Microbiology Course Material

Semester – IV, Paper – CC9

S-IV/CC9/U-5

Unit 5: Microbial Bioremediation

By

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B.Sc (HONOURS) MICROBIOLOGY (CBCS STRUCTURE)

CC-9: ENVIRONMENTAL MICROBIOLOGY (THEORY)
SEMESTER – IV

SYLLABUS: UNIT 5: MICROBIAL BIOREMEDIATION

- Principles and degradation of common pesticides,
- Organic (hydrocarbons, oil spills) and inroganic (metals) matter,
- Biosurfactants

Hydrocarbon

Hydrocarbon is a class of organic chemical compounds composed only of the elements carbon (C) and hydrogen (H). The carbon atoms join together to form the framework of the compound, and the hydrogen atoms attach to them in many different configurations. Many hydrocarbons occur in nature. In addition to making up fossil fuels, they are present in trees and plants, as, for example, in the form of pigments called carotenes that occur in carrots and green leaves. More than 98 percent of natural crude rubber is a hydrocarbon polymer, a chainlike molecule consisting of many units linked together. The structure and chemistry of individual hydrocarbons depend on the types of chemical bonds that link the atoms together to form their constituent molecules.

Nineteenth-century chemists classified hydrocarbons as either aliphatic or aromatic on the basis of their sources and properties. Aliphatic described hydrocarbons derived by chemical degradation of fats or oils. Aromatic hydrocarbons constituted a group of related substances obtained by chemical degradation of certain pleasant-smelling plant extracts. The terms *aliphatic* and *aromatic* are retained in modern terminology, but the compounds they describe are distinguished on the basis of structure rather than origin.

Hydrocarbons are the principal constituents of petroleum and natural gas. They serve as fuels and lubricants as well as raw materials for the production of plastics, fibres, rubbers, solvents, explosives, and industrial chemicals.

❖ Types of Hydrocarbons

As defined by IUPAC nomenclature of organic chemistry, the classifications for hydrocarbons are:

- Saturated hydrocarbons are the simplest of the hydrocarbon species. They are composed entirely of single bonds and are saturated with hydrogen. The formula for acyclic saturated hydrocarbons (i.e., alkanes) is C_nH_{2n+2}. The general form of saturated hydrocarbons is $C_nH_{2n+2(1-r)}$, where r is the number of rings. Those with exactly one ring are the cycloalkanes. Saturated hydrocarbons are the basis of petroleum fuels and are found as either linear or branched species. Substitution reaction is their characteristics property (like chlorination reaction to form chloroform). Hydrocarbons with the same molecular formula but different structural formulae are called structural isomers. Chiral saturated hydrocarbons constitute the side chains of biomolecules such as chlorophyll and tocopherol.
- Unsaturated hydrocarbons have one or more double or triple bonds between carbon atoms. Those with double bond are called alkenes and have the formula C_nH_{2n} (assuming non-cyclic structures). Those containing triple bonds are called alkyne and have the formula C_nH_{2n-2}.
- Aromatic hydrocarbons, also known as arenes that have at least one aromatic ring.
- Hydrocarbons can be gases (e.g. methane and propane), liquids (e.g. hexane and benzene), waxes or low melting solids (e.g. paraffin wax and naphthalene) or polymers (e.g. polyethylene, polypropylene and polystyrene).

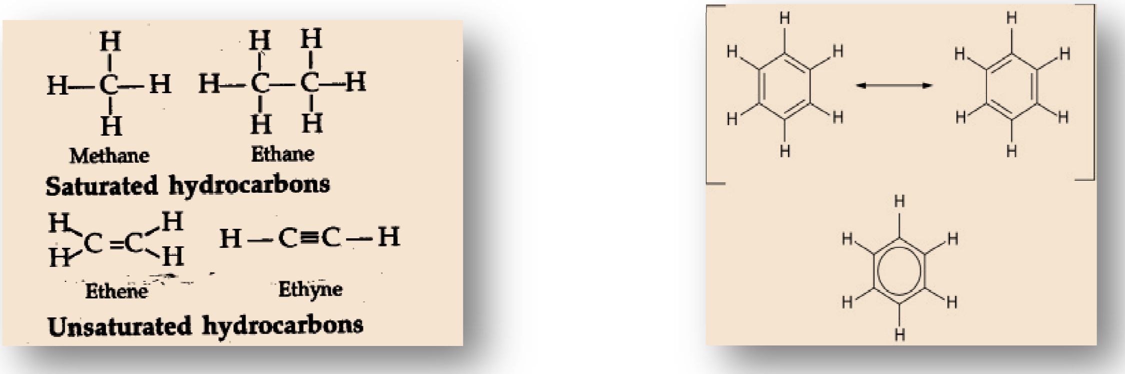

Figure 1(a) Figure 1(b)

Figure 1: (a) Structures showing Saturated and Unsaturated Hydrocarbons, (b) Structure showing formation of Aromatic Hydrocarbons

- Uses:
- Hydrocarbons are a primary energy source for current civilizations.
- The predominant use of hydrocarbons is as a combustible fuel source.
- In their solid form, hydrocarbons take the form of asphalt (bitumen).
- Mixtures of volatile hydrocarbons are now used in preference to the chlorofluorocarbons as a propellant for aerosol sprays, due to chlorofluorocarbons' impact on the ozone layer.
- Methane (CH_4) and ethane (C_2H_6) are gaseous at ambient temperatures and cannot be readily liquefied by pressure alone.
- Propane (C_3H_8) is however easily liquefied, and exists in 'propane bottles' mostly as a liquid.
- Butane (C_4H_{10}) is so easily liquefied that it provides a safe, volatile fuel for small pocket lighters.
- Pentane (C_5H_{12}) is a colorless liquid at room temperature, commonly used in chemistry and industry as a powerful nearly odorless solvent of waxes and high molecular weight organic compounds, including greases.
- Hexane (C_6H_{14}) is also a widely used non-polar, non-aromatic solvent, as well as a significant fraction of common gasoline.

- The C_6 through C_{10} alkanes, alkenes and isomeric cycloalkanes are the top components of gasoline, naphtha, jet fuel and specialized industrial solvent mixtures. With the progressive addition of carbon units, the simple non-ring structured hydrocarbons have higher viscosities, lubricating indices, boiling points, solidification temperatures, and deeper color.

At the opposite extreme from methane lie the heavy tars that remain as the *lowest fraction* in a crude oil refining retort. They are collected and widely utilized as roofing compounds, pavement composition, wood preservatives and as extremely high viscosity shear-resisting liquids.

- **Petroleum:**

Extracted hydrocarbons in a liquid form are referred to as petroleum or mineral oil, whereas hydrocarbons in a gaseous form are referred to as natural gas. Petroleum and natural gas are found in the Earth's subsurface with the tools of petroleum geology and are a significant source of fuel and raw materials for the production of organic chemicals. The extraction of liquid hydrocarbon fuel from sedimentary basins is integral to modern energy development. Hydrocarbons are mined from oil sands and oil shells, and potentially extracted from sedimentary methane hydrates. These reserves require distillation and upgrading to produce synthetic crude and petroleum.

Economically important hydrocarbons include fossil fuels such as coal, petroleum and natural gas, and its derivatives such as plastics, paraffin, waxes, solvents and oils. Hydrocarbons – along with NO_x and sunlight – contribute to the formation of tropospheric ozone and greenhouse gases.

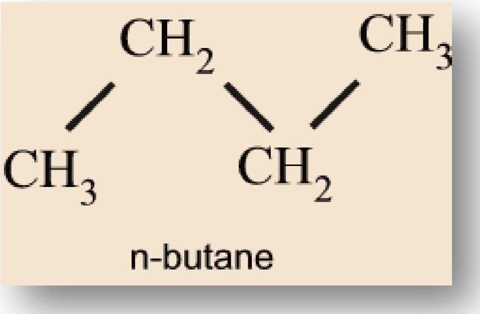

Figure 2: Chemical structure of Petroleum

Poisoning:

- Hydrocarbon poisoning such as that of benzene and petroleum usually occurs accidentally by inhalation or ingestion of these cytotoxic chemical compounds.
- Intravenous or subcutaneous injection of petroleum compounds with intent of suicide or abuse is an extraordinary event that can result in local damage or systemic toxicity such as tissue necrosis, abscess formation, respiratory system failure and partial damage to the kidneys, brain and nervous system.
- Many hydrocarbons are highly inflammable; therefore, care should be taken to prevent injury. If hydrocarbons undergo combustion in tight areas, toxic carbon monoxide can form.
- Benzene and many aromatic compounds are possible carcinogens, and proper safety equipment must be worn to prevent these harmful compounds from entering the body.
- Hydrocarbons should be kept away from fluorine compounds due to the high probability of forming toxic hydrofluoric acid.
- Burning hydrocarbons as fuel, producing carbon dioxide and water, is a major contributor to anthropogenic global warming.
- Hydrocarbons are introduced into the environment through their extensive use as fuels and chemicals as well as through leaks or accidental spills during exploration, production, refining, or transport. Anthropogenic hydrocarbon contamination of soil is a serious global issue due to contaminant persistence and the negative impact on human health.

Biodegradation occurs naturally because hydrocarbons have always been present in the environment, released from seeps and reservoirs through various geologic processes. Natural attenuation of hydrocarbon contamination can be monitored by a number of analytical methods, including gas chromatography and infrared spectroscopy. Absolute proof of degradation versus dilution or other non-degradative processes can be obtained using compound specific isotope analysis (CSIA), where enrichment in the natural abundance of heavier isotopes of carbon and hydrogen in the parent hydrocarbons confirms **biotransformation**. Often **biostimulation** - the addition of limiting nutrients - may be desired in sites where attenuation occurs slowly or not at all.

Many bioremediation efforts involve injection and circulation of oxygen to subsurface environments in order to increase the availability of oxygen as both reactant and electron acceptor. Common methods include using a vacuum pump to promote air circulation, **bioventing** or **biosparging** that add air to the subsurface, water circulation above ground to add oxygen, and the introduction of substances that release oxygen like peroxides or ozone. Remediation may also involve introduction of bacterial strains (**bioaugmentation**) as well as nutrients to stimulate hydrocarbon degradation. This strategy is most often used at sites with mid-weight or heavier petroleum products, as lighter compounds volatilize.

- **Aerobic Degradation**

Hydrocarbons are readily degraded under aerobic conditions. Bacteria, fungi, and algae are all capable of aerobic hydrocarbon degradation. In general, alkenes (hydrocarbons containing double bonds) and short-chain alkanes (hydrocarbons containing only single bonds) are the most easily degraded, followed by branched alkanes (alkanes with side chains) and then aromatics (hydrocarbons in a stable ring structure). However, degradation rates vary based on environmental parameters and decrease as hydrocarbon complexity increases. Reported degradation rates vary considerably because hydrocarbon composition depends on the source of the petroleum and age of the spill. For example, compound degradation varies from 5% to 30% in 28 days while up to 100% degradation occurs with nitrogen addition. Degradation rates by fungal species reportedly range from ~ 30-100% degradation over 28 days or less. The primary rate-limiting factor in aerobic biodegradation is delivery of oxygen. Oxygen availability is dependent on the ability of oxygen to move or diffuse through the site environment as well as on the uptake rate by microorganisms. Addition of oxygen can increase degradation rates several orders of magnitude over naturally occurring rates.

- **Alkanes and Alkenes**

Alkanes containing 14 carbons or fewer are prone to volatilization, while alkanes containing more carbons are less volatile. Regardless, alkanes and alkenes, with the exception of cyclic alkanes (alkanes in a ring structure), are the most readily degraded hydrocarbons with reported degradation of alkanes containing up to 44 carbons. Both alkanes and alkenes are degraded with addition of molecular oxygen. Oxygen availability and the initial step of degradation are rate-limiting. In the initial steps of aerobic alkane degradation, enzymes called oxygenases add molecular oxygen to the hydrocarbon molecules, forming alcohols that are further oxidized to fatty acids that are subsequently metabolized to acetyl-CoA and finally to CO_2 and H_2O. Many mono- and di-oxygenases and other oxidases have a wide substrate range and act readily on a variety of hydrocarbons.

- **Aromatic Hydrocarbons**

Aromatic hydrocarbons are generally more difficult to degrade than shorter alkanes and alkenes due to their greater toxicity, yet they are readily degraded aerobically by many bacteria and fungi. Degradability decreases with increasing number of rings and increased molecular size, due to increased hydrophobicity and adsorption capacity. The median primary degradation rate of benzene, toluene, ethylbenzene, and xylene (BTEX compounds) ranges from 0.05-0.2 day^{-1}. The general pathway for degradation of aromatic compounds begins with the addition of O_2 by mono and di-oxygenases. This yields key intermediate products such as benzyl alcohol, phenol or catechol, protocatechuate, and gentisate. These intermediates then undergo ring cleavage, also by a variety of oxygenases, resulting in carboxylic acid formation. Degradation then continues to acetyl-CoA and succinyl-CoA that enter into central metabolism. Fungal degradation occurs by non-specific extracellular oxidizing enzymes that form radical intermediates, although many reactions are similar to those found in bacteria.

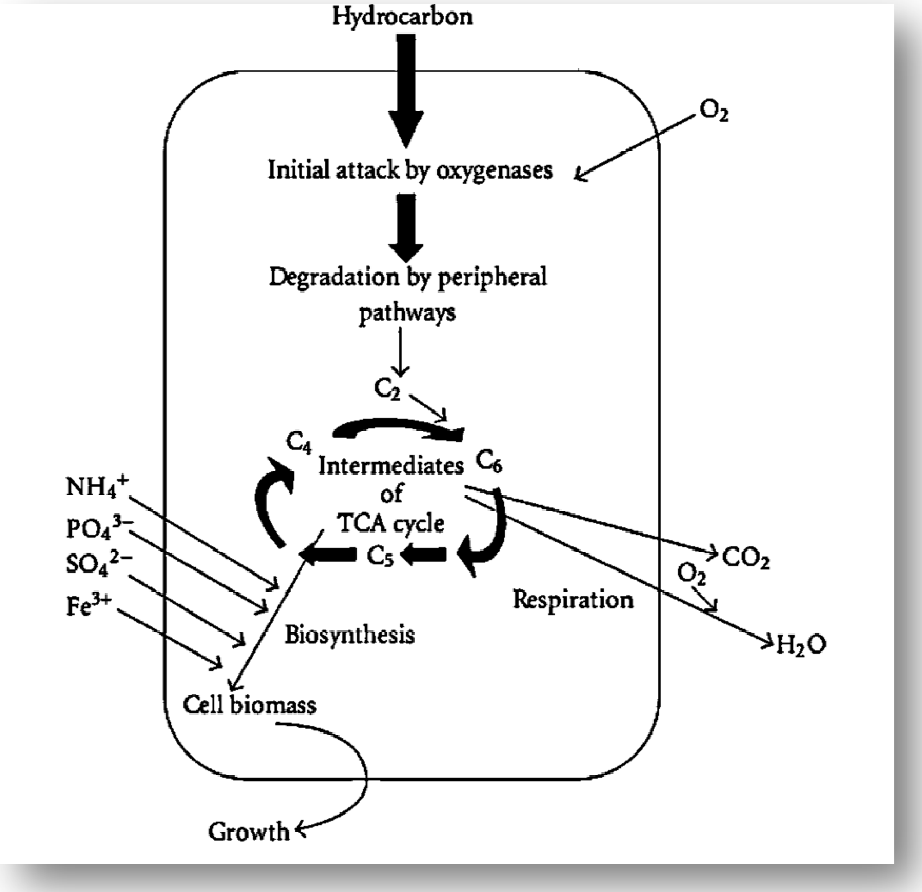

Figure 3: Diagram showing the mechanism of Aerobic Degradation of Hydrocarbons

- **Anaerobic Degradation**

Hydrocarbon degradation under anaerobic conditions is often slower compared to aerobic degradation, due to less favorable reaction energetics with alternate electron acceptors. Despite this limitation, both facultative and obligately anaerobic bacteria and archaea are known to degrade hydrocarbons without oxygen. Such microorganisms develop readily at hydrocarbon-impacted sites owing to rapid consumption of oxygen, and therefore anaerobic processes significantly impact the fate of hydrocarbons in the environment. Initial steps in anoxic hydrocarbon degradation that involve adding an oxidized functional group to activate the molecule are typically rate-limiting. Doubling time for anaerobic hydrocarbon degraders ranges from days to months. Despite slow growth rates, complete degradation of many different types of hydrocarbons occurs in the absence of oxygen. Anaerobic microbes use terminal electron acceptors other than oxygen in respiration, including compounds such as nitrate, sulfate, carbon dioxide, oxidized metals, or even certain organic compounds. At a contaminated site, microbes tend to use electron acceptors sequentially as a function of decreasing reduction potential in the order of oxygen, nitrate, ferric iron, sulfate, and H_2 (Fig. 2). In a few cases, specific species of denitrifying or sulfate reducing microorganisms have been shown to metabolise certain hydrocarbons completely to CO_2 and water. However, anaerobic degradation of hydrocarbons more often occurs via **syntrophy**, where the degradation of a substrate by one microbe is dependent on the activity of another microbe responsible for keeping intermediate products such as formate and H_2 at low concentrations. Low product concentrations drive otherwise thermodynamically unfavorable reactions. Syntrophy is more common under anaerobic conditions because the use of oxygen as a terminal electron acceptor is more energetically favorable. Syntrophic processes are absolutely necessary for complete degradation to methane and carbon dioxide, since methanogens (archaea that produce methane) are only able to metabolize simple substrates like acetate and hydrogen. Multiple syntrophic relationships may be present in any given environment, based on available substrates and conditions. In methanogenic environments, where all other electron acceptors are used up, primary degraders such as *Peptococcaceae* and *Clostridium* degrade hydrocarbons to intermediates like H_2 and acetate, which are consumed by methanogens.

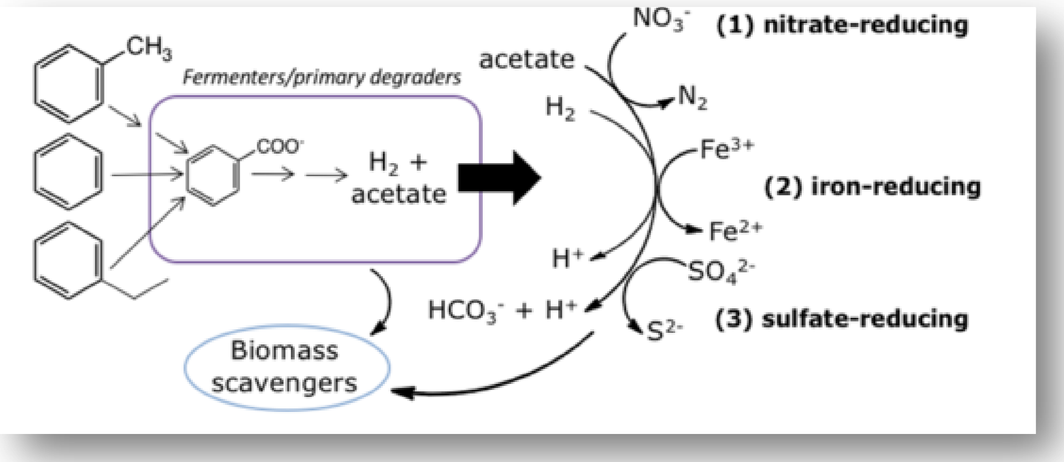

Figure 4: Diagram showing Anaerobic Degradation of Hydrocarbons

- **Summary**

Hydrocarbon remediation occurs more quickly under aerobic conditions than anaerobic. The primary concern in in situ aerobic remediation is oxygen delivery and mixing, which may be achieved by a number of previously-established methods including land-farming, sparging, groundwater recirculation, and peroxide addition. However, aerobic remediation is not feasible in all environments, particularly in low permeability soils. In these cases, anaerobic bioremediation may be preferred. Important considerations for biodegradation include the nature of the suite of hydrocarbons present (light or heavy hydrocarbons), bioavailability, microorganism community composition, nutrient availability, soil permeability, and pH. Biostimulation through addition of nutrients such as nitrogen, phosphorus, and iron is often helpful. Bioaugmentation by addition of actively degrading microbial cultures and nutrients may accelerate biotransformation of particularly recalcitrant hydrocarbons like benzene and polycyclic aromatic hydrocarbons (PAHs), but site conditions must first be assessed to predict and ensure their effectiveness. Knowing the degradation pathways and responsible organisms is useful to:

a) assess potential for natural and enhanced remediation, and
b) track biodegradation at a site by monitoring functional gene biomarkers and degradation intermediates.

Oil is a mixture of hydrocarbon compounds, which are the decayed remains of marine animals and plants, died and drifted to the bottom. For the past 600 million years, under intense pressure and temperatures, these remains changed into complicated hydrocarbons called petroleum. Crude oil is a mixture of gas, naphtha, kerosene, light gas, and residuals, which causes hazardous health effects if consumed by any life forms.

Contamination of seawater due to an oil pour, as a result of an accident or human error, is termed as an **oil spill**. Oil is among the most important energy sources in the world and because of its uneven global distribution, it is transported by ships across the oceans and by pipelines across the land. This has resulted in several accidents in the past while transferring the oil to vessels during transportation and breaking of pipelines while drilling in the earth's crust. While massive and catastrophic spills receive most of the attention, smaller and chronic ones occur on a regular basis. These spills contaminate the coasts and estuaries and can cause serious health problems to human beings.

The production of petroleum products rose from 500 million tons in 1950 to over 2,500 million tons in the mid-1990s, which resulted in massive transportation and associated oil spills. The number has been increasing with the increasing rate of oil transportation, ageing of oil tankers, as well as an increase in the size of oil tankers. Oil accounts for over half the tonnage of all sea cargo. It is estimated that approximately 706 million gallons of waste oil enter the ocean every year, with over half coming from land drainage and waste disposal; for example, from the improper disposal of used motor oil. Offshore drilling and production operations and oil spills or leaks from ships or tankers typically contribute less than 8 percent of the total. The remainder comes from routine maintenance of ships (nearly 20 percent), hydrocarbon particles from onshore air pollution (about 13 percent) and natural seepage from the seafloor (over 8 percent).

- **Harmful Effects of Oil spills:**

1. Public health impacts include illnesses caused by toxic fumes or by eating contaminated fish or shellfish.
2. Loss and disruptions of commercial and recreational fisheries, seaweed harvesting, boating, and a variety of other uses of affected water.
3. Any nation or tourist destination close to oil drilling or shipping lanes is at high risk of experiencing economic collapse and the disastrous environmental effects of oil spills and immediate needs to be properly prepared to clean up.
4. Oil destroys the insulating ability of fur-bearing mammals, such as sea otters, and the water repellency of a bird's feathers, thus exposing these creatures to the harsh elements. Without the ability to repel water and insulate from the cold water, birds and mammals will die from hypothermia.
5. Juvenile sea turtles can also become trapped in oil and mistake it for food.
6. Dolphins and whales can inhale oil, which can affect lungs, immune function and reproduction.
7. Many birds and animals also ingest oil when they try to clean themselves, which can poison them.

8. Fish, shellfish, and corals may not be exposed immediately, but can come into contact with oil if it is mixed into the water column — shellfish can also be exposed in the intertidal zone.
9. When exposed to oil, adult fish may experience reduced growth, enlarged livers, change in heart and respiration rates, fin erosion, and reproduction impairment.
10. Fish eggs and larvae can be especially sensitive to lethal and sublethal impacts. Even when lethal impacts are not observed, oil can make fish and shellfish unsafe for humans to eat.

- **Preventive measures:**

To prevent oil spills is the topmost priority; and the responsibility lies equally on individuals as well as on governments and industries because the sources of oil waste in the ocean is due to carelessness, rather than an accident.

- Integration of preventive measures in an industrial process, operation, or product should be a part of the cost of daily operations.
- Before starting any fueling, de-fueling or internal transfer operation, all machinery and piping systems should be properly checked for tightness and for signs of leaking glands, seals and gaskets.
- While changing oil or adding oil to machinery, proper care should be taken to avoid oil spills.

- **Largest Oil Spill**

Dreadful oil spill results in great damage to the marine environment and sometimes ends up with destroying a complete species. It is said that the largest known oil spill incident was not an accident but a result of a preplanned war. As per statistics, there was a recorded discharge of billions of gallons of oil from the largest oil spill.

We all are familiar with “the Gulf War” of 1991 in which the forces from Iraq quit their invasion of Kuwait. After that, for blocking the forces from the United States, the Iraqi forces left the oil pipelines and wells of oil open and set it to fire. The fire was so massive that it begins in January and ended after four months in April. In this incident, as per reports, more than 200 million gallons of oil was thrown in “the Persian Gulf”. This oil spill incident not only harmed marine species but also destroyed major oil reserve in the world, thus diminishing oil availability. This oil spill not only resulted in marine water pollution but also fouled the coastlines, damaged the fisheries, killed the aquatic life especially non-mobile life and ruined the aesthetics of the place and thus reduced the capital generation from tourism.

Picture: Oil Spill during the Gulf War, 1991

It had a long-lasting effect on the marine life of the area. The after-effects of this oil spill were even more terrific, as it even took months to clear the affected area and bring it back to its normal condition. Due to such destruction caused by this oil spill, it became a burning issue and a matter of grave concern for almost all the countries around the globe. On the basis of outcome and reasons for these incidents, maritime authorities are becoming more careful in handling oil with the latest technical advancements.

Bioremediation is any process that uses decomposers and green plants, or their enzymes, to improve the condition of contaminated environments. Bacteria can be used to clean up oil spills in the ocean through bioremediation. Bioremediation can also be used in other water systems like rivers, streams, and estuaries. Few examples of such microorganisms or biological agents to break down or remove oil are *Alcanivorax* or *Methylocella silvestris*.

Since the contaminants of concern in crude oil are readily biodegradable under appropriate conditions, the success of oil- spill bioremediation depends on our ability to establish those conditions in the contaminated environment. Bioremediation relies on stimulating the growth of certain microbes that utilize contaminants like oil, solvents, and pesticides for sources of food and energy. These microbes convert contaminants into small amounts of water, as well as harmless gases like carbon dioxide.

• Types of Bioremediation

There are two different types of bioremediation, *in situ and ex situ.*

• In situ Bioremediation

In situ bioremediation involves the treatment of the contamination on site. In the case of soil contamination, in situ bioremediation involves the addition of mineral nutrients. These nutrients increase the degradation ability of the microorganisms that are already present in the soil. Sometimes new microorganisms are added to the contaminated area. In this case, microorganisms can sometimes be genetically engineered to degrade specific contaminants keeping in mind about the conditions at the site. An example of a microorganism that has been genetically engineered is *Pseudomonas fluorescens* (strain: *HK44*). The approach taken depends upon the relationship between the type of contamination and the type(s) of microorganisms already present at the contamination site. For example, if the microorganisms already present are appropriate to break down the type of contamination, cleanup crews may only need to "feed" these microorganisms by the addition of fertilizers, nutrients, oxygen, phosphorus, etc.

There are two frequently used methods of supplying oxygen to the microorganisms:

A. Bioventing - This consists of blowing air from the atmosphere into the contaminated soil. First, injection wells must be dug into the contaminated soil. How many wells, how close together they go, how deep they are dug all depends on the factors affecting the rate of degradation (type of contamination, type of soil, nutrient levels, concentration of contaminants, etc). Once all of the injection wells are dug, an air blower is used to control the supply of air that is given to the microorganisms. These injection wells can also be used to add nitrogen and phosphorus, maximizing the rate of degradation.

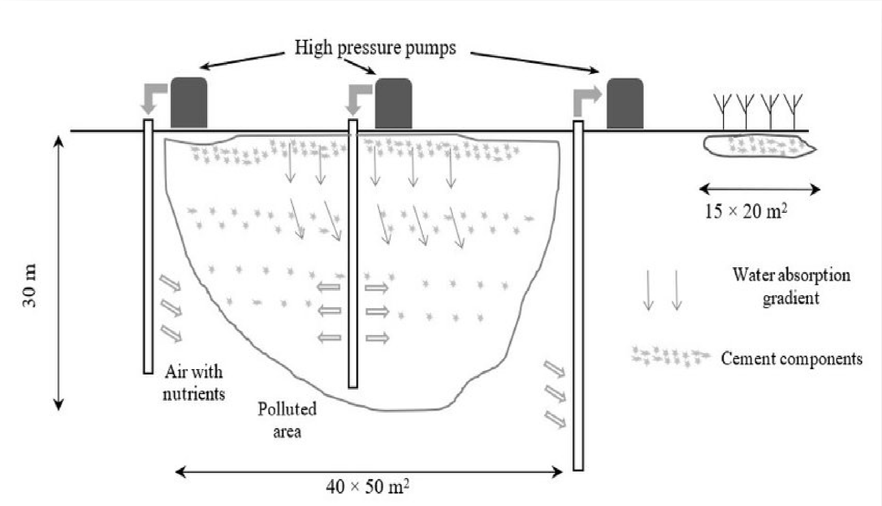

Figure 5: Mechanism of Bioventing

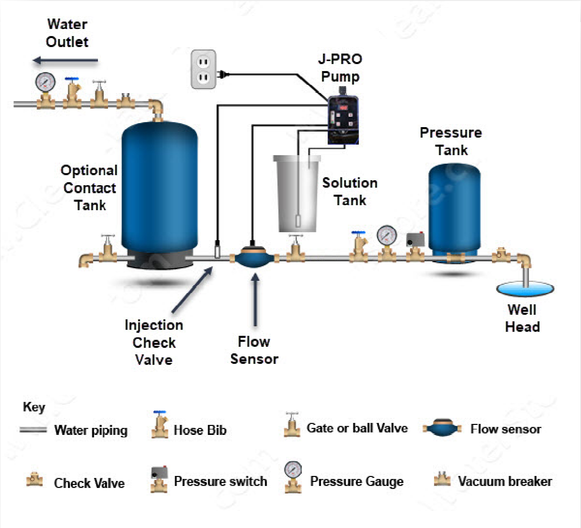

Figure 6: Mechanism of Hydrogen Peroxide Injection

A. Hydrogen Peroxide Injection - In cases in which the contamination has already reached the groundwater, bioventing will not be very successful. Instead, hydrogen peroxide is used. It functions much the same as bioventing, using the hydrogen peroxide instead of air blowers to deliver oxygen to the microorganisms. If the soil is shallow (the groundwater is fairly close to the surface) the hydrogen peroxide can be administered through sprinkler systems. If the groundwater is fairly deep beneath the surface, injection wells are used.

- **Ex Situ Bioremediation**

Ex situ bioremediation involves the physical extraction of the contaminated media to another location for treatment. If the contaminants are just in the soil, the contaminated soil is excavated and transported for treatment. If the contamination has reached the groundwater, it must be pumped and the surrounding contaminated soil must also be removed. One major thing that this removal of the contaminants does right away is to stop the spread of the contamination. Provided that the cleanup crew does a good job in the excavation process, there should ideally be no remaining contaminants, but it is also usually alright to have a minimal amount of contaminants remaining. If minimal contaminants do remain in the soil, they can likely be broken down by the naturally occurring microorganisms already present.

There are two main types of ex situ bioremediation. They are referred to as phases:

1. Solid Phase - Solid phase treatment consists of placing the excavated materials into an above ground enclosure. Inside this enclosure, the contaminated soil is spread onto a treatment bed. This treatment bed usually has some kind of built-in aeration system. Using this system,

cleanup crews are able to control the nutrients, moisture, heat, oxygen and pH. This allows them to maximize the efficiency of the bioremediation. The soil can also be tilled like farmland, helping to provide oxygen and enable additional aerobic biodegradation of the contamination. Solid phase treatment is especially effective if the contaminants are fuel hydrocarbons. However, it does require a lot of space and sometimes it cannot be used for that very reason. There are three solid phase bioremediation techniques. They are: landfarming, biopiling, and composting.

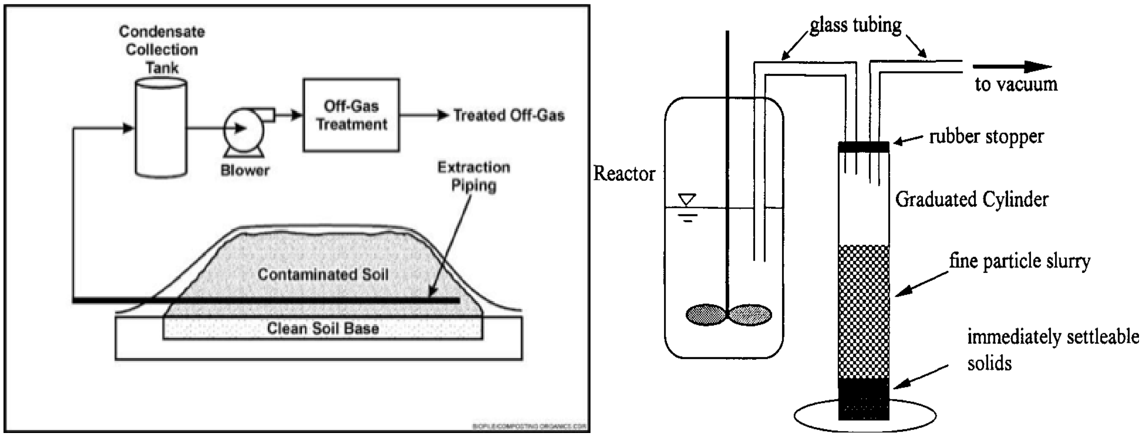

Figure 7 (a) Figure 7 (b)

Figures 7: (a) Mechanism of Solid Phase Bioremediation, (b) Mechanism of Slurry Phase Bioremediation

2. Slurry Phase - the contaminated soil is excavated and removed from the site as much as possible. The contaminants are then put into a large tank which is known as a bioreactor. Cleanup crews use this bioreactor to mix the contaminants and the microorganisms. This mixing process keeps the microorganisms in constant contact with the contaminants. Water, oxygen, and nutrients are added. Since the cleanup crews have complete control of the conditions in the bioreactor, they can adjust things until they achieve the optimal conditions for the degradation of

the contaminants. Since the degradation can be kept at or very close to optimal conditions, it does not take very long to break down the contaminants.

- **Comparison of the processes:**

- In fact, slurry phase bioremediation is much faster than many other bioremediation techniques.
- It is very useful in cases in which the contaminants need to be broken down very quickly.
- Another advantage to slurry phase bioremediation is the fact that it can be a permanent solution to the problem.

However, it is not always a permanent solution. Its success is highly dependent upon the chemical properties of the soil and contaminations. Slurry phase bioremediation definitely has some disadvantages.

- The rate of treatment is limited by the size of the bioreactor. That is, if a small bioreactor is being used, the rate of degradation will be very slow.
- Also, additional treatment of the wastewater is required. After the additional treatment, the waste water must then somehow be disposed of. These things add quite a bit of cost. They are part of the reason that slurry phase bioremediation has a high operating cost as well as a fairly high capital cost.

Bioremediation has been successfully used to clean up pollutants including crude oil, gasoline, pesticides, sewage, and chlorinated solvents used in cleaning supplies. The benefits of bioremediation include lower costs and less disruption of the contaminated environment when compared to other cleanup methods.