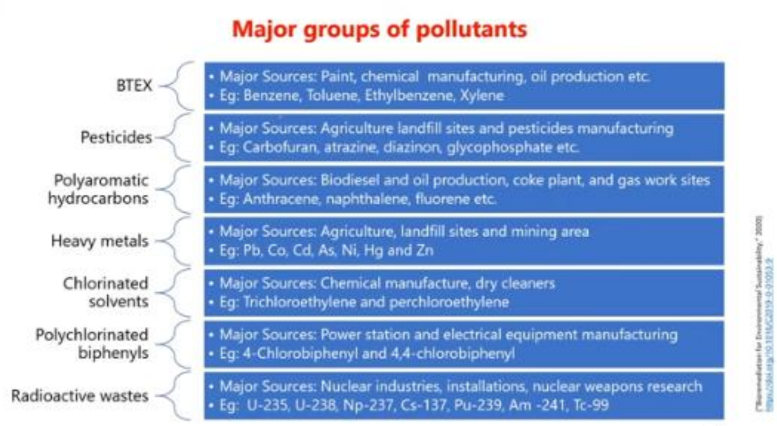


The major groups of pollutants can be categorized as follows: Number one is BTEX, which stands for benzene, toluene, ethylbenzene, and xylene. Mostly, these are pollutants originating from the paint industry, chemical manufacturing, and oil production. Then we have pesticides, which mostly come from agricultural landfill sites and pesticide manufacturing units. Some examples include carbofuran, atrazine, diazinon, and glyphosate.

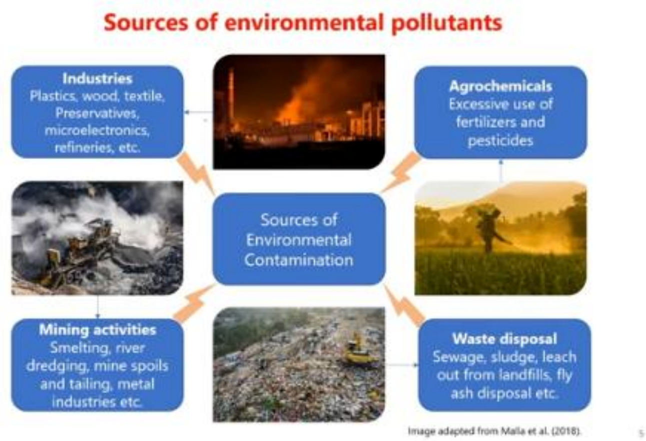
Then we have the polyaromatic hydrocarbons. These are basically obtained from the biodiesel oil production process, coke plants, and gas worksites. Some examples include anthracene, naphthalene, fluorene, etc. Then there are the heavy metals, which are mostly obtained from agricultural activities, landfill sites, and mining activities. Some examples are lead, cobalt, cadmium, and so on.

Then we have the chlorinated solvents. These are mostly the outcome of chemical industries and the dry cleaning process. Some examples include trichloroethylene and perchloroethylene. Then we have the polychlorinated biphenyls, which are mostly sourced from power stations and the electrical equipment manufacturing industry. Some examples include 4-chlorobiphenyl and 4,4'-chlorobiphenyl.

Finally, one of the most dangerous ones is radioactive waste, which comes from nuclear industries, nuclear installations, and nuclear weapons research, etc. This includes nuclear power production plants. There are different isotopes of uranium and other metals, which are very harmful to all of us. Now, what are the sources of environmental pollutants, or what causes environmental contamination? As we saw in the earlier slide, they mostly come from industries like the plastic industry, as well as the wood and textile industries.



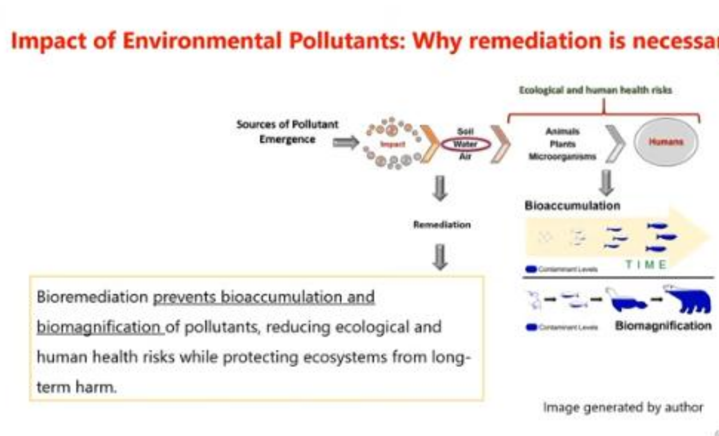
The textile industry uses a lot of dyes, preservatives, microelectronics, refineries, etc. We have also found that agriculture is one of the point sources of environmental pollutants, mostly agrochemicals. The excessive use of fertilizers, pesticides, and weedicides are some of the reasons why our environment is getting polluted. Then, waste disposal in cities as well as rural areas, sewage, large leachate from landfills, and fly ash disposal, etc. All contribute to environmental pollution, and one of the major contributors is the mining industry. Here, smelting, river dredging, mine spoils, tailings, and the metal industry, etc.



Contribute to environmental pollution. Now, what is the impact of environmental pollutants, and why do we require bioremediation? Here, we now know the various sources of environmental pollution, as discussed in the earlier slide, and they actually impact the biological world and the environment. They may pollute soil, water, and air. They pose ecological and human health risks, affecting animals, plants, microorganisms, and ultimately, human life. One problem with environmental pollutants is bioaccumulation.

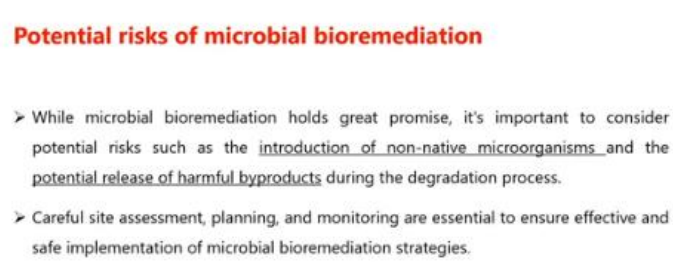
You can see that small organisms or small pieces are affected first, which are consumed by larger ones, and again by even larger ones. This is just a symbolic picture, but in the food network, smaller organisms are always consumed by larger organisms. When many smaller organisms with small contaminations are consumed by a comparatively larger one, bioaccumulation occurs. Then, finally, it gets biomagnified. So, you can see that From one organism to another, there is a flow of these pollutants due to the food network, and you can see here the contamination level, which is shown by the size and color.

Do remediation—I mean, treat these pollutants in the source or in the environment—we can actually stop these from entering the food network or ecological niche, and thereby we can actually save the environment. Bioremediation prevents the bioaccumulation and biomagnification of pollutants by reducing ecological and human health risks while protecting ecosystems from long-term harm. So that is why remediation is necessary. Now, what are the advantages of using microbes for remediation or microbial bioremediation? Number one is safety.

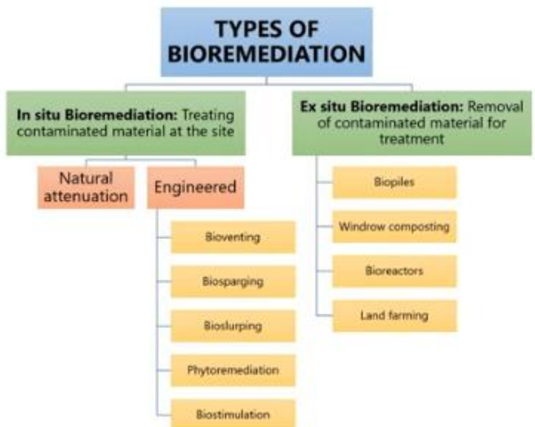


It reduces risks to human health and the environment. Then, this is an environmentally friendly process. It reduces pollution without harming ecosystems. It is cost-effective, lowering remediation costs significantly. And it is also a natural process that is enhanced.

So, it boosts natural degradation processes, then there is minimal disruption, thereby preserving existing ecosystems during the remediation process. And it has long-term effectiveness, sustaining remediation over extended periods, and it's a versatile process applicable to various contaminants and environments. Now, what are the potential risks of microbial bioremediation? Despite all the advantages, we have to consider these potential risks, such as the introduction of non-native microorganisms, and the potential release of harmful byproducts during the degradation process. Careful site assessment, planning, and monitoring are essential to ensure effective and safe implementation of microbial bioremediation strategies.

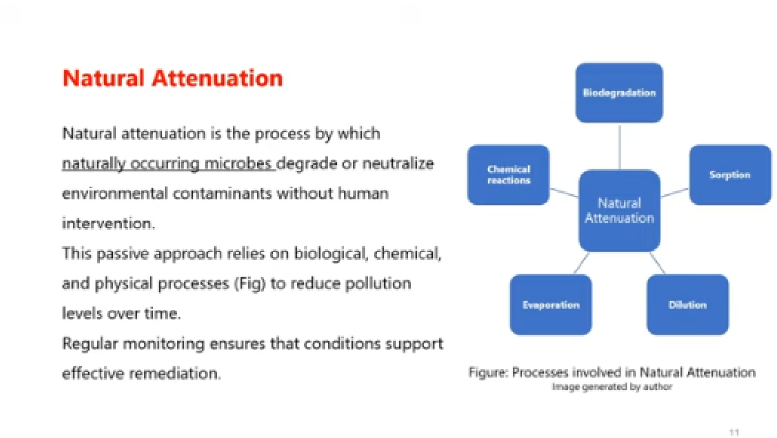


Now, what are the different types of bioremediation we can use for our purpose? The first one is in-situ bioremediation, where treating contaminated material is done on the site itself. So, this may be a natural attenuation process or an engineered process where we go for bioventing, biosparging, bioslurping, phytoremediation, and biostimulation. Or, it may be ex-situ bioremediation, where removal of contaminated material for treatment is done at a site away from the original location. So, this may be done by biopiles, windrow composting, bioreactors, and land farming.

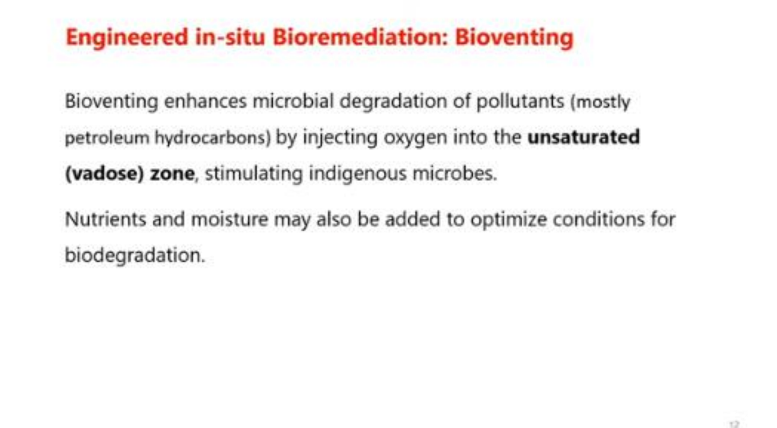


Let us now discuss in-situ bioremediation, which is basically a process of treating contaminated soil, groundwater, or sediments directly at the site of pollution without removing the contaminated material. It utilizes natural or introduced microorganisms to degrade or detoxify pollutants in place or at the location itself. So, number one is natural attenuation, which is the process by which naturally occurring microbes degrade or neutralize environmental contaminants without human intervention. This passive approach relies on biological, chemical, and physical processes to reduce pollution levels over time.

So, it includes biodegradation or simply sorption, or we may also dilute the pollution, which is, of course, not a very scientific process. Then, by the process of evaporation and also chemical reactions. However, regular monitoring ensures the conditions that support effective remediation are very essential. So, let us now discuss bioventing, which is basically a method of engineered in-situ bioremediation. Bioventing enhances microbial degradation of pollutants, mostly petroleum hydrocarbons.



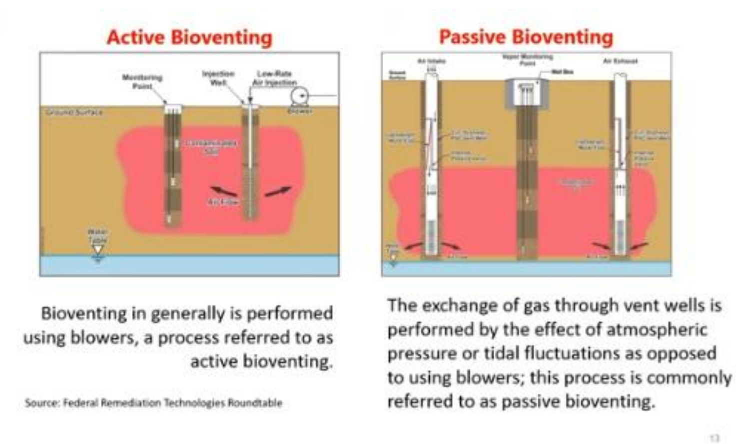
By injecting oxygen into the unsaturated overdose zone, stimulating indigenous microbes to grow and degrade the pollutants in the site or location. Nutrients and moisture may also be added to optimize conditions for biodegradation. There can be two types of bioventing. One is active bioventing, where bioventing is generally performed using blowers. As you can see, this is blowing air through this pipe, which is deep into the soil.



So, since it uses the blowing of air with the help of blowers, we call it active bioventing. Here, you see this is the ground surface, this is the water table, and this is the contaminated soil. We will inject or blow air through this duct with the help of a blower. We also have a monitoring system in place, which will keep observing whether the biodegradation is progressing satisfactorily or not. This blowing, of course, is very slow, so we do low-rate air injection in the case of active bioventing.

The whole idea is to facilitate the growth of the naturally occurring microorganisms over there. In passive bioventing, we do not use a blower. Here, air intake happens passively through the passage we have built. There is also an exhaust passage, as you can see here. There is a vapor monitoring point to keep continuous observation of the progress.

So, here in passive bioventing, the exchange of gas happens through vent wells, which is basically due to the effect of atmospheric pressure, barometric effect, or sometimes tidal fluctuations. We do not use blowers, and because of the absence of blowers, we call it passive bioventing. So, biomethane is commonly used for light petroleum spills. Its effectiveness depends on air injection rates, with lower rates often supporting better microbial activity. So, if we blow with very high speed, that will not help us achieve more efficient bioremediation. So, bioventing helps degrade volatile organic compounds.



High airflow can sometimes lead to the release of these volatile organic compounds into the atmosphere, requiring additional treatment like biotrickling filters, which we have discussed earlier. Then there is biosparging, which is similar to bioventing, involving injecting air into the subsurface to stimulate microbial activity for pollutant removal. However, unlike bioventing, air is injected into the saturated zone, causing volatile organic compounds to move upward into the unsaturated zone for biodegradation. So, the effectiveness of biosparging depends on soil permeability and pollutant biodegradability. Biosparging is used primarily for treating petroleum-contaminated aquifers and effectively shifts conditions from anaerobic to aerobic, promoting biodegradation of pollutants like benzene, toluene, ethylbenzene, and xylene, briefly called BTEX.

Engineered in-situ Bioremediation: Bioventing (contd.)

Bioventing is commonly used for light petroleum spills, its effectiveness depends on air injection rates, with lower rates often supporting better microbial activity.

While it helps degrade volatile organic compounds (VOCs), high airflow can lead to the release of VOCs into the atmosphere, requiring additional treatment like biotrickling filters (discussed in previous lecture).

14

Engineered in-situ Bioremediation: Biosparging

Biosparging, similar to bioventing, involves injecting air into the subsurface to stimulate microbial activity for pollutant removal.

However, unlike bioventing, air is injected into the **saturated zone**, causing volatile organic compounds (VOCs) to move upward into the unsaturated zone for biodegradation.

15

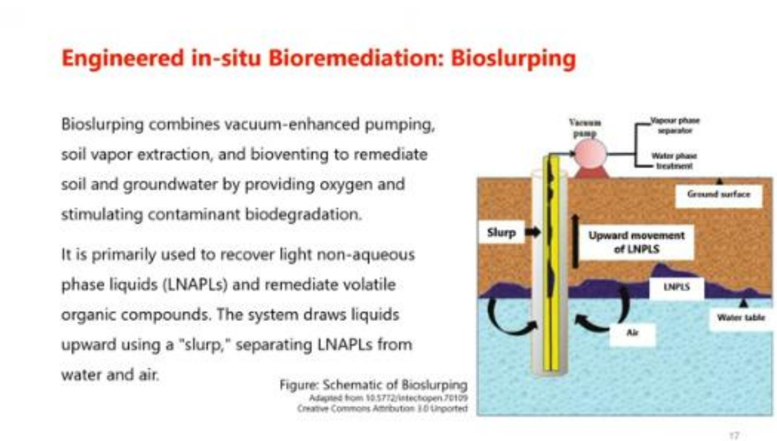
Biosparging (contd.)

Its effectiveness depends on soil permeability and pollutant biodegradability. Biosparging is used primarily for treating petroleum-contaminated aquifers and effectively shifts conditions from anaerobic to aerobic, promoting biodegradation of pollutants like BTEX (Benzene, Toluene, Ethylbenzene, Xylene).

16

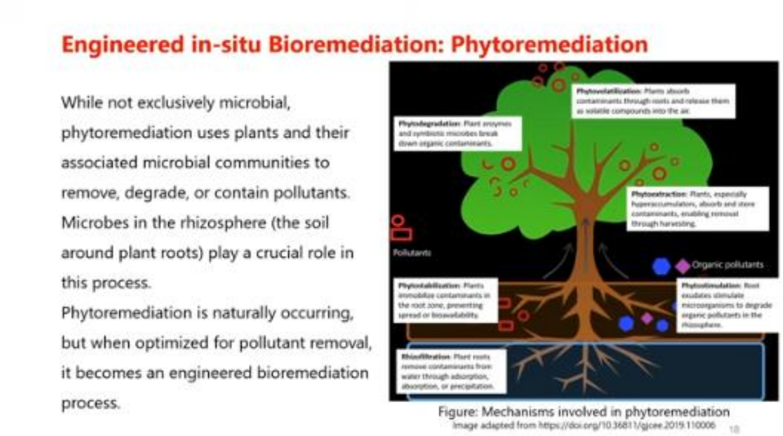
Then there is another kind of bioremediation. We call it bioslurping. This combines vacuum-enhanced pumping, soil vapor extraction, and bioventing to remediate soil and groundwater by providing oxygen and stimulating contaminant biodegradation. So, here you can see this vacuum pump, and then there is the upward movement of these LNAPLs, which are actually entrapped over here. This is the ground surface, and this is the water table. So, these are basically used to recover the LNAPLs, or the light non-aqueous phase liquids, and remediate volatile organic compounds.

The system draws liquids upward using a slurp, separating the LNAPLs from the water and the air. So, let us now see another process, which we call phytoremediation. This is not exclusively microbial in nature. Phytoremediation uses plants and their associated microbial communities to remove, degrade, or contain pollutants. Microbes in the rhizosphere around the plant roots in the soil play a crucial role in this process.



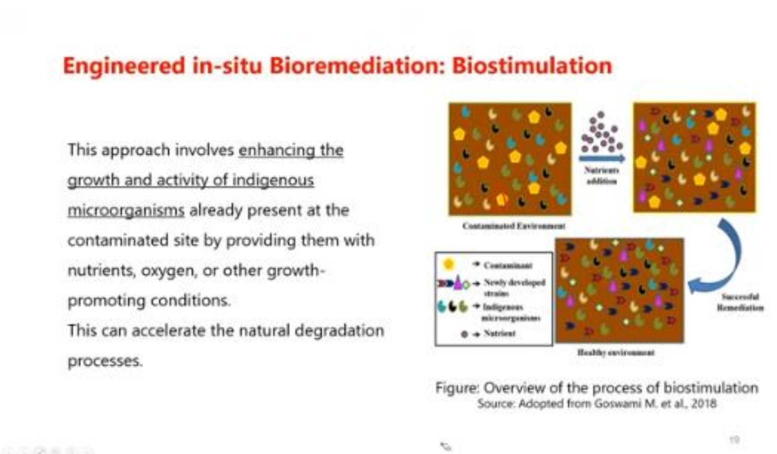
So, here we have rhizofiltration happening. The plant roots remove contaminants from water through absorption or precipitation. Then we have phytostimulation. Plants immobilize contaminants in the root zone, preventing spread or bioavailability. Then we have phytostimulation, where root exudates stimulate microorganisms to degrade organic pollutants in the rhizosphere.

Then we have phytoextraction, where plants, especially hyperaccumulators, absorb and store contaminants, enabling removal through harvesting. Then we have phytodegradation, where plant enzymes and symbiotic microbes break down organic contaminants. Then we have phytovolatilization, where plants absorb contaminants from roots and release them as volatile compounds into the air. Briefly, phytoremediation is naturally occurring, but when optimized for pollutant removal, it becomes an engineered bioremediation process. So, let us now study the engineered in-situ bioremediation process, which we call biostimulation.

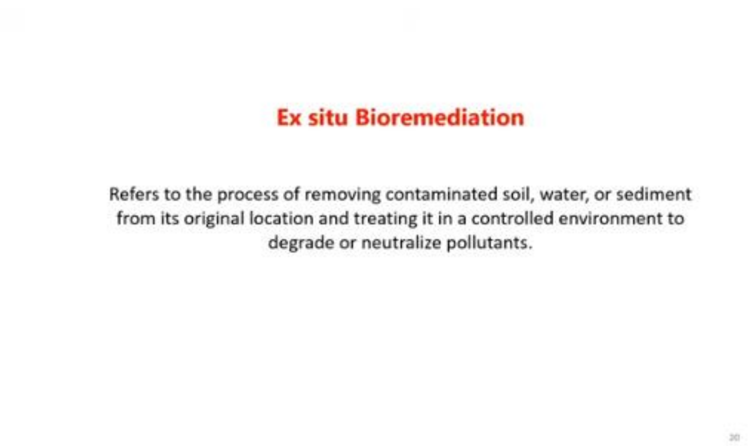


This involves enhancing the growth and activity of indigenous microorganisms already present in the contaminated site by providing them with nutrients, oxygen, or other growth-promoting conditions, and these accelerate natural degradation processes. So, this is the contaminated environment, and here we will add nutrients. This will help the microbes to grow or multiply and, therefore, also carry out the bioremediation, thereby degrading the pollutants in the contaminated sample or site, and then we get a healthy environment. So, these are some representatives. These are the contaminants.

Then, these are some of the newly developed strains you can see over here. And these are indigenous microorganisms. So, we may use both of them. And then, these are the nutrients which we have added to enhance this process. So, this is, in simple terms, the process of biostimulation.

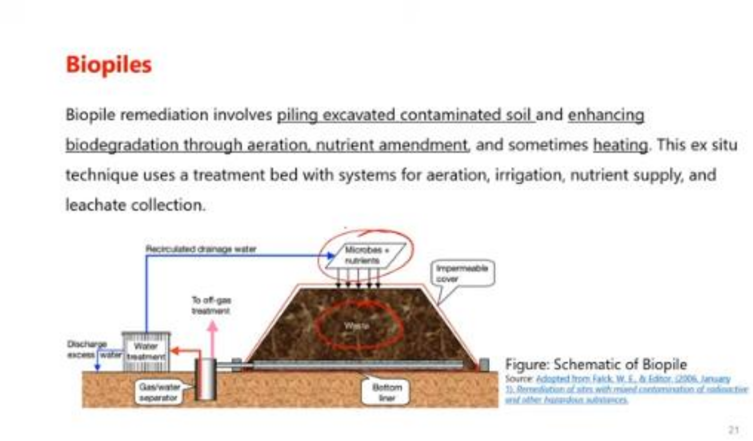


Now, another approach for bioremediation is the ex situ bioremediation, which basically refers to the process of removing contaminated soil, water, or sediment from its original location. It is treated in a controlled environment to degrade or neutralize pollutants. So, one of the methods is biopiling. Biopile remediation involves piling excavated contaminated soil and enhancing biodegradation through aeration, nutrient amendment, and sometimes heating. This ex situ technique uses a treatment bed with a system for aeration, irrigation, nutrient supply, and leachate collection.



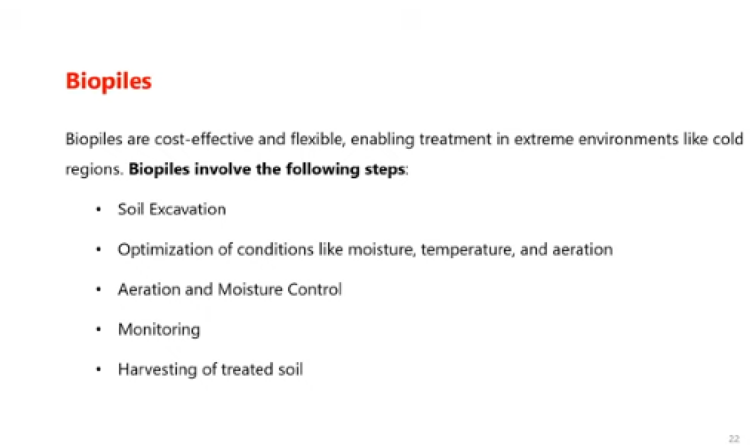
So, here in this figure, we can see a scheme of biopiling. So, this is the waste, and we have an impermeable cover which is actually covering this waste from both the upper side and the bottom side. And then there is this gas-water evaporator, which will actually try to suck in the gas or the water, and that mixture will be separated. Water will be sent to a water treatment plant, and the gas will go to a gas treatment or gas capture unit. And this water, after treatment, is, you know, maybe available for different uses.

And the water, which is the waste and could not be purified, a part of it will be recirculated into the waste, and we also add microbes and nutrients into this waste from time to time or at the beginning itself. So, this is, in simple terms, a biopile, and this waste was actually excavated from or transported from another location and brought into this unit. So here, basically, we may use indigenous microbes, or naturally occurring microbes, and sometimes engineered microbes. So, these biopiles are cost-effective and flexible.

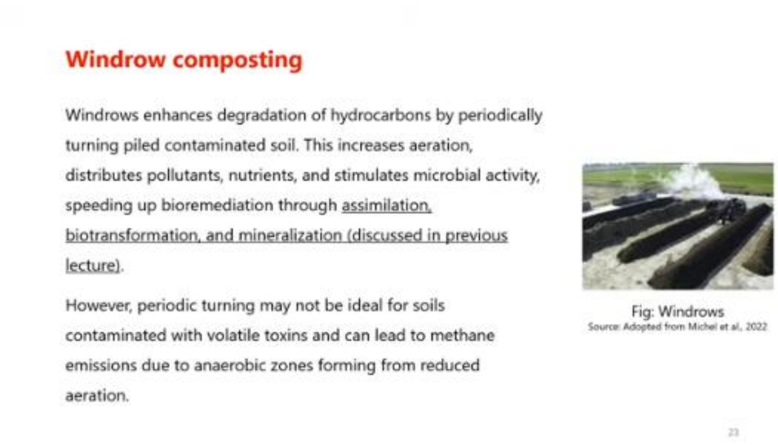


These enable treatment in extreme environments, like cold regions. Biopiles involve the following steps. Number one is the soil excavation. Second is the optimization of conditions like moisture, temperature, and aeration, and also the control of these various conditions, like air and moisture.

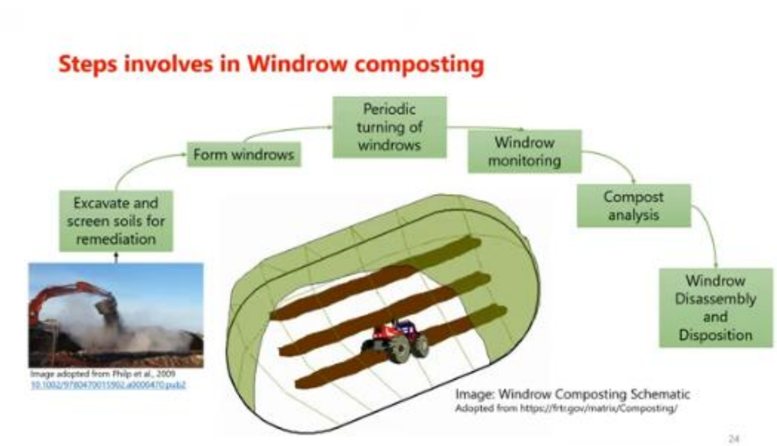
Then, continuous monitoring and harvesting of the treated soil. Once the soil is bioremediated, we will harvest it and use it for different economic purposes. Another method is windrow composting. So, we can see here the rows—these are basically rows of deposited waste that has been transported or excavated from one site and brought here. And we actually arrange them in parallel heaps, large heaps.



So, windrows enhance the degradation of hydrocarbons. By periodically turning piled contaminated soil, this increased aeration distributes pollutants and nutrients, stimulating microbial activity and speeding up bioremediation through assimilation, biotransformation, and mineralization. However, periodic turning may not be ideal for soils contaminated with volatile toxins and can lead to methane emissions due to anaerobic zones forming from reduced aeration. So, some of the steps involved in windrow composting are: We excavate and screen the soils for bioremediation.

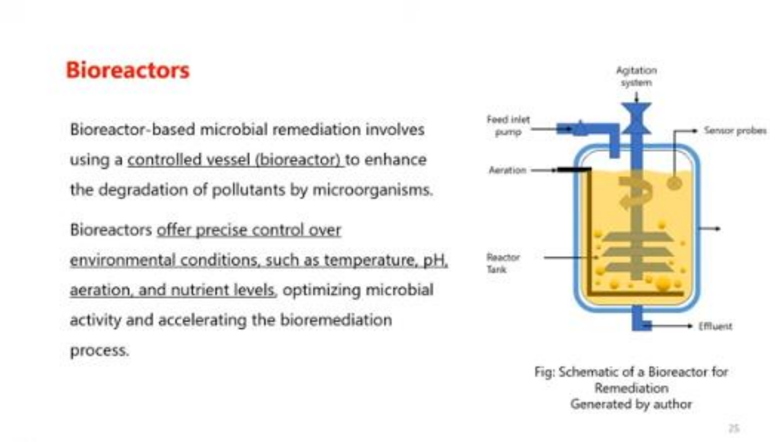


Then, we form windrows, periodically turn them, monitor the windrows, and analyze the compost. Then, the windrow will be disassembled and disposed of. And basically, the soil is now ready to be utilized for some economic activity. Another approach is using bioreactors. This bioreactor-based microbial remediation involves using a controlled vessel or bioreactor to enhance the degradation of pollutants by microorganisms.

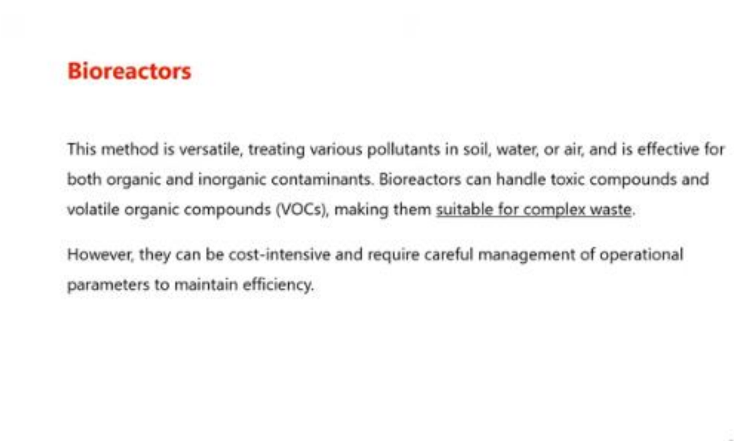


Bioreactors offer precise control over environmental conditions such as temperature. pH, aeration, and nutrient levels, optimizing microbial activity and accelerating the bioremediation process. So here we have this bioreactor unit with an agitation system. Then we have a channel for aeration, sending in air or oxygen. And we also have a feed inlet pump.

Through these, we will be sending in the waste to be bioremediated in this reactor tank. Then we also have sensor probes for monitoring the bioremediation from time to time. And once the treatment is over, the effluent is obtained through a passage at the bottom of this reactor tank. Bioreactors are a versatile method. They can treat various pollutants in soil, water, or air.

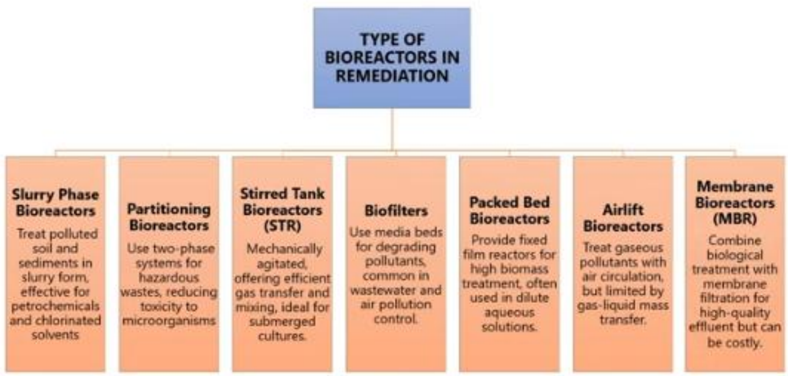


And they are effective for both organic and inorganic contaminants. Bioreactors can handle toxic compounds and volatile organic compounds, making them suitable for treating complex waste. However, they can be cost-intensive and require careful management of operational parameters to maintain efficiency. So, let us now look into the different types of bioreactors used in bioremediation. Number one is the slurry-phase bioreactor.



Here, the polluted soil is treated, and the sediment is obtained in slurry form. This is effective for petrochemicals and chlorinated solvents. Then, we have the partitioning bioreactor, which uses two-phase systems for hazardous wastes, reducing toxicity to the microorganisms. Then, we have the stirred tank bioreactors, or STRs, which are mechanically agitated, offering efficient gas transfer and mixing, ideal for submerged cultures. Then, we have the biofilters.

These use media beds for degrading pollutants and are common in wastewater and air pollution control. Then, we have the packed-bed bioreactors, which provide fixed-film bioreactors for high biomass treatment, often used in dilute aqueous solutions. Then, we have the airlift bioreactors, which treat gaseous pollutants with air circulation but are limited by gas-liquid mass transfer. Then, we have the membrane bioreactors, which combine biological treatment with membrane filtration for high-quality effluent but can be very costly. Another method is land farming.



Land farming is a simple and cost-effective bioremediation technique used for treating hydrocarbon-polluted soils. Land farming involves excavating and spreading polluted soil from contaminated sites on a surface where native microorganisms degrade the contaminants through aerobic degradation. So, this is one schematic diagram of land farming. So, here we have the contaminated soil, and then we do tilling for soil aeration. Then, we have porous cup lysimeters and a groundwater monitoring well because the leachates may enter the groundwater sometimes. The leachate is collected and treated.

Of course, that can also be optional, but it is always good to treat the leachate, which may have a lot of harmful pollutants. Land farming involves tillage for aeration, nutrient addition, and irrigation, which stimulate microbial activity. It is effective for removing hydrocarbons via biodegradation and volatilization. And it can be applied in various climates with minimal environmental impact. However, land farming requires large space, is less effective for inorganic pollutants, and faces challenges with toxic volatiles and extreme climates.

Land farming

Land farming is a simple and cost-effective bioremediation technique used for treating hydrocarbon-polluted soils.

It involves excavating and spreading polluted soil from contaminated site on a surface where native microorganisms degrade the contaminants through aerobic degradation.

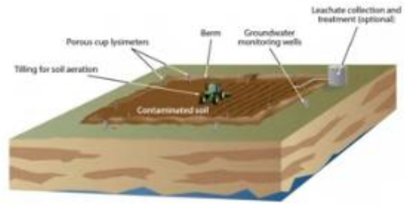


Figure: Schematic of Land Farming
Source: U.S. Environmental Protection Agency 1994

28

Land farming (contd..)

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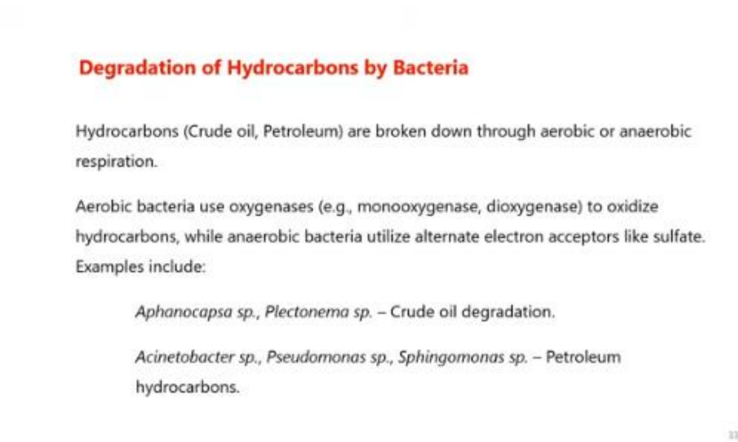
However, land farming requires large space, is less effective for inorganic pollutants, and faces challenges with toxic volatiles and extreme climates.

29

Let us now move to section 2, where we will discuss the various microorganisms used in bioremediation, like bacteria, fungi, and microalgae, and the different factors that affect bioremediation. So, the microorganisms used in bioremediation include bacteria, algae, and fungi. So, let us discuss bacteria first, which are crucial for microbial remediation, effectively degrading various pollutants. They assist in wastewater treatment by breaking down organic matter and heavy metals. They also degrade hydrocarbons in oil spills and synthetic dyes and are effective in breaking down pesticides and detoxifying heavy metals.

Degradation of hydrocarbons by bacteria. Hydrocarbons, crude oil, and petroleum are broken down through aerobic or anaerobic respiration. Aerobic bacteria use oxygenases, such as monooxygenase or dioxygenase, to oxidize hydrocarbons, while anaerobic bacteria utilize alternate electron acceptors like sulfate. Some examples include Aphanocapsa species or Plectonema species for crude oil degradation. Then we have Acinetobacter and Pseudomonas, and also Sphingomonas, for petroleum hydrocarbon degradation.



Degradation of Hydrocarbons by Bacteria

Hydrocarbons (Crude oil, Petroleum) are broken down through aerobic or anaerobic respiration.

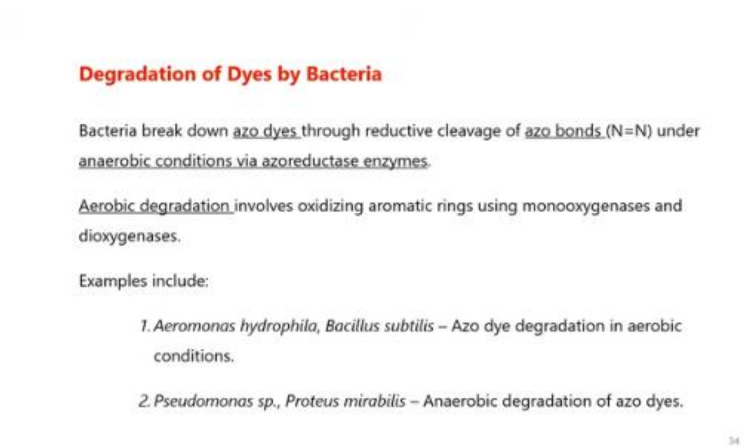
Aerobic bacteria use oxygenases (e.g., monooxygenase, dioxygenase) to oxidize hydrocarbons, while anaerobic bacteria utilize alternate electron acceptors like sulfate. Examples include:

Aphanocapsa sp., Plectonema sp. – Crude oil degradation.

Acinetobacter sp., Pseudomonas sp., Sphingomonas sp. – Petroleum hydrocarbons.

33

Then we have certain bacteria which can degrade dyes, particularly azo dyes, through reductive cleavage of the azo bonds under anaerobic conditions via the enzyme called azoreductase. Aerobic degradation of the dyes involves oxidizing aromatic rings using monooxygenases and dioxygenases. Some examples include Aeromonas hydrophila, Bacillus subtilis, and azo dye degradation in aerobic conditions. Then there are Pseudomonas and Proteus mirabilis, where anaerobic degradation of azo dyes takes place. The bacteria are also used for the degradation of pharmaceuticals and personal care products.



Degradation of Dyes by Bacteria

Bacteria break down azo dyes through reductive cleavage of azo bonds (N=N) under anaerobic conditions via azoreductase enzymes.

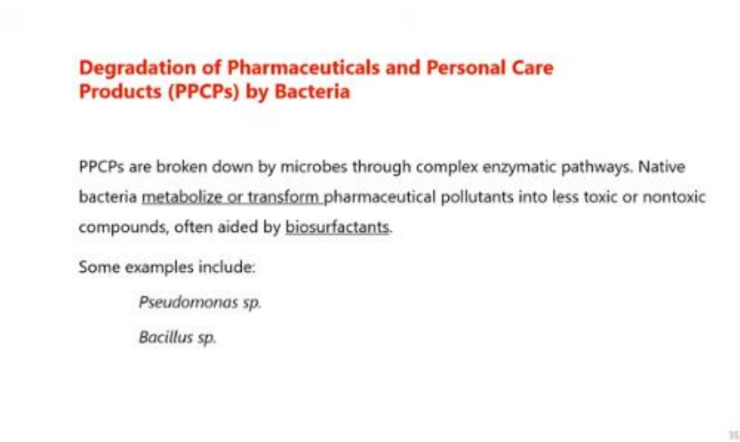
Aerobic degradation involves oxidizing aromatic rings using monooxygenases and dioxygenases.

Examples include:

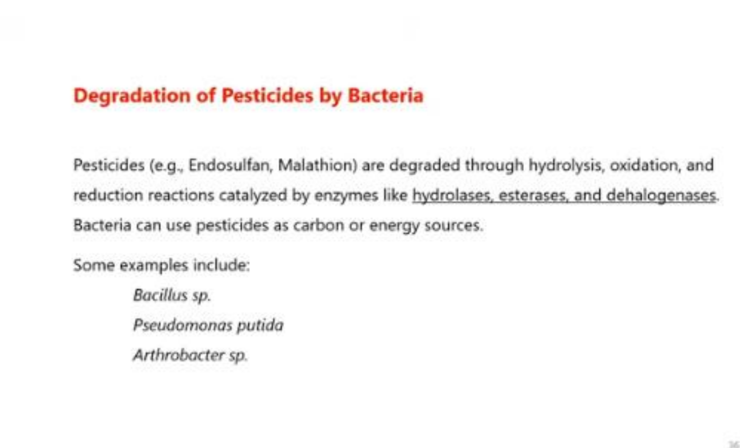
1. *Aeromonas hydrophila, Bacillus subtilis* – Azo dye degradation in aerobic conditions.
2. *Pseudomonas sp., Proteus mirabilis* – Anaerobic degradation of azo dyes.

34

These are broken down by microbes through complex enzymatic pathways. Native bacteria metabolize or transform pharmaceutical pollutants into less toxic or non-toxic compounds, often aided by biosurfactants. Some examples include Pseudomonas and Bacillus. Bacteria can also degrade pesticides, particularly endosulfan and malathion, through hydrolysis, oxidation, and reduction reactions catalyzed by enzymes like hydrolases, esterases, and dehalogenases. Bacteria can use pesticides as carbon or energy sources.



Some of the bacteria that can degrade pesticides include Bacillus, Pseudomonas, and Arthrobacter. Some heavy metals are detoxified by bacteria, particularly lead, cadmium, and mercury, via biosorption, bioaccumulation, and reduction. Biosorption involves binding metal ions to cell wall structures, while the reduction process converts toxic metals like chromium-4 to chromium-3 into less toxic forms. Some examples include Desulfovibrio species and Geobacter species, where heavy metal reduction takes place, and Escherichia species and Pseudomonas species, where methylation of mercury occurs. Let us now discuss the role of fungi in bioremediation.



Dedetoxification of Heavy Metals by Bacteria

Bacteria detoxify heavy metals (Lead, Cadmium, Chromium, Mercury) via biosorption, bioaccumulation, and reduction. Biosorption involves binding metal ions to cell wall structures. Reduction processes convert toxic metals (e.g., Cr(VI) to Cr(III)) into less toxic forms.

Some examples include:

1. *Desulfovibrio sp., Geobacter sp., Vibrio harveyi* – Heavy metal reduction (e.g., lead, chromium).
2. *Escherichia sp., Pseudomonas sp.* – Methylation of mercury (Hg).

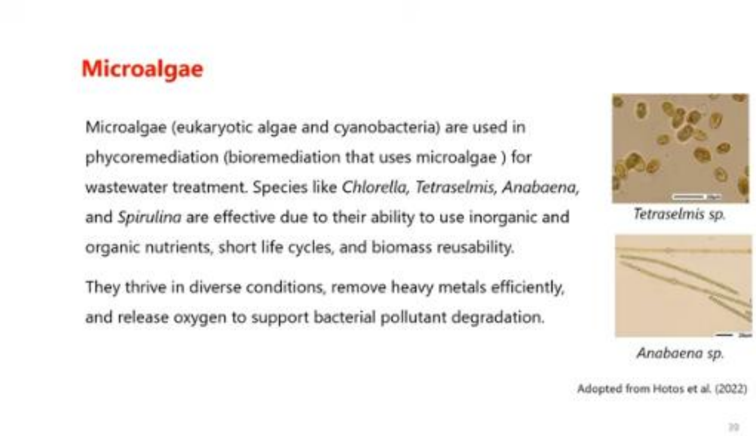
They are crucial in pollutant removal, especially heavy metals. By transforming them into less toxic forms, they are easy to cultivate, produce significant biomass, and degrade contaminants like dyes, pharmaceuticals, and hydrocarbons through extracellular enzymes and protective hyphae. Certain fungi used in wastewater treatment include Pleurotus, Pulmonarius, Aspergillus parasitica, and Stachybotrys species. Then we have the lignolytic fungi with enzymes like laccases and peroxidases, which degrade complex synthetic dyes. Also, yeast like Saccharomyces cerevisiae and Candida krusei absorb and degrade heavy metals or detoxify them.

Fungi

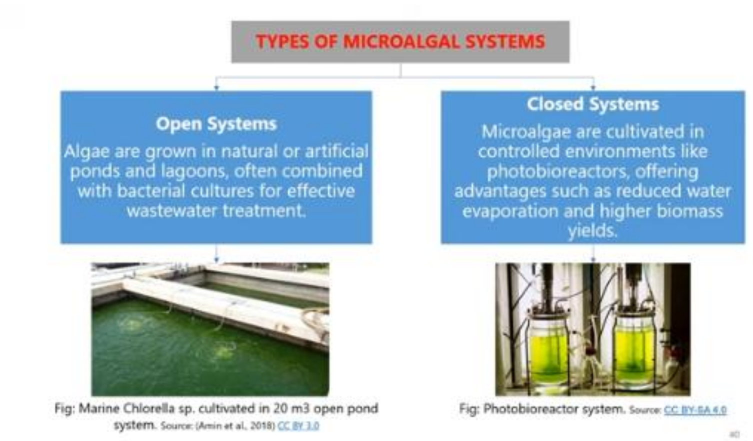
Fungi are crucial in pollutant removal, especially heavy metals, by transforming them into less toxic forms. They are easy to cultivate, produce significant biomass, and degrade contaminants like dyes, pharmaceuticals, and hydrocarbons through extracellular enzymes and protective hyphae.

Certain fungi in wastewater treatment include *Pleurotus pulmonarius, Aspergillus parasitica*, and *Stachybotrys sp.* Ligninolytic fungi, with enzymes like laccase and peroxidases, degrade complex synthetic dyes, while yeasts such as *Saccharomyces cerevisiae* and *Candida krusei* absorb and degrade heavy metals.

Then we have microalgae, which are used in phycoremediation, a form of bioremediation that uses microalgae for wastewater treatment. Species like Chlorella, Tetraselmis, Anabaena, and Spirulina are effective due to their ability to use inorganic and organic nutrients, their short life cycles, and their reusable biomass. So, these are some of the species, as shown here—Tetraselmis and Anabaena—which are used in the bioremediation process. These microalgae can thrive in diverse conditions, remove heavy metals efficiently, and release oxygen to support bacterial pollutant degradation. So, what are the types of microalgal systems used for bioremediation?

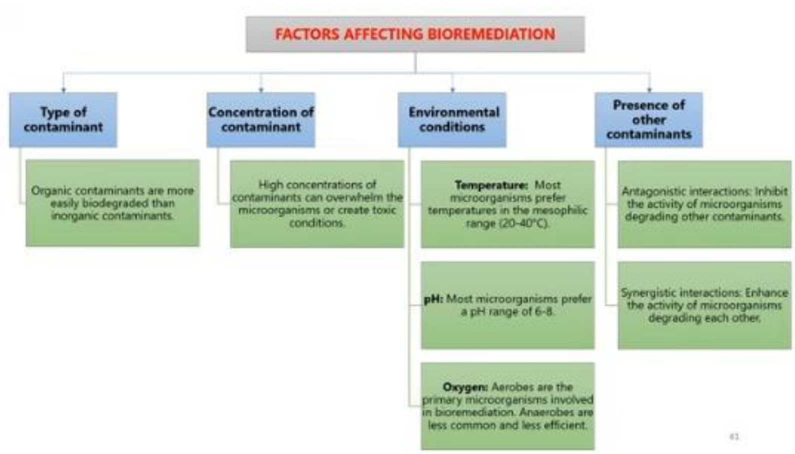


We have open systems where algae are grown in natural or artificial ponds and lagoons, often combined with bacterial cultures for effective wastewater treatment. Here, we can see the marine Chlorella species cultivated in an open pond over here. Then we have closed systems where microalgae are cultivated in controlled environments like photobioreactors, offering advantages such as reduced water evaporation and higher biomass yields. So, this is a photobioreactor system. Now, let us discuss the various factors that affect bioremediation.



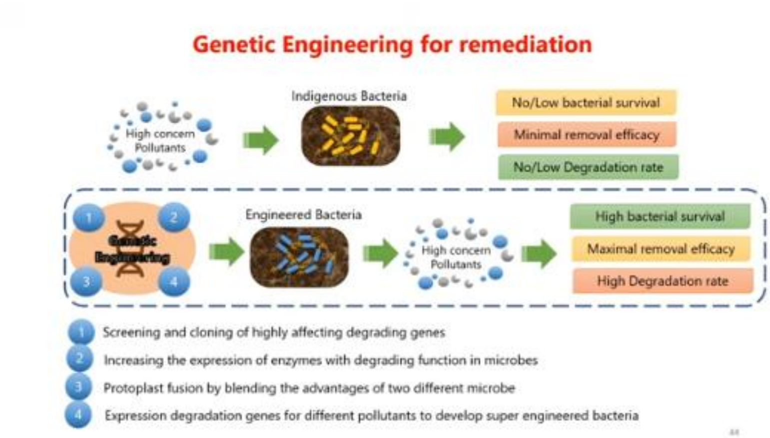
It actually depends on the type of contaminant. Organic contaminants are more easily biodegraded than inorganic contaminants. The concentration of the contaminant also matters. High concentrations of contaminants can overwhelm the microorganisms or create toxic conditions, and environmental conditions like temperature affect the bioremediation process. Most microorganisms prefer temperatures in the range of 20 to 40 degrees centigrade and a pH of around 6 to 8. Oxygen is essential, as aerobes are the primary microorganisms involved in bioremediation, while anaerobes are less common and less efficient in this process. The presence of other contaminants also affects the bioremediation process.

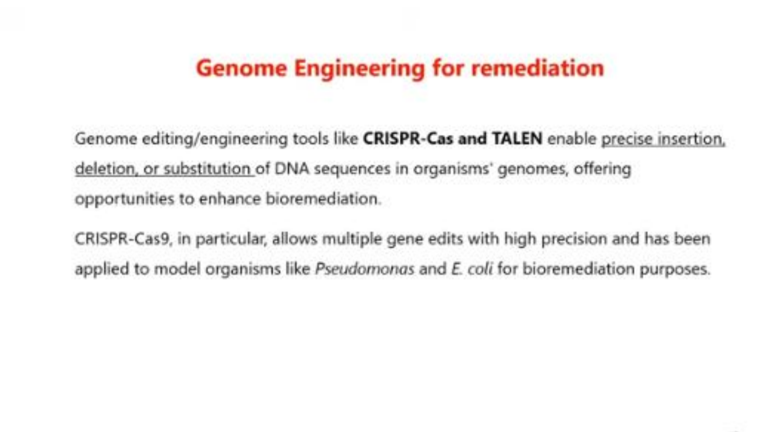
Antagonistic interactions inhibit the activity of microorganisms degrading other contaminants. There are also synergistic interactions, which enhance the activity of microorganisms degrading those contaminants. Let us now move to section 3, where we will discuss recent trends in bioremediation and also the risk assessment of genetically engineered microbes. Genetic engineering is becoming very important for bioremediation. Let us first examine the bioremediation process when using indigenous bacteria.



Here, we have pollutants of high concern, but these indigenous bacteria may not survive or may have low survival rates in the presence of high-concern pollutants, making them unable to remove the contaminants effectively. Thus, efficacy is minimal, and the degradation rate will be low or entirely absent. In contrast, engineered bacteria, produced through genetic engineering, may achieve high bacterial survival, maximal removal efficacy, and high degradation rates. Now, what are the four steps of genetic engineering or engineered bacteria deployment? The first step is screening and cloning highly effective degrading genes in specific microbes to be used for this process.

Increasing the expression of enzymes with degrading functions in microbes, then protoplast fusion by blending the advantages of two different microbes, then expressing degradation. Genes for different pollutants, expression of degradation genes for different pollutants to develop super-engineered bacteria. We can also use genome engineering for bioremediation. Particular tools like CRISPR-Cas and TALEN enable precise insertion, deletion, or substitution of DNA sequences in organisms. Genome offering opportunities to enhance bioremediation. CRISPR-Cas9, in particular, allows multiple gene edits with high precision and has been applied to model organisms like Pseudomonas and Escherichia coli for bioremediation purposes.





These techniques can also be adapted for non-model organisms such as Achromobacter and Comamonas to express specific genes relevant to the bioremediation process. Metabolic engineering is another way to optimize microbial pathways for contaminant degradation. Enzymes like oxidases, esterases, and phenol oxidases play key roles in bioremediation, as seen in organisms like Bacillus species and fungi such as Phanerochaete chrysosporium. Then we have recombinant enzyme production and immobilization, which enhances enzyme stability and efficiency, making enzymatic bioremediation a green, effective method for degrading persistent xenobiotics like PAHs, TNTs, and PCBs. We can also use genetic engineering for heavy metal remediation.

Genome Engineering for remediation

Genome editing/engineering tools like **CRISPR-Cas and TALEN** enable precise insertion, deletion, or substitution of DNA sequences in organisms' genomes, offering opportunities to enhance bioremediation.

CRISPR-Cas9, in particular, allows multiple gene edits with high precision and has been applied to model organisms like *Pseudomonas* and *E. coli* for bioremediation purposes.

These techniques can also be adapted for non-model organisms such as *Achromobacter* and *Comamonas* to express specific genes relevant to bioremediation.

45

Genetic Engineering for remediation

Metabolic engineering optimizes microbial pathways for contaminant degradation. Enzymes like **oxidases, esterases, and phenoloxidases** play key roles in bioremediation, as seen in organisms like *Bacillus sp.* and fungi like *Phanerochaete chrysosporium*.

Recombinant enzyme production and immobilization enhance enzyme stability and efficiency, making enzymatic bioremediation a green, effective method for degrading persistent xenobiotics like PAHs, TNT, and PCBs.

PAHs: Polycyclic Aromatic Hydrocarbons, TNT: Trinitrotoluene and PCBs: Polychlorinated Biphenyls

46

Genetic engineering improves the bioremediation efficiency of heavy metals by inserting metal-binding proteins and peptides into the extracellular space, screening for microorganisms with strong absorption capabilities, and chemically altering the electrophilic groups on their outer surface. Genetically engineered microbes have been created with channels, secondary carriers, and primary active transporters to absorb heavy metals. In this list table, we can see some of the genetically engineered microbes that have been used for heavy metal remediation. And then we can see in this last column the various metals which are remediated, and here we can see the different types of genes that have been either inserted into the microorganism or modified. In E. coli, you have these SpPCS metalloregulatory protein, then ArsR organomercurial lyase, then mercury transporter, then we have the MICE metallothionein gene.

Genetic engineering for heavy metal (HM) remediation

- Genetic engineering improves the bioremediation efficiency of HM by inserting metal-binding proteins and peptides into their extracellular space, screening for microorganisms with strong adsorption capabilities, and chemically altering the electrophilic groups on their outer surface.
- GEMs have been created with channels, secondary carriers, and primary active transporters to absorb HMs.

47

So, these are used for bioremediation of cadmium, arsenic, and mercury. So, you can go through this table and read more about the various genetically engineered microbes and the genes that have been inserted or modified to target certain heavy metals. Another approach is the omics approach for microbial bioremediation. So here, we have these microbes from which we can extract DNA, RNA, proteins, and metabolites. The corresponding fields that deal with these molecules are DNA (metagenomics), RNA (transcriptomics), proteins (proteomics), and metabolites (metabolomics). So, we conduct bioinformatics studies on secondary metabolites, functional genes, and enzymes. Then, we understand the metabolic pathways and try to modify and optimize them to produce engineered microbes that degrade pollutants in the environment.

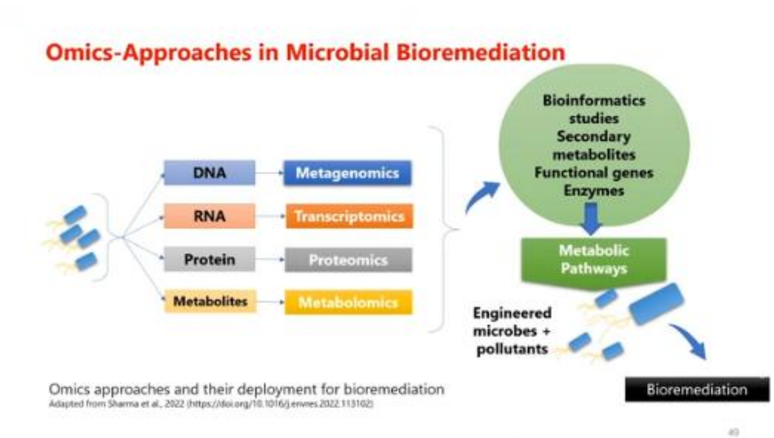
Genetically engineered microbes for HM remediation

GEMs	Gene inserted/modified	HM remediation
Escherichia coli	SpPCS; metalloregulatory protein ArsR; organomercurial lyase; mercury transporter; Mice metallothionein (pMT-Thio gene)	Cd^{2+}; As; Hg; Hg
Escherichia coli	Mice metallothionein (*pMT-Thio* gene)	Pb^{2+}, Cd^{2+}
Corynebacterium glutamicum	ars operons overexpression	As
Methylococcus capsulatus	CrR	Cr^{6+}
Pseudomonas putida strain	Chromate reductase (ChrR)	Cr
Deinococcus radiodurans	*merA* gene	Cd^{2+}; Hg
Pseudomonas K-62' *Achromobacter* sp AO22	organomercurial lyase gene; *mer* gene	Hg; Hg

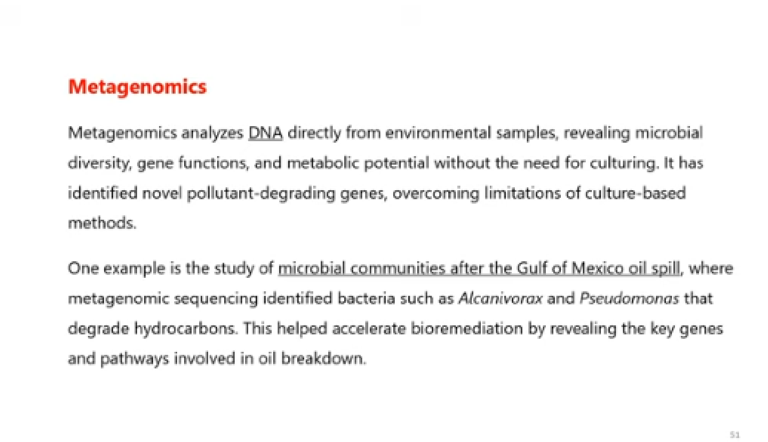
(Yadav & Sharma, 2023

48

Therefore, we achieve bioremediation through this omics approach. Omics studies have revolutionized metabolic engineering for bioremediation by providing comprehensive datasets that allow for the systematic optimization of microorganisms to enhance pollutant degradation. In the case of metagenomics, it involves the analysis of DNA directly from environmental samples or the total DNA of all microorganisms, helping reveal microbial diversity, gene functions, and metabolic potential without the need for culturing. This approach is used to understand the non-culturable part of the microbial population. It has identified novel pollutant-degrading genes, overcoming the limitations of culture-based methods, as only a very small percentage of microbes are culturable.



One example is the study of microbial communities after the Gulf of Mexico oil spill, where metagenomic sequencing identified bacteria such as Alcanivorax and Pseudomonas that degraded hydrocarbons. This helped accelerate bioremediation by revealing the key genes and pathways involved in oil breakdown. Transcriptomics focuses on the active genes expressed within microbial communities by studying the mRNA profile. This helps in understanding the functional responses of microorganisms to pollutants, as well as identifying key metabolic pathways involved in biodegradation. For example, if a particular pollutant, such as hydrocarbon, is present in the environment,



That hydrocarbon will impact the expression of genes of the overall microbes in that population. And many of them will be trying to express certain genes to produce certain enzymes or proteins, which will be able to degrade those pollutants. So, through transcriptomics, we can understand that overexpression or underexpression, as the case may be. Some of the examples include the case of Polaromonas, where transcriptome analysis identified genes elevated in response to cis-dichloroethene, including those for antioxidant proteins and transporters. Similarly, in the case of proteomics, we examine the protein expression profiles of microorganisms that are exposed to pollutants.

Transcriptomics

Transcriptomics focuses on the active genes expressed within microbial communities by studying mRNA. This helps in understanding the functional response of microorganisms to pollutants, as well as identifying key metabolic pathways involved in biodegradation.

Example: In *Polaromonas sp.* JS666, transcriptomic analysis identified genes elevated in response to cis-dichloroethene (cDCE), including those for antioxidant proteins and transporters.

52

It identifies key proteins and enzymes involved in pollutant breakdown, offering insights into microbial physiological responses and potential pathways for enhanced bioremediation. It is important to remember that the transcriptomic profile and the proteomic profile may be different. Not the entire transcriptome may actually be reflected in the final proteome due to various events. So, proteomic analysis of Mycobacterium vanbaalenii revealed the induction of dioxygenases and catalase peroxidase when exposed to polycyclic aromatic hydrocarbons.

Proteomics

Proteomics examines the protein expression profiles of microorganisms exposed to pollutants. It identifies key proteins and enzymes involved in pollutant breakdown, offering insights into microbial physiological responses and potential pathways for enhanced bioremediation.

Example: Proteomic analysis of *Mycobacterium vanbaalenii* revealed the induction of dioxygenases and catalase-peroxidase when exposed to polycyclic aromatic hydrocarbons (PAHs).

53

Then we have the Metabolomics, which studies the changes in metabolite concentrations within microbial communities exposed to environmental contaminants. It reveals metabolic shifts, aiding in the understanding of microbial activity and environmental impacts of pollutants. Some examples, like metabolomics, have linked changes in metabolite levels to microbial responses to toxic substances such as hydrocarbons and pesticides. Now, let us go into a brief discussion of the use of immobilized microorganism technology in bioremediation.

Metabolomics

Metabolomics studies changes in metabolite concentrations within microbial communities exposed to environmental contaminants. It reveals metabolic shifts, aiding in the understanding of microbial activity and the environmental impact of pollutants.

Example: Metabolomics linked changes in metabolite levels to microbial responses to toxic substances like hydrocarbons and pesticides.

This method enhances bioremediation by stabilizing biological cells, preventing competition with local species. It provides a controlled environment where microorganisms can break down contaminants over extended periods, thus offering higher degradation rates compared to free-living microbial systems. So, we have various cell immobilization techniques. The number one may be the chemical method. The first one is adsorption, and the second one is covalent bonding.

Immobilized microorganism technology

This method enhances bioremediation by stabilizing biological cells, preventing competition with local species.
This approach provides a controlled environment where microorganisms can break down contaminants over extended periods, thus offering higher degradation rates compared to free-living microbial systems.

Then we have the physical immobilization techniques, where we may entrap the microbial cells or encapsulate them. So, let us discuss adsorption first. The immobilization of cells through adsorption is accomplished by moving the target cells from the bulk to the support surface or the matrix surface. Here, these cells or biocatalysts are adsorbed on the surface of the matrix and not inside the matrix. Adsorption basically relies on the physical attachment of microorganisms on the surface of a water-insoluble carrier, mediated by weak molecular forces such as Van der Waals interactions, ionic forces, and hydrogen bonds.

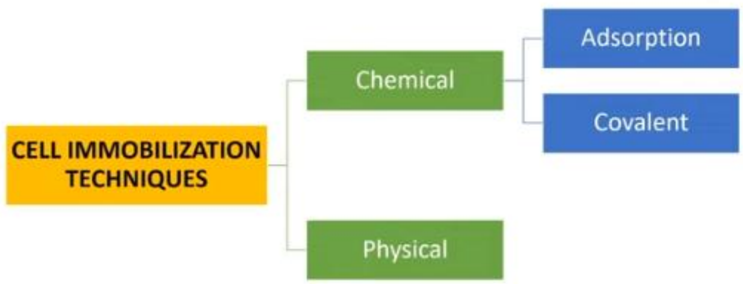


Figure: Classification of cell immobilization methods based on interaction modes or bonds involved between cells and support carriers (physical or chemical)
Source: Bouabidi, Z.B. et al., 2019

These weak forces help maintain the natural structure of the immobilized cells without causing structural changes. In the case of covalent immobilization, We have an active matrix or surface where we may have a spacer molecule and a linker molecule through which the cells are immobilized by covalent bonds. This is a cost-effective method of immobilization that relies on creating a covalent link between the target cells and the support matrix in the presence of a binding agent. The functional group or groups on the cell's surface are covalently bonded to the support material directly or through a linker.

Adsorption

- The immobilization of cells through adsorption is accomplished by moving the target cells from the bulk to the support's surface.
- This technique relies on the physical attachment of microorganisms/biocatalysts to the surface of a water-insoluble carrier, mediated by weak molecular forces such as Van der Waals interactions, ionic forces, and hydrogen bonds.
- These weak forces help maintain the natural structure of the immobilized cells without causing structural changes.

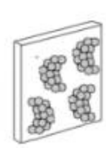


Fig: Biocatalysts adsorption on the carrier.
Source: Adopted from Bouabidi, Z.B. et al, 2019

Then we have the physical methods of immobilization, the number one being the entrapment, as we can see here in the left figure and the encapsulation in the right figure. Cell entrapment is an irreversible immobilization technique that traps particles or cells inside of a support matrix to provide the cells with a protection from outside aggressions. Encapsulation is a form of entrapment in which the cell or enzyme is contained within a semi-permeable membrane where immobilized cells are free to float within the core space. The membrane semi-permeability permits the movement of unrestricted substrates and nutrients. What are the advantages of immobilized microbes over free microbes?

Entrapment (left)

- Cell entrapment is an irreversible immobilization technique that traps particles or cells inside of a support matrix to provide the cells with protection from outside aggressions.

Encapsulation (right)

- Encapsulation is a form of entrapment in which the cell or enzyme is contained within a semipermeable membrane where immobilized cells are free to float within the core space.
- The membrane's semi-permeability permits the movement of unrestricted substrates and nutrients.

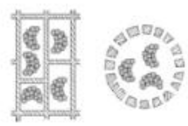
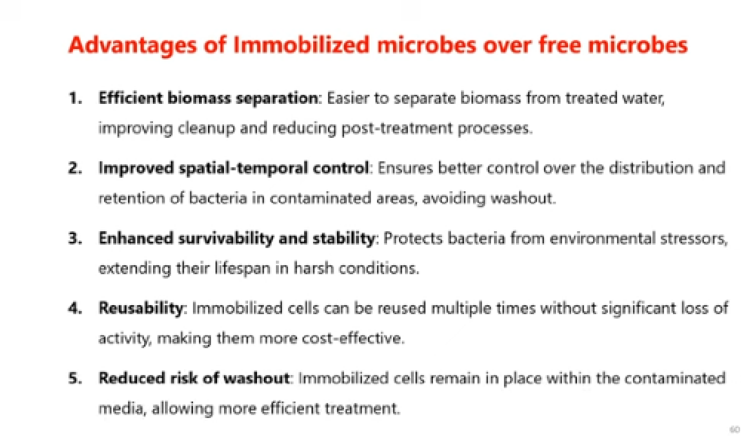


Fig: Entrapment of biocatalysts into the carrier (left), Encapsulation of biocatalysts immobilization (right)
Source: Adopted from Bouabidi, Z.B. et al., 2019

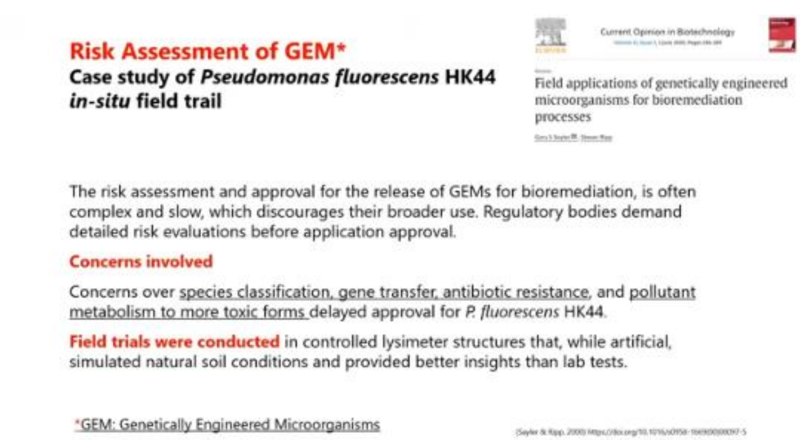
Number one is it helps in the separation of biomass efficiently, easier to separate biomass from treated water, improving cleanup and reducing post treatment processes. Then we have improved spatial temporal control, which ensures better control over the distribution and retention of bacteria in contaminated areas, avoiding washout. Then we have the enhanced survivability and the stability. This protects bacteria from environmental stressors, extending their lifespan in harsh conditions. Then we have the advantage of reusability.

Immobilized cells can be reused multiple times without significant loss of activity until and unless they are viable making them more cost effective. Then there is reduced risk of washout. Immobilized cells remain in place within the contaminated media allowing more efficient treatment. So, we have seen that genetically engineered microbes can be very efficient in the bioremediation process, but since these are modified genetically and they are synthetic, we need to do the risk assessment. So, in this case, we can see here the field applications of genetically engineered microorganisms for bioremediation.



which involves the species pseudomonas fluorescens HK44 and it was done in situ. The risk assessment approval for the release of GEMs for bioremediation is often complex and slow, which discourages their broader use. Regulatory bodies demand detailed risk evaluations before application approval. Of course, we should not be frustrated. This is very, very important for our own long-term applications.

So, certain concerns over species classifications in transfer antibiotic resistance and pollutant metabolism to more toxin forms delays approval in this particular case of Pseudomonas fluorescens. Then field trials were conducted in control lysimeter structures that while artificial simulated natural soil conditions and provided better insights than the lab tests. So, what was the outcome of this risk assessment of these genetically modified pseudomonas? In general, it is often assumed that GEMS face survival challenges due to the energy burden of foreign genes. In this case, pseudomonas strain did not show such burden and survived in the field.



However, predicting genetically modified microorganisms or genetically engineered microorganism survival in in-situ remediation remains difficult due to unpredictable environmental factors. And due to this, the use of genetically engineered microbes widely approved for large-scale commercial use in bioremediation is still limited. So ,with this, we come to the end of this lecture. Thank you for your kind attention. Amen.