REVIEW ON BIOREMEDIATION OF OIL SPILLS USING MICROBIAL APPROACH

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ABSTRACT

This review paper is designed to overview the role of microbes in bioremediation of oil spills. The type of bioremediation that uses fungi, for removing or degrading pollutants from the soil and water surfaces with their metabolic power it is called mycoremediation. *Penicillium chrysogenum* is used in mycoremediation and other fungi are found to oxidize aromatics hydrocarbons using mono-oxygenases, forming a trans-diol, and also has ability to degrade hydrocarbons, and other xenobiotics compounds e.g. heavy metals into non toxic form. *Penicilium chrysogenum* alone is found to degrade monocyclic aromatic hydrocarbons (benzene, toluene, ethylbenzene and xylene (BTEX). Despite of the abundance of fungi in wastes, *Penicillium* species, especially *Penicillium chrysogenum* receives little attention in bioremediation and biodegradation studies of hydrocarbons and other xenobiotics, e.g. heavy metals, even at low concentrations, can be toxic to humans and other forms of life. Accidental or intentional oil spills has a deep impact on the environmental pollution.Oil spills can have disastrous effects on the various environment, economy, and health of animals and plants. Global pollution is increasing due to the variations in natural and anthropogenic activities leading to contamination of various terrestrial and aquatic ecosystems with heavy metals, inorganic and organic compounds. Controlled and Uncontrolled discharge of solid and liquid wastes, use of agricultural fertilizers, herbicides, insecticides and pesticides sewage disposal and accidental spillage are some of the main contributors of alarmingly increased content of various contaminants in the biosphere .

Key words: Bioremediation, Biodegradation, Mycoremediation, *Penicillium chrysogenum*, Oil spills

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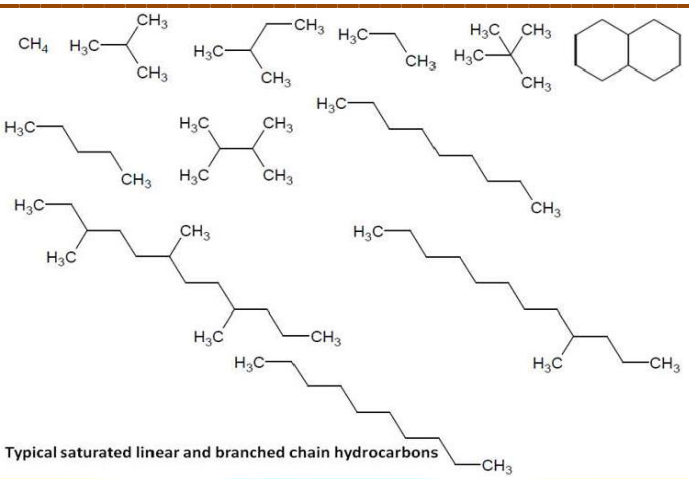
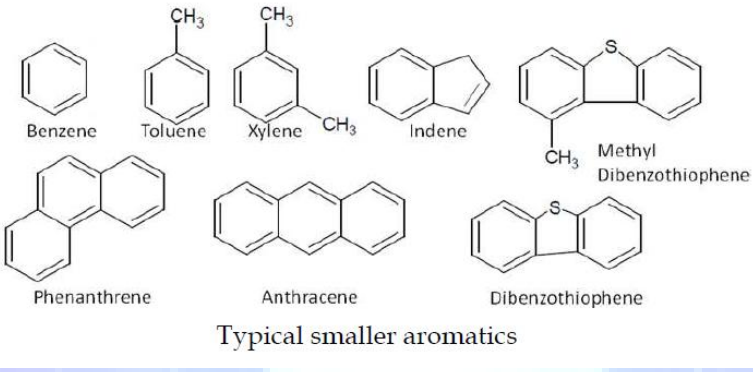
INTRODUCTION

Global pollution is increasing,due to the variations in natural and anthropogenic activities leading to contamination of various terrestrial and aquatic ecosystems with heavy metals,inorganic and organic compounds. Controlled and uncontrolled discharge of solid and liquid wastes, use of agricultural fertilizers, herbicides, insecticides and sewage disposal, explosives and accidental or intentional spillages, are some of the main contributors of alarmingly increased content of various contaminants in the biosphere. Industries such as textiles, electroplating, tannaries and refinaries are recognised as a serious environmental threat all over the world(Tariq *et al.*, 2005).

Accidental or intentional oil spills has a deep impact on the environmental pollution(Narayani, 2010). Oil spills from oil tankers and from distant oil spills,have been recognized as a major environmental hazard. The spilled oil is believed to destroy the habitat of seabirds, marine mammals and fish.The thick and gummy crude oil discharges can cause immediate harm to fish and wildlife, degrade oceans and coastal habitats, and over time, even threaten human health(Agarwal, 2002). Oil spills cause severe damage to the ecosystems and pose threats of; Fire, ground water pollution due to percolation and air pollution due to evaporation(Singh, 2007**).**

Environmental contamination by crude oil is relatively common because of its widespread use and its associated disposal operations and accidental spills. The term petroleum is referred to an extremely complex mixture of a wide variety of low and high molecular weight hydrocarbons. This complex mixture contains saturated alkanes, branched alkanes, alkenes, napthenes (homo-cyclic and hetero-cyclic), aromatics (including aromatics containing hetero atoms like sulfur, oxygen, nitrogen, and other heavy metal complexes), naptheno-aromatics, large aromatic molecules like resins, asphaltenes, and hydrocarbon containing different functional groups like carboxylic acids, ethers, etc. Crude oil also contains heavy metals and much of the heavy metal content of crude oil is associated with pyrrolic structures known as porphyries.





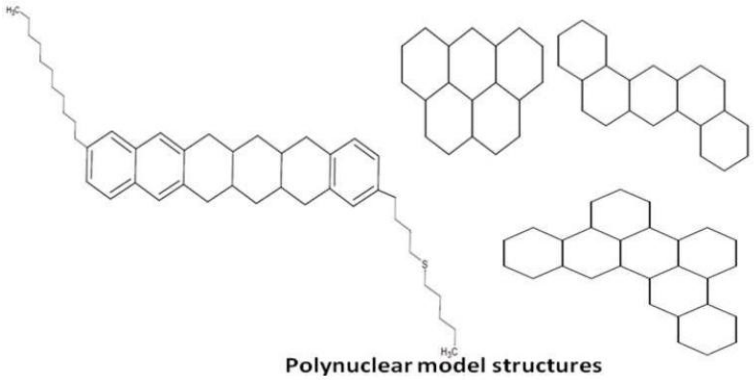


Fig.1: Some of the representative molecules in the light and heavy fractions found in Petroleum.

Living matter is exposed to petroleum in many ways directly or indirectly. Some by-products, formed during petroleum refining and processing which are used for the manufacturing of other

products are highly toxic. Constantly, these toxic compounds are inadvertently released into the environment and if this effect is connected to the effect of accidental crude oil spills Worldwide, and then these combined sources of unrestricted hydrocarbons constitute the major cause of environmental pollution. The toxicity of the hydrocarbon molecules and their availability for microbial metabolism depend on their chemical and physical nature. Petroleum is toxic and can be lethal depending upon the nature of the petroleum fraction, the way of exposure to it, and the time of exposure. Chemicals and dispersants in crude oil can cause a wide range of health effects in people and wildlife, depending on the level of exposure and susceptibility. The highly toxic chemicals contained in crude oil can damage any organ system in the human body like the nervous system, respiratory system, circulatory system, immune system, reproductive system, sensory system, endocrine system, liver, kidney, etc. and consequently can cause a wide range of diseases and disorders. Individuals more susceptible to harm by the toxic effects of crude oil are as follows;

- Infants, children, and unborn babies.
- Pregnant women.
- People with pre-existing serious health problems.
- People living in conditions that impose health stress.

The damage caused by the toxicity of crude oil to organ systems may be immediate or it may take months or years. In addition, oil refineries generate huge quantities of oily sludge, a hydrocarbon waste. The US Environmental Protection Agency and the Exxon Company used microorganisms (by a process called Bioremediation) to clean up Alaskan beaches contaminated by the Valdez oil spill (EPA, 2006; Singh, 2007).

There are many different ways that oil spills can be cleaned. The methods chosen to clean up an oil spill are determined based upon the type of oil spilled, the location and its proximity to sensitive environments, and other environmental factors (EPA, 2006). There are mechanical, chemical, and biological methods. Mechanical methods include booms (see Figure 4 and 5 below), skimmers, and truck vacuums. Chemical methods include dispersants, surface washing agents, and surface collecting agents. Biological methods are the use of microbiological cultures, enzyme additives, and nutrient additives to increase the rate of biodegradation of the contaminants (EPA, 2006). In India, a consortium of bacterial species has been developed to

convert oil spills and oily sludge, the inoculant is aptly called Oil zapper. Inoculation with oil zapper reduced oily sludge contamination in soil to merely 0.5% in 360days from the initial 13.41%.In contrast; it declined to only 11.35% in the uncontaminated land. Oil zapper has been effective in relatively large-scale field trials as well (Singh, 2007).

Oil spills:

An oil spill is a leakage from oceangoing tankers, pipelines or other oil sources. It occurs very frequently and causes enormous ecological harm (Medha, 2013).

Oil spills in the oceans

The spilling of oil in the oceans destroys not only the earth's ecosystem but also an extremely negative impact or living organisms. The toxic substances, in fact, threaten the mere existence of marine life.

Most of us know about oil rigs and how oil is extracted from the sea bed. This same oil is used for various purposes - from transportation, construction, to processes in chemical industries. Unfortunately, oil spilled by tankers during loading/unloading, discharging, ballasting, tank cleaning, or near offshore platforms, drilling rigs and wells, are the foremost causes responsible for ocean pollution. Fundamentally, an oil spill is the release of liquid petroleum hydrocarbon into the environment on account of human activity.

Pollution of Ocean water also depends upon what kind of oil was accidentally or deliberately dumped into the ocean. Oil can be of any type - crude oil, by-products of petroleum, or refined petroleum products like gasoline or diesel fuel, oil mixed in waste, or oily refuse. If light oil like diesel gets spilled, then this oil does not stay in the environment for a long time. It gets evaporated very easily, though it is toxic and highly inflammable (Medha, 2013).

World worst oil spills:

Persian Gulf, Kuwait: Between 400 - 450 million gallons of oil was spilled into the ocean deliberately during the Gulf War, in the year 1991. It is considered as the world's worst and the only planned oil spill ever.

Gulf of Mexico: In the year 2010, the Deepwater Horizon oil spill, also called the BP oil disaster, was considered as the world's largest offshore oil spill ever, which spilled approximately 4.9 million barrels of oil.

Bay of Campeche, Mexico: In 1979, around 140 million gallons of oil was blown out, when an oil well was drilled. Sparks ignited an accidental explosion, and it took nearly a year to stop the continuous oil leak.

Off the coast of Trinidad and Tobago, West Indies: In the year 1979,around 90 million gallons of oil leaked from the Atlantic Empress tanker, that collided with the Aegean captain, as it was trapped in a tropical storm.

Kolva River, Russia: A poorly maintained pipeline was leaking for eight months. In 1979,a barrier was constructed to contain oil, until sudden cold storms caused the barrier to collapse, which in turn lead to a massive oil leak.

Arabian Gulf, Iran: In the year 1983, around 80 million gallons of oil was spilled after a tanker collided with an oil platform. The leak was not covered quickly because of the ongoing war between Iran and Iraq.

Saldanha Bay, South Africa: This oil spill occurred in the year 1983, after the oil tanker Castillo de Bellver caught fire and broke into two parts, spilling around 79 million gallons of oil.

Ports all, France: In 1978, due to a severe winter storm, crude carrier Amoco Cadiz broke into half after the rudder got damaged, and spilled around 69 million gallons of oil into the English Channel.

Off the coast of Angola: In 1991, between 50-80 million gallons of oil was discharged into the ocean, after oil tanked exploded. This formed a large slick, which kept burning for three days, before the tanker sank completely.

Genoa, Italy: In the year 1991, the tanker M/T Haven was put back into operation, even after being in poor state of repair and hit by a missile during the Iran-Iraq war. This tanker sank into the Mediterranean Sea and leaked oil for nearly twelve year (Medha, 2013).

Figure 6-shows some of the ocean's oil spills

Figure 2: Some of Ocean oil spills

Bioremediation for oil spills:

Bioremediation for oil spills is a technique that eliminates contamination of hydrocarbons from water and soil. Oil spillage takes place mostly from ships, posing hazards to the aquatic life to a large extent. Oceans get polluted with harmful chemicals due to seepage of oil (including petrol, diesel and other types of hydrocarbons) from ship wrecks, mishandling and accidents. The contaminated water when comes in contact with the soil, further pollutes it. The process of removing the toxic compounds from oceans and soil is quite tedious and expensive at the same time. Bioremediation is one such effective method of cleaning oils from soil and water, thereby making them safe for aquatic and terrestrial species (Saptakee, 2011).

Bioremediation methods for oil spills:

Different techniques are applied either in situ or ex situ for eliminating toxic substances from the soil. The application of techniques depends on the nature and the intensity of the pollution. The microbes breakdown the chemicals with the help of the enzymes secreted by them. Thus, the soil or water becomes clear when the chemicals are taken up by them (Saptakee, 2011).

Using bacterial species:

Pseudomonas species are potent bacteria that are capable of degrading hydrocarbons from petrol and diesel, thereby reducing the impact of oil spills. *P. alcaligenes* is capable of breaking down polycyclic aromatic hydrocarbons while *P. mendocina* and *P. putida* can remove toluene. *P. veronii* can degrade large number of aromatic organic compounds. These oil based compounds are eaten up by the bacteria as they utilize them as substrates for carrying out metabolism. These microorganisms occur in abundance in water bodies and soil and are effective in cleansing oil spills. With an increase in density of these microorganisms, the process of bioremediation is also accentuated. Other Bacteria that help in bioremediation are *Achromobacter, Flavobacterium, Acinetobacter,* etc. (Saptakee, 2011).

Using fungal species:

Penicillium species are commonly found in food, indoor air, and soils. Particularly, *Penicillium chrysogenum* has been found on dried cereals, salted meat, and many other low water activity foods, but is also common in indoor air environments and salty soils and water (marine water). Strains of the genus *Penicillium* have been reported as good hydrocarbon-assimilating and there are many reports showing their ability to transform xenobiotics compounds like phenol into less mutagenic products. Phenol is a typical contaminant of the environment generated by many industries (Erika *et al*., 2011).

Penicillium chrysogenum strain has the ability to degrade phenol compounds, heavy metals (e.g. lead, nickel and iron), and monocyclic aromatic hydrocarbons (benzene, toluene, ethyl benzene and xylene) (Pedro *et al.*, 2014).

BTEX are the low molecular weight aromatics and most soluble oil components, volatile aromatics compounds, most toxic in crude and refined oils, and are known as carcinogen. Although many of them may be removed through evaporation (i.e. by natural method).They makes up 20-50% of most crude oils, about 75% of fuel oil and about 100% of gasoline and kerosene. Mono aromatic hydrocarbons are toxic to some microorganisms due to their solvent action on cell membranes, but in low concentrations they are easily biodegradable under aerobic conditions.

Penicillium chrysogenum and other fungi normally oxidize aromatics hydrocarbons using mono-oxygenases, forming a trans-diol. The low molecular weight aromatics are more soluble at a

higher temperature, the highest degradation rates generally occur in the range of 30-40^{0}c in soil environment, 20-30^{0}c in some freshwater environments and 15-20^{0}c in marine environment.

Impacts of oil spills:

Oil spills can have disastrous effects on the marine and coastal environment. The following provides information about the various ill effects of oil spills on various environments, on the economy, and health of animals and plants (Narayani, 2010).

Environmental Impact:

An oil spill, be it intentional or accidental, has a deep impact on environment. During an oil spill in the ocean, the viscous liquid spreads on the sea surface, forming a cohesive oil slick. The volatile organic compounds evaporate and about 20-40% of the mass is lost in this process. A very small percentage may also dissolve in water. What remains behind is oil residue, which over a period of time, forms a thick mousse on the water surface. Now some of the oil residue may congeal into sticky tar balls, which can be found floating in the water. And some of the oil waste disintegrates in sunlight (photolysis) and decomposes (biodegradation) into the sea waters. This oil, when reaches the coastlines, further interacts with the beach sand, rocks, boulders, vegetation, and the terrestrial habitat of animals and plants, which is no less than contamination of the living habitat. One of the prime impacts of oil spills is toxic acid rains and erratic climate change, caused by oil vapours in the atmosphere (Narayani, 2010).

Economic Impact:

Apart from the environment, economy of a country also experiences a severe setback. Losing tons of oil, which is not only a natural source of energy but also a depleting source, has a severe impact on a country's economy. Besides this, the tourism industry also gets affected. This is because, after the spill, many recreational activities like scuba diving, boating, snorkelling, and fishing are prohibited until clean up is complete. The fisheries bear the maximum brunt of these oil spills. Fish and other sea food sources are contaminated, which pose a health hazard. Also, fishing areas remain closed until the clean up, as many of the fishing boats and equipment get damaged due to the oil slick. Also operations of industries like power plants, desalination plants, and nuclear central stations are affected badly, as they use sea water for many of their commercial activities (Narayani, 2010).

Health Impact:

Oil, i.e. crude oil, contains dangerous chemical compounds like benzene, propylene glycol, polypropylene glycol butyl ether, and hydro-treated light petroleum distillates. And exposure to these compounds may be harmful to health. It has been observed that people working in such polluted areas experience severe headaches, back pain, dizziness, dermatitis, watery eyes, and strep throat. Also, the toxic compounds in oil damage the red blood cells in animals and cause severe respiratory problems. These compounds also have factored genetic toxicity, which is even more dangerous. It is not just the air, but also the contaminated sea food which is a tangible reason for many cases of food poisoning near beaches where such accidents have occurred. In some cases, it has also been observed that the mental health of people living in these area gets severely affected as an aftermath of an oil spill. So, be it plant, animal, or human life, ill effects of an oil spill are lethal in nature. The most susceptible victims are aquatic life, vegetation by coastlines, and cleaners who clear up the debris. Such man-made disasters that aggravate environmental pollution can surely be averted to prevent such cataclysmal consequences. (Narayani, 2010).

Impact on Marine life:

Depending on the type of oil, it can stick to the body of the fish and marine animals, forming a thick layer. Here are a few pointers which discuss the same into detail;

- Damage to the feathers of birds, destroying their beneficial properties.
- Fur seals get affected by oil spills, as the oil sticks to their fur. Whales and dolphins remain safe from any external affects, as their slippery body does not allow the oil to stick.
- Drowning of marine animals if the oil sticks on the flipper is observed.
- Ulcers and other internal problems in the body, leading to diseases and death.
- Loss of appetite and weight loss problems in birds, wildlife, and marine life.
- Problems of the digestive system, leading to fragile body.
- A sense of alienation among the community members, as the smelling ability of the seals and other marine animals is reduced, which makes them feel lost as they are unable to detect their fellow beings.
- Reduces the bird's ability to fly, as the feathers get entangled in a dense mass of thick layers of oil (Kundan, 2009).

Impact on birds:

As far as aquatic birds are concerned; the oil seeps down and disturbs its structure, feathers, and the insulating capacity. This makes them more vulnerable to temperature changes, and affects their capability to float in water. In addition to this, the flight of these birds is hindered, making them helpless when it comes to escaping from predators. Further, when they clean themselves or preen, they tend to take in the oil covering their feathers completely with it. As a result, they experience altered liver functioning, kidney damage, and irritation of the digestive tract. These problems, in tandem with restricted foraging capacity, leads to dehydration and metabolic imbalances. Ultimately, if there is no human intervention, these birds die (Medha, 2013). See figure 7 below.

Impact on aquatic fauna and flora:

The dangers of oil spills are mostly observed with regards to smaller marine organisms, which dwell at the bottom of the ocean. Larval fish, plankton, seaweed, mussels, oysters, dolphins, turtles, algae, and fish, are all considerably affected by it. Marine plants too are affected badly, as the layer of oil on the top creates a hindrance for sunlight to pass through, which then does not let the process of photosynthesis take place. One of the most disturbing facts is that, this decreases the amount of flora and fauna, and disrupts the entire marine food chain.

- **Sea Otters:**

  When the fur of otters gets covered in oil, it obstructs them from creating air pockets or air bubbles. These bubbles not only act as insulators and help them survive the cold weather, but also protect and help them to float. When oil builds-up on their dense fur, they are unable to adjust or maintain the necessary air pockets or protect themselves from the change, which, in turn, leads to their death. Sometimes, these otters are taken out and placed under observation, to treat them from the oil, disease, or broken bones (Medha, 2013).

- **Whales:**

  There is the possibility that the oil that has been spilled is consumed by whales either directly or indirectly. Either ways, it reaches their blowhole, which helps them breathe. Consuming oil blocks the blowhole and restricts them from breathing, which, in turn, leads to their death. In many cases, it also happens that whale's gobble up small fish that

have swum through the oil before becoming their food. The oil reaches the whale's system, poisons it, and eventually leads to its death.

Fig 3: Effects of oil spills on some organisms.

Advantages of bioremediation:

Bioremediation has many advantages over traditional cleanup methods of marine oil spills;

- One of the major advantages of bioremediation is the savings in cost and also the savings in the time put forth by workers to clean a contaminated site. The financial savings of bioremediation, when used properly, have tremendous benefits compared to traditional cleanup processes. After the Exxon Valdez spill, the cost to clean 120 km of shoreline by bioremediation was less than cost to provide physical washing of the shore for one day Another way that bioremediation allows for savings, is that unlike traditional methods, bioremediation continues to clean the contaminated site without the constant need of workers. This saves a great deal of money which would be spent on labour hours, and it also allows for time to be spent performing further research on bioremediation.
- Bioremediation is also advantageous due to its environmentally friendly approach. Unlike chemical methods, no foreign or toxic chemicals are added to the site.
- It is also environmentally friendly because it does not require any disruption to the natural habitat which often occurs from physical and chemical methods of cleanup.

- Bioremediation allows for natural organisms to degrade the toxic hydrocarbons into simple compounds which pose no threat to the environment, and this also eliminates the need to remove and transport the toxic compounds to another site. This loss of a need to transport the oil and contaminated soils lowers further risk of additional oil spills, and also saves energy and money which would be put forth in the transportation process. These environmental benefits also make bioremediation a positively viewed method by the general public. With the limited resources in today's world, this is a very much supported technology, which pleases the public and hence is given political support and funding for further research (EPA, 2006).

Disadvantages of bioremediation:

One of the greater downsides of bioremediation for marine oil spills is that it is a slow process.

- Oil spills can pose a great threat to many different habitats, environments, and industries, and depending upon the urgency of cleanup, bioremediation may not always be the best available option.
- Also there are many variables that affect whether bioremediation is capable and practical for the cleanup of different oil spills depending on where the spill takes place and the conditions of the water there, it may be very difficult to provide proper nutrient concentrations to the oil degrading microorganisms.
- If an oil spill occurs offshore, there is typically much more energy and waves, and this can cause for the quick loss and dilution of nutrients provided by bio stimulation.
- In the case of bio augmentation, there are problems which occur, particularly the competition that will develop between the native and foreign microbes, making this an unsuccessful method of bioremediation.
- Another disadvantage of bioremediation is that it is a very difficult process to conduct field tests on. This is due to the many factors and conditions which cannot be controlled in the field, but only in laboratory tests (EPA, 2006).

CONCLUSION

Microorganisms, most especially fungi and bacteria have the capacity of bioremediating and biodegradating of hydrocarbons which are the major constituents of petroleum oil and other xenobiotics, e.g. heavy metals, which even at low concentrations, can be toxic to humans and other forms of life. Bioremediation is also advantageous due to its environmentally friendly approach, time and cost saving. Unlike chemical methods, no foreign or toxic chemicals are added to the site.

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