SEM V: MI-305.1 Environmental Microbiology
Unit 4 B: Bioremediation of Petroleum Hydrocarbons

- Biological = living organisms
- Remediation = Solution to Environmental pollutants (Harmful to humans and dangerous to environment)
- Bioremediation accelerate the natural fate of biodegradable pollutants and hence natural or **"green solution"** to the problem of oil pollutants that causes minimal, if any additional ecological effects.
- Bioremediation can be defined as utilization of microbes to remove (degrade) pollutants from the environment.
- Bioremediation is necessary and cost effective means of removing certain environmental pollutants that adversely affect human health or environment quality.
- The enormous natural capacity of diverse microbes to degrade numerous organic compounds ranging from petroleum hydrocarbons to chlorinated solvents and to transform various inorganic substances including metal forms basis for bioremediation.
- Bioremediation explore the potential of metabolic activities of microbes to change an undesirable chemical into one that has less objectionable properties.

$$\text{Petroleum Hydrocarbons} \xrightarrow{\textbf{Microbes}} CO_2 + H_2O$$

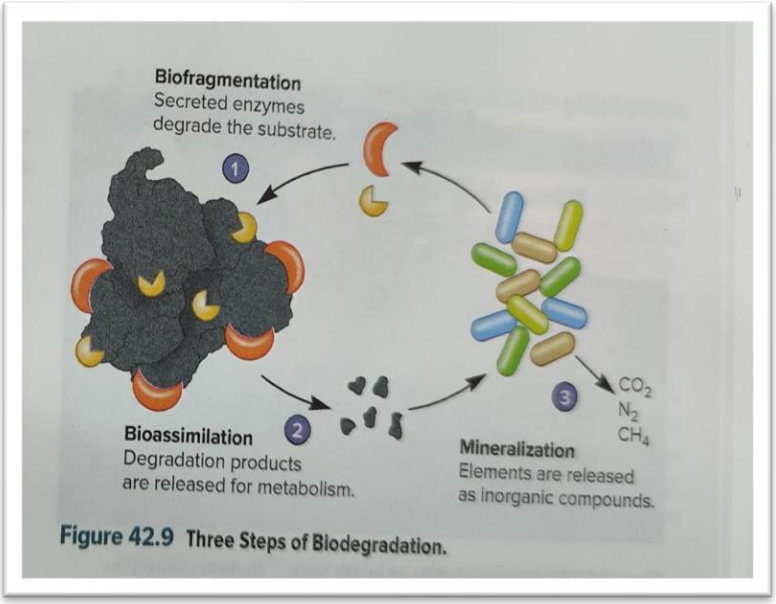


Figure 42.9 Three Steps of Biodegradation.

- Bioremediation relied on naturally occurring microbes, often the indigenous microbes at a contaminated site.

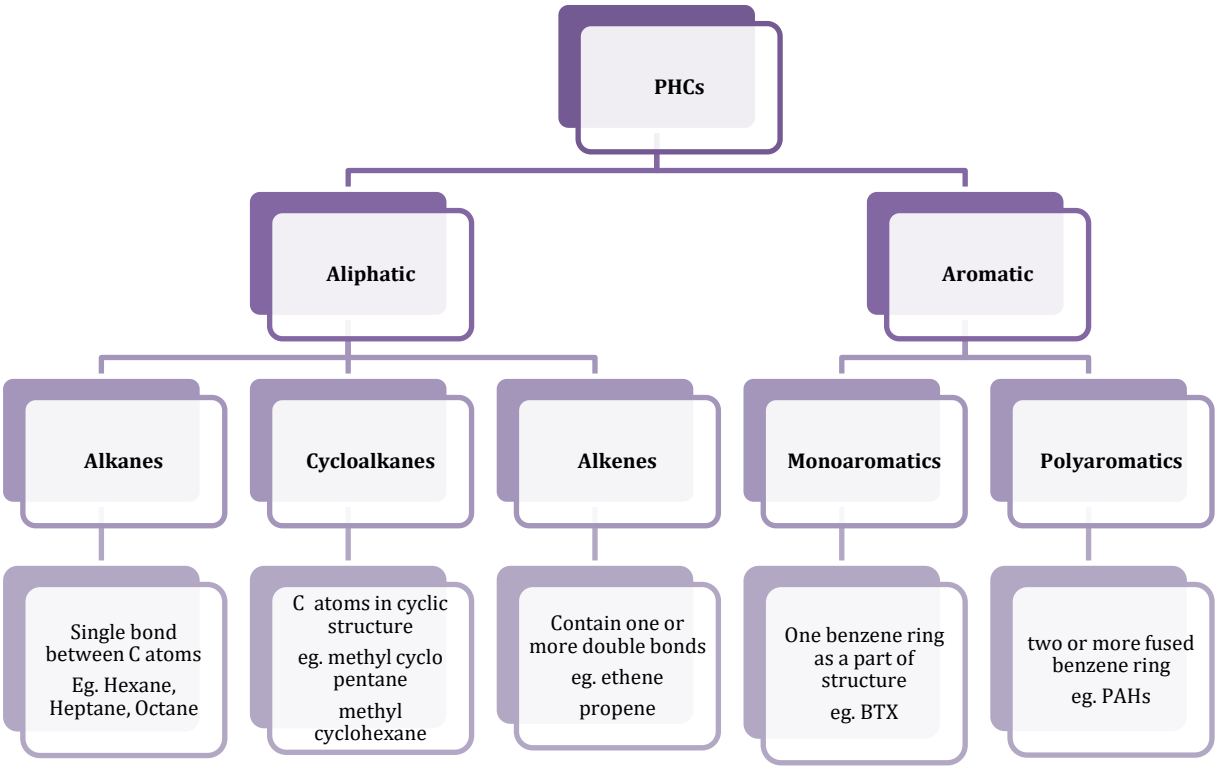
Contaminated Sites: Soil (variety of soil nearby contaminated area), Marine water, Freshwater or underground water

Role of Naturally Occurring Microbes (Indigenous):

- Number of microbes is less in natural environment. Environmental conditions may not be favorable.
- Natural rate of degradation is very slow.
- Bioremediation process in that increasing the number of indigenous organisms and addition of organisms (exogenous) to stimulate the biodegradation process.
- Petroleum hydrocarbons (PHCs) – chemicals comprise of oil and products, refined from oil such as gasoline and diesel.

Occurrence

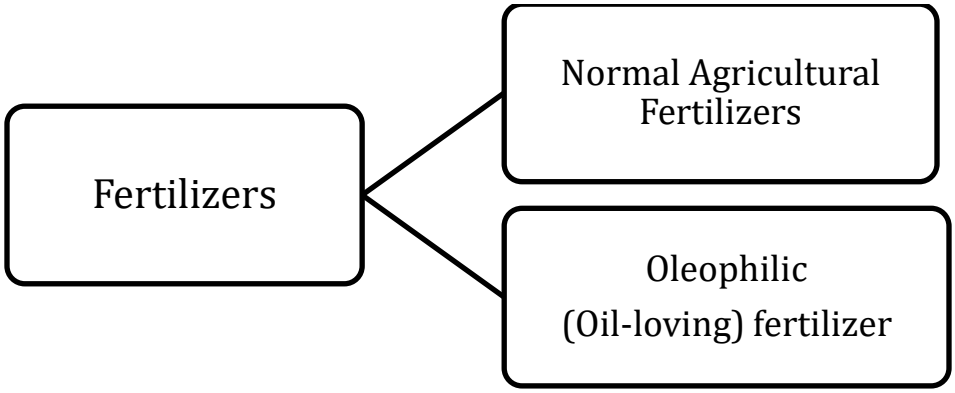
- PHCs are widespread environmental pollutants.
- Amenable to removal by bioremediation
- Hundreds of thousands of underground storage tanks leaked gasoline containing toxic benzene, toluene, xylene (BTX) into soil and ground water.
- Oil spills have contaminated coastal marine environments with tons of crude oil.



Microbes

- Indigenous microbes present that can degrade many PHCs but at a slower rates.
- Populations of PHCs degraders are less than 1% total microbes in unpolluted environments but increase to 1 to 10% in environments exposed to petroleum pollutants.
- Mixed cultures of non-genetically engineered microbes are commonly proposed as inocula for seeding to bioremediate oil contaminated soils and waters.
- A genetically engineered hydrocarbon-degrading *Pseudomonas* was first organism patented in a landmark decision of US Supreme Court.
- Pollution it is unnecessary to seed with any hydrocarbon-degrading culture because there already is sufficient concentration of indigenous hydrocarbon degraders.
- Deliberate environmental release of genetically engineered microbes is again controversy.
- Bioremediation of oil pollutants generally relies on modifying the environment so that the growth of indigenous hydrocarbon-degrading microbes is stimulated.
- Microbes require nitrogen, phosphorus, and other mineral nutrients for incorporation into biomass, the availability of these nutrients within the area of hydrocarbon are critical.
- Nitrogen and phosphorus limit microbial hydrocarbon degradation.
- Various fertilizer formulations are used to supply these necessary nutrients.

Types of Fertilizers



- Addition of N and P containing fertilizer overcomes the nutritional limitation for microbial growth because petroleum contains concentration of these substances well below those needed for microbial growth.
- Typical ratios of carbon to nitrogen in a microbial cell are 10:1 and of carbon to P 30:1
- Rapid hydrocarbon biodegradation requires molecular O_2 because initial steps in the biodegradation of hydrocarbon by most microbes such as *Pseudomonas* spp. Involves the direct incorporation of oxygen by oxygenase.
- Tilling, forced aeration and addition of peroxides are used in soils and ground waters to provide favorable environmental conditions for hydrocarbon biodegradation.
- Bioremediation is rather simple and low cost methods used to clean up major oil spills such as Exxon Valdez spill occurred in 1987 in Alaska and numerous contaminated soils and aquifers.
- Not all hydrocarbons are biodegraded in this process.
- Exxon Valdez spill formed the basis for a major study on bioremediation through **fertilizer application** and was the largest application of this emerging technology.
- **Inipol** (Oleophilic microemulsion with urea as nitrogen source)
    - Laureth phosphate as a 'phosphate' source
    - Oleic acid as 'carbon' source
- **Customblen** (Slow release fertilizer)
    - Calcium phosphate
    - Ammonium phosphate
    - Ammonium nitrate
    - (Polymerized vegetable oil coating)
- Within approximately 2 to 3 weeks oil on surface of shorelines treated with Inipol and Customblen was degraded to that these shorelines were visibly cleaner than non-bioremediated shorelines.
- Monitoring of the oil-degrading microbial populations and measuring the rates of oil degradation activities by a joint Exxon US Environmental Protection Agency (USEPA) and State of Alaska Department of Conservation team showed that 5 fold increase in rates of oil biodegradation typically followed fertilizer application.

- The addition of fertilizer caused no eutrophication, no acute toxicity to sensitive test species and didn't release of undergraded oil residues from the beaches.
- Because of its effectiveness, bioremediation became the major treatment method for removing oil pollutants from the polluted sites.
- Bioremediation is cleanup strategy developed for future oil spills.

Stimulating Biodegradation

- Bioremediation involves stimulating the degradative activities of microbes already present in contaminated waters or soils.
- Natural microbial communities may not be able to carry out biodegradation processes at a desired rate due to limiting physical or nutritional factors.
- Biodegradation is limited by low O_2 levels.
- N, P and other nutrients needed may be limiting.
- It is necessary to determine limiting factors and supply the needed material to modify environment.
- Monitoring and recovery wells are put into place so that the nutrient status and rates of biodegradation can be determined by periodic sampling.
- Addition of easily metabolized organic matter such as glucose increases biodegradation of recalcitrant compounds that are usually not used as carbon and energy source by microbes. This process termed **Co-metabolism**.

Addition of Glucose & Cellulose + Compound to be degraded (**Recalcitrant**) ⟶ **Co-metabolism**

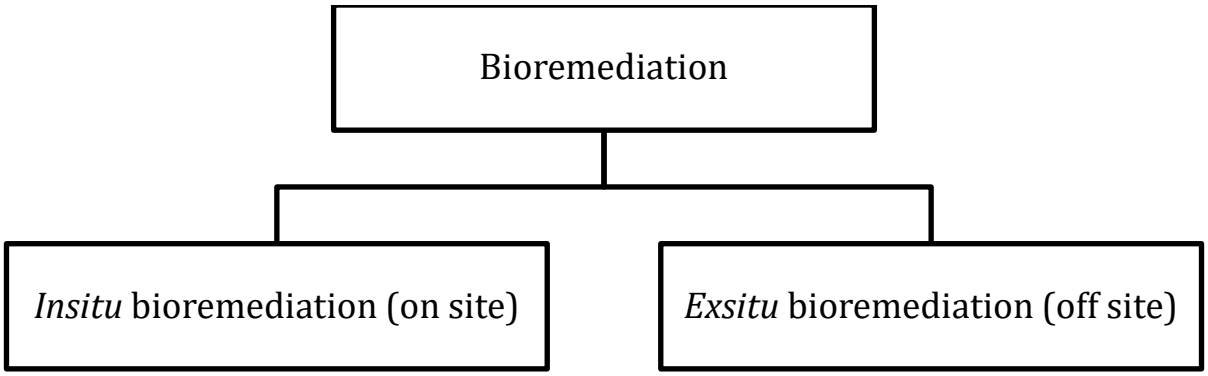
- Stimulating hydrocarbon degradation in water and soil:
- Oil spills in marine environments illustrates hydrocarbon.
- Dispersed hydrocarbons in the ocean, contact between microbes, hydrocarbon substrate, and other essential nutrients must be maintained.
- To achieve this, pellets containing nutrients and oleophilic (hydrocarbon soluble) preparation are used.

- This technique accelerates the degradation of different crude oil slicks by 30-40% in comparison with control oil slicks where additional nutrients are not available.
- Approaches to increase biodegradation nutrient additions, biosurfactant, chemical dispersants, additional use of high-pressure steam, use of microbially produced glycolipid emulsifier proved useful.
- Bioremediation approaches are used in soils and sediments.

Factors affecting Bioremediation

- The existence of a microbial population.
- Available contaminants.
- Environmental factors a) type of soil, b) temperature, c) soil reaction (neutral or alkaline), d) presence of O_2 and nutrients

Bioremediation strategies:



- *In situ* bioremediation applied to soil and ground water at the site with minimal disturbance.
- On the site oxygen and nutrients are being supplied.
- **Bioventing**: involves supplying low air flow rates and providing oxygen in necessary amounts for the biodegradation while minimizing volatilization and release of contaminants to the atmosphere.
- This will stimulate growth of indigenous bacteria and works for simple hydrocarbons and used where contamination is deep under surface.
- **Biosparging** involves injection of air under pressure below the water to increase oxygen concentration and enhance rate of biological degradation.
- It increases the mixing in the saturated zone and thereby increases the contact between soil and groundwater.

- **Bioaugmentation:** technique involve addition of microbes that degrade pollutants. It involves both indigenous as well as exogenous microbes. Sometimes exogenous population can be able to compete with indigenous microbes.
- In soil mostly indigenous microbes degrade pollutant effectively under good management.

Microbes:

1. **Aerobic microbes:** *Pseudomonas, Sphingomonas, Rhodococcus, Mycobacterium*
2. **Ligninolytic Fungi:** *Phanerochaete chrysosporium*
3. **Methylotrophs:** uses methane as 'C' and 'E' source

***Ex situ* Bioremediation:**

- Applied to soil and groundwater which are removed from site and dumped elsewhere.
- Various *Ex situ* measure described as under:

1. **Land farming:**

- Contaminated soil is excavated and spread over a prepared bed and periodically tilled until pollutants are degraded.
- The goal is to facilitate aerobic degradation of contaminants.
- Practice is limited to the treatment of superficial 10-35 cm soil.
- Reduce monitoring and maintenance as well as cleanup cost.

2. **Composting Technique:**

- Contaminated soil is mixed with organic amendments.
- These organic materials are used as energy source by microbes.

Bio-piles

- Hybrid farming and composting.
- In this engineered cell are constructed as aerated composted piles.
- Bio-piles provide favorable environment for indigenous aerobic and anaerobic microbes.
- Control physical losses of contaminants by leaching and volatilization.

Bioreactor

- Bioreactors are used for *ex situ* treatment of contaminated soil or water.
- It involves processing of contaminated solid material or water through an engineered contaminated system.

Advantages of Bioremediation:

- Natural process and perceived as an acceptable waste treatment process.
- Microbial population sustains naturally using contaminants and decrease pollution.

- Degraded products are harmless (CO_2, H_2O, biomass).
- Useful for complete destruction of wide variety of contaminants.
- Transform hazardous compounds into harmless products.
- Complete destruction of target pollutants is possible.
- Can be carried without disrupting normal activities.
- Potential threats to human health and the environment are less.
- Less expensive process.

Disadvantages:

- Highly specific and limited to biodegradable compounds.
- End products may be more persistent or toxic than the parent compound.
- Presence of metabolically capable microbial population, environmental conditions, and levels of nutrients determines extent of degradation which is normally not controllable.
- Difficult to extrapolate pilot scale studies to full scale operations.
- Research needed to develop bioremediation technologies for different sites.
- Takes longer time than other treatment.
- Require pretreatment such as excavation washing or physical extraction before being placed in a bioreactor.

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