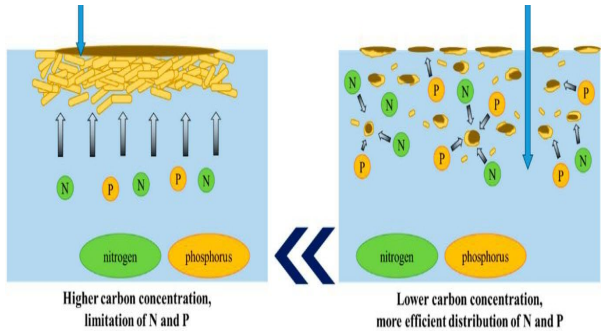
Mustansiriyah University
College of Science
Biology department \ Microbiology

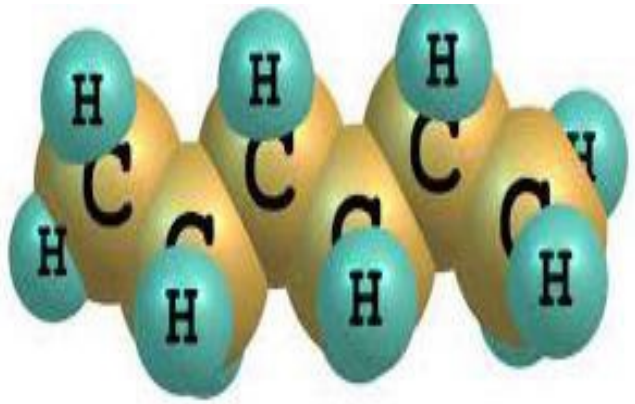
Bioremediation



Lecture Three

By

Dr. Zaid Shaker Naji



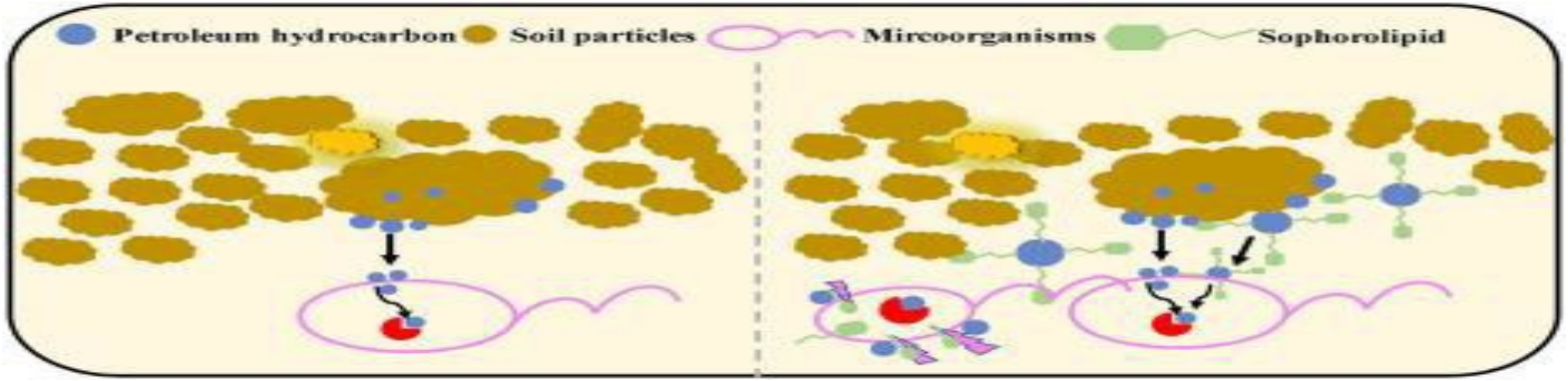
Microbial Degradation of Petroleum:

Petroleum-based products are the major source of energy for industry and daily life. Leaks and accidental spills occur regularly during the exploration, production, refining, transport, and storage of petroleum and petroleum products.

Petroleum hydrocarbons can be divided into four classes:

- The saturates
- The aromatics
- The asphaltenes (phenols, fatty acids, ketones, esters, and porphyrins)
- The resins (pyridines, quinolines, carbazoles, sulfoxides, and amides)

Release of hydrocarbons into the environment whether accidentally or due to human activities is a main cause of water and soil pollution. Soil contamination with hydrocarbons causes extensive damage of local system since accumulation of pollutants in animals and plant tissue may cause death or mutations



According to the Environmental Protection Agency (EPA), oil releases threaten public health and safety by contaminating drinking water, causing fire and explosion hazards, diminishing air and water quality, compromising agriculture, destroying recreational areas, wasting nonrenewable resources, and costing the economy millions of dollars. Microbial biodegradation of petroleum hydrocarbon pollutants employs the enzyme catalytic activities of microorganisms to enhance the rate of pollutants degradation. This article provides an overview about bioremediation for petroleum hydrocarbon pollutants.

One of the most efficient bacteria degrade crude oil is Acinetobacter baunannii

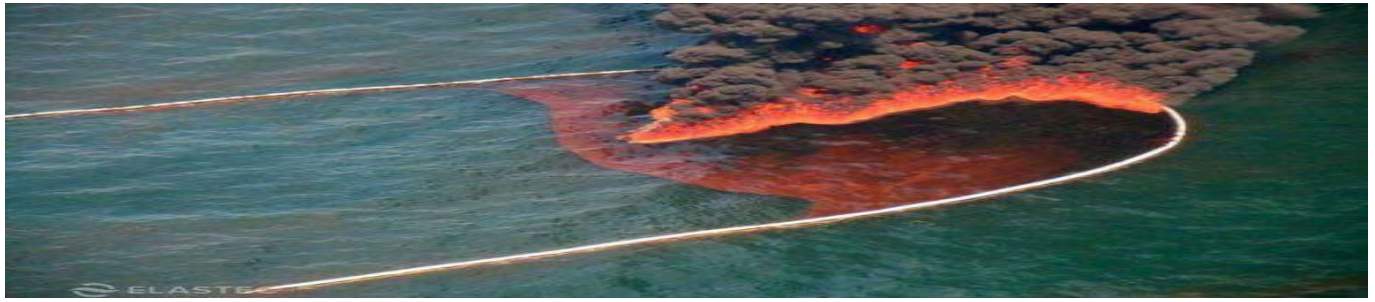
Acinetobacter is a genus of gram-negative bacteria belonging to the wider class of Gammaproteobacteria. Acinetobacter species are oxidase-negative, exhibit twitching motility, and occur in pairs under magnification power.

Causes of oil spills

Oil spills may originate in natural or anthropogenic causes:

1- Natural causes - such as oil that seeps from the bottom of oceans which enters the marine environment. Crude oil is formed during long periods of time through natural processes involving organic matter from dead organisms. Thus, oil exists in many environments and may be naturally spilled due to various factors (including climatic conditions, disturbance, etc.). Such natural oil spills may occur in oceans, due to eroding of sedimentary rocks from the bottom of the ocean (the effect may be similar with that of an accidental oil spill from human drilling in oceans such as the recent BP (British Petroleum) oil spill from the Gulf of Mexico).

2- Anthropogenic causes - including accidental oil spills (such as the recent BP oil spill in the Gulf of Mexico) as well as leaks and spills due to a large variety of human activities related to oil refining, handling and transport, storage and use of crude oil and any of its distilled products.



Accidental spills may occur in various circumstances, most often during the following activities:

- **Storage** - oil and oil products may be stored in a variety of ways including underground and aboveground storage tanks such containers that might develop leaks over time.
- **Handling** - during transfer operations and various uses.
- **Transportation** - these could be **large oil spills** (up to million and hundreds of million gallons) on water or land through accidental rupture of big transporting vessels (e.g., tanker ships or tanker trucks) or **smaller oil spills**, through pipelines and other devices also happens and their impact is mainly due to a large number of usually minor spills.

3-Intentional Oil Discharges

Intentional oil discharges are not necessarily done by purpose as most of them occur in the following circumstances:

- **Through drains or in the sewer system**. This include any regular activities such as changing car oil if the replaced oil is simply discharged into a drain or sewer system
- **Indirectly through the burning of fuels**, including vehicle emissions; they release various individual components of oils and oil products, such as a variety of hydrocarbons (out of which benzene and PAHs could pose serious health risks) Polycyclic Aromatic Hydrocarbons.

•**Offshore drilling** we have recently experienced the massive oil spill in the Gulf of Mexico, with its terrible consequences on the environment, marine life and humans as the spill continues since April 22, 2010, and it may take a while until a solution is implemented

•**Routine maintenance activities** - such as cleaning of ships may release oil into navigable waters This may seem insignificant; however, due to the large number of ships even a few gallons spilled per ship maintenance could build up to a substantial number when all ships are considered

•**Road runoff** - oily road runoff adds up especially on crowded roads. With many precipitation events, the original small amounts of oil from regular traffic would get moved around and may build up in our environment.

In order to get rid of oil spill we need to focus on biodegradation by natural populations of microorganisms represents one of the primary mechanisms by which petroleum and other hydrocarbon pollutants can be removed from the environment and is cheaper than other remediation technologies.

The success of oil spill bioremediation depends on

1) Ability to establish and maintain conditions that favor enhanced oil biodegradation rates in the contaminated environment .
2) Presence of microorganisms with the appropriate metabolic capabilities.
3) Sufficient concentrations of nutrients and oxygen are present.
4) The physical and chemical characteristics of the oil and oil surface area are also important to determinants the bioremediation success.

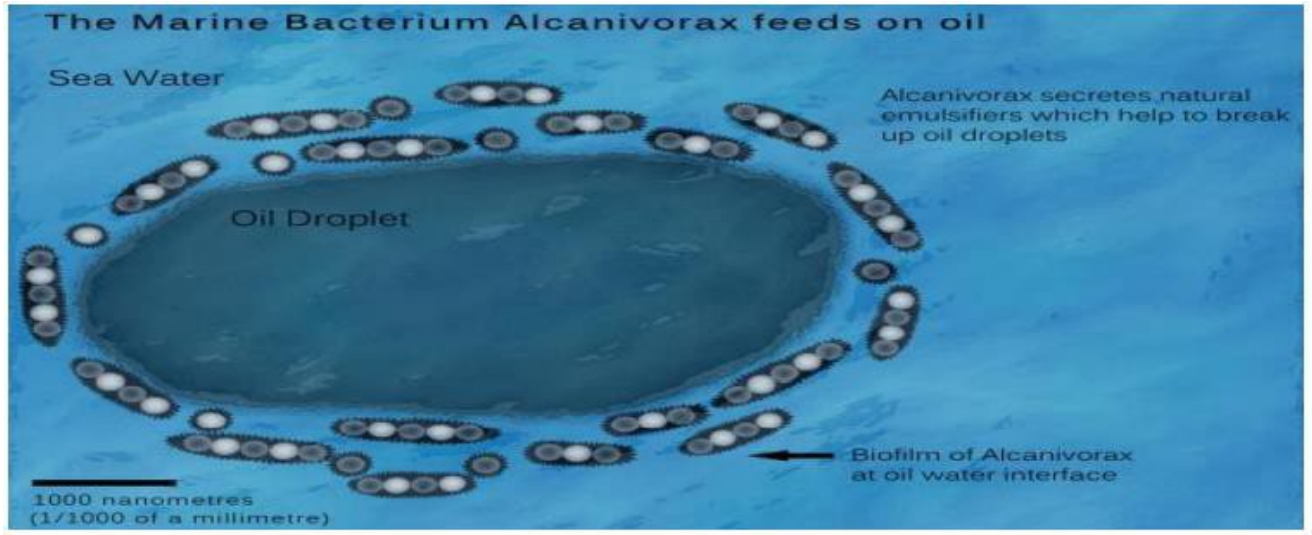
There are two main methods to treat oil spills through bioremediation:

(a) bioaugmentation, in which known oil degrading bacteria are added to supplement the existing microbial population.
(b) biostimulation, in which the growth of indigenous oil degraders is stimulated by the addition of nutrients or other growth-limiting factors.

The susceptibility of hydrocarbons to microbial degradation can be generally ordered as follows: linear alkanes > branched alkanes > small aromatics > cyclic alkanes Hydrocarbons in the environment are biodegraded primarily by bacteria, yeast, and fungi. The efficiency of biodegradation ranged from 6% to 82% for soil fungi, 0.13% to 50% for soil bacteria, and 0.003% to 100% for marine bacteria.

Bacteria are the most active agents in petroleum degradation, and they work as primary degraders of spilled oil in environment. Several bacteria are even known to feed exclusively on hydrocarbons *Pseudomonas fluorescens, P. aeruginosa, Bacillus subtilis, Bacillus sp., Alcaligenes sp., Acinetobacter lwoffi, Flavobacterium sp., Micrococcus roseus, and Corynebacterium sp.* were isolated from the polluted stream which could degrade crude oil.

Acinetobacter sp. was found to be capable of utilizing n-alkanes of chain length C10–C40 as a sole source of carbon. Also Alcanivorax borkumensis used for cleaning oil was is a marine bacteria can absorb and digest linear and branched alkanes. It is a gram negative bacteria, has an outer membrane of lipopolysaccharides. It occurs naturally in unpolluted waters all over the world. Borkumensis creates enzymes both are alkane hydroxylases hydroxylation.

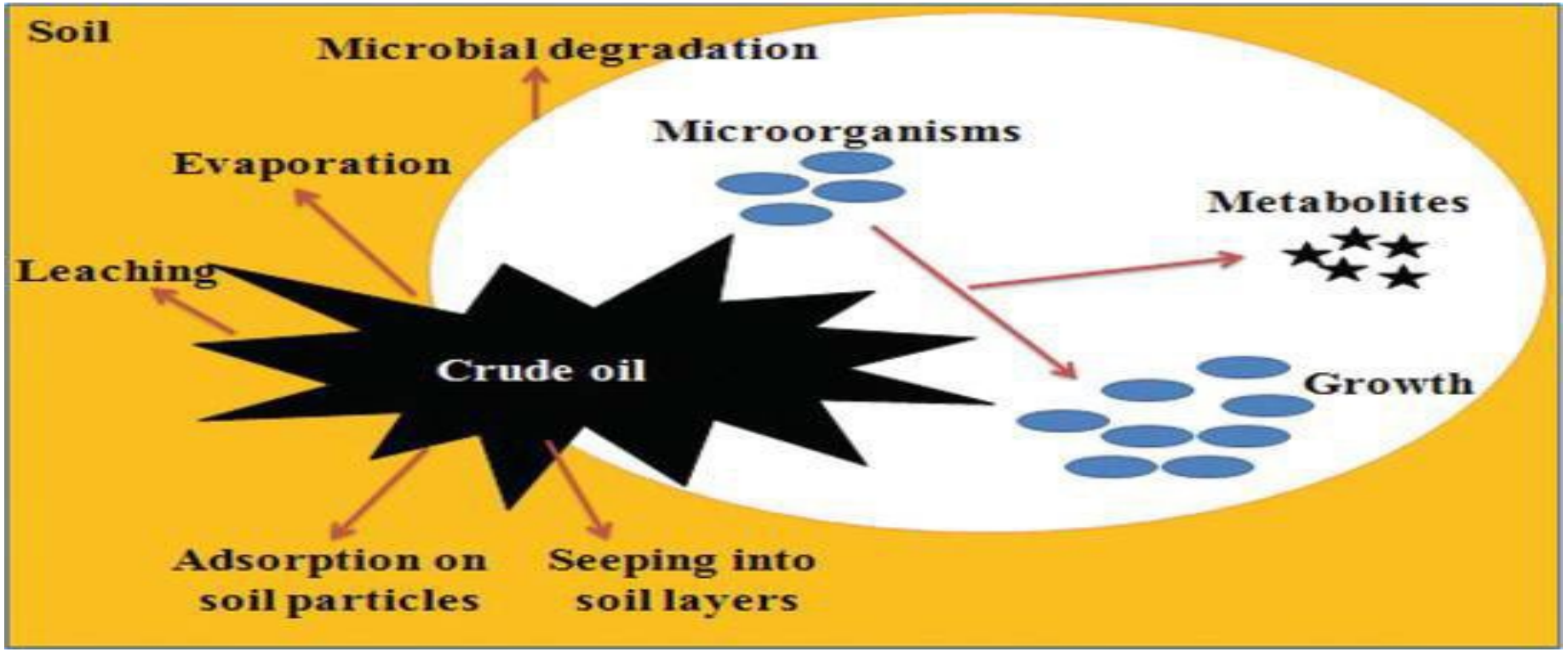


Fungal genera, namely, *Amorphoteca, Neosartorya*, and yeast genera, namely, Candida were isolated from petroleum contaminated soil and proved to be the possible organisms for hydrocarbon degradation. Also algae is important members of the microbial community in both aquatic and terrestrial ecosystems reported of capable utilizing crude oil and a mixed hydrocarbon substrate and exhibited extensive degradation of n-alkanes and iso-alkanes as well as aromatic hydrocarbons. Protozoa, by contrast, had not been shown to utilize hydrocarbons.

Factors Influencing petroleum hydrocarbon degradation

1) Temperature: plays an important role in biodegradation of hydrocarbons by directly affecting the chemistry of the pollutants as well as affecting the physiology and diversity of the microbial flora. Low temperature increased the viscosity of the oil, while the volatility of the toxic low molecular weight hydrocarbons were reduced, delaying the start of biodegradation. Temperature also affects the solubility of hydrocarbons. Although hydrocarbon biodegradation can occur over a wide range of temperatures, the rate of biodegradation generally decreases with the decreasing temperature, highest degradation rates that generally occur in the range 30–40◦C in soil environments, 20–30◦C in some freshwater environments and 15–20◦C in marine environments.

2) Nutrients : are very important components for successful biodegradation of hydrocarbon pollutants especially nitrogen, phosphorus, and in some cases iron. Some of these nutrients could become limiting factor thus affecting the biodegradation processes. So additions of nutrients were necessary to enhance the biodegradation of oil pollutant. On the other hand, excessive nutrient concentrations can also inhibit the biodegradation activity. Many scientist reported the negative effects of high NPK {nitrogen (N), phosphorus (P) and potassium (K)} levels on the biodegradation of hydrocarbons especially on aromatics.



Mechanism of petroleum hydrocarbon degradation by microorganisms

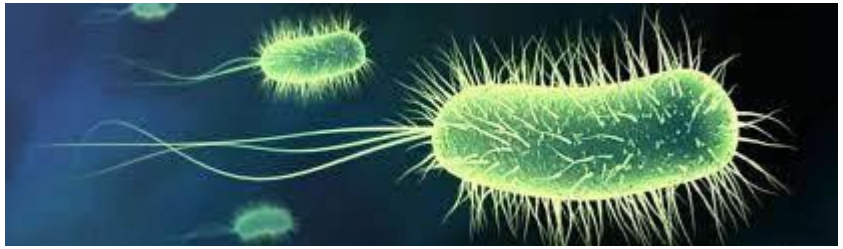
1The degradation of petroleum hydrocarbons can be mediated by specific enzyme system for example oxygenase.

2Attachment of microbial cells to the substrates: Immobilized cells have been used for the bioremediation of numerous toxic chemicals. Immobilization of bacterial cells enhanced the biodegradation rate of crude oil compared to free living cells. Immobilization can be done in batch mode as well as continuous mode. Packed bed reactors are commonly used in continuous mode to degrade hydrocarbons.

3Production of biosurfactants which are heterogeneous group of surface active chemical compounds produced by a wide variety of microorganisms ex: Pseudomonads sp.

4 Phytoremediation for the treatment of petroleum hydrocarbon contamination.

5Genetically modified bacteria: Applications for genetically engineered microorganisms (GEMs) in bioremediation have received a great deal of attention to improve the degradation of hazardous wastes under laboratory conditions. As a result the microbial degradation can be considered as a key component in the cleanup strategy for petroleum hydrocarbon remediation.



The Lecture is over

Thank you