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Review on Comparative Study on Bioremediation for Oil Spills Using Microbes.

Safiyanu I[1]*, Abdulwahid Isah A[2], Abubakar US[3], and Rita Singh M[4].

[1]Department of Biotechnology, Sharda University, Greater Noida, UP, INDIA.
[2]Department of Biotecghnology, Sharda University, Greater Noida, UP, INDIA.
[3]Bioresources Development Centre, Kano. National Biotechnology Development Agency (NABDA), Abuja, Nigeria.
[4]Department of Biotechnology, Sharda University, Greater Noida, UP, INDIA.

ABSTRACT

This review paper is designed to overview and compares the role of microbes in bioremediation for 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. Bioremediation for oil spills is a technique that uses microbes to eliminate contamination of hydrocarbons from water and soil, thereby making them safe for aquatic and terrestrial species. Bioremediation can be used by bacterial species, fungal species (a process called mycoremediation) and plant species (by a process called phytoremediation). In this review paper, we only considered and compared the activities of a bacterial species(Pseudomonas putida) and fungal species(Penicillium chrysogenum), where the Pseudomonas putida is found to have the dominant role in marine ecosystems and the Penicillium chrysogenum becoming more important in freshwater and terrestrial environments. There are three methods to clean up oil spills; physical, chemical and biological (bioremediation), but Bioremediation is advantageous due to its time and cost saving than physical method, also unlike chemical method, no foreign or toxic chemicals are added to the site.

Keywords: Oil Spills, Hydrocarbons, Bioremediation, Pseudomonas putida and Penicillium chrysogenum.

Corresponding author

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 [1,2].

Accidental or intentional oil spills has a deep impact on the environmental pollution.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 [1, 3, 4].

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 sulphur, 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 [1, 5].

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 [1, 5-7].

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 contaminate by the Valdez oil spill [1, 5].

There are mechanical, chemical, and biological methods. Mechanical methods include booms, skimmers, and truck vacuums. Chemical methods include dispersants, surface washing agents, and surface collecting agents. Biological methods (Bioremediation) are the use of microbiological cultures, enzyme additives, and nutrient additives to increase the rate of biodegradation of the contaminants [1, 5, 6].

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 [1, 8].

Hydrocarbons in the environment are primarily biodegraded by bacteria and fungi. The reported 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. Many scientists reported that mixed populations with overall broad enzymatic capacities are required to degrade complex mixtures of hydrocarbons such as crude oil in soil, fresh water and marine environments. Bacteria are the most active agents in petroleum degradation, and they work as primary degraders of spilled oil in environment [6].

Bioremediation can be used by bacterial species, plant species (a process called *phytoremediation)* and fungal species (a process called *mycoremediation*) [1, 9, 10].

Bioremediation using bacterial species can include using *Pseudomonas species* which 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* [1, 6, 9, 10].

Pseudomonas putida has the ability to degrade and remove toluene and other monocyclic aromatic hydrocarbons, e.g. benzene, toluene and xylene. The bacterial degradation of aromatic hydrocarbons, normally involves the formation of a diol followed by cleavage of aromatic ring and formation of diacid such as *cis-cis* muconic acid [9, 10].

Mycoremediation is the process of degrading or removing toxicants from the environment using fungi. Fungi are important decomposers in the natural environment. They create enzymes to degrade the plant polymers cellulose and lignin, two very durable compounds that give plants their structure. Using similar mechanisms, fungi can also break down certain toxic substances [1, 11].

Mycoremediation has been applied to contaminated soil, oil spills, industrial chemicals, contaminated surface water, and farm waste. Some specific examples include:

• *Lentinus edodes* (shiitake mushroom) can degrade pentachlorophenol (PCP), a broad-spectrum biocide that is more toxic than DDT.

• *Pleurotus pulmonarius* (lung or Italian oyster mushroom) can degrade atrazine, a herbicide that is contaminating groundwater in many Midwestern US states.

• *Phanerochaete chrysosporium*, a white rot fungus, can degrade compounds such as biphenyl and triphenylmethane [11].

Penicillium species belongs to the Phylum *Ascomycota* in the kingdom *Fungi* and 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 [12].

Penicillium chrysogenum strain has the ability to degrade monocyclic aromatic hydro carbons (e.g. benzene, toluene, ethyl benzene and xylene; BTEX), phenol compounds and heavy metals (e.g. lead, nickel and iron).The *Penicillium chrysogenum* and other fungi normally oxidize aromatics hydrocarbons using mono-oxygenases, forming a *trans-diol* [13, 14].

Petroleum

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 sulphur, 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 [1, 5, 6].

Environmental Contamination by Petroleum (crude oil)

Environmental contamination by crude oil is relatively common because of its widespread use and its associated disposal operations and accidental spills. 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 [1, 5-7].

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 [1, 7].

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 [1, 7].

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 [1, 9].

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. Bioremediation can be used by bacterial species, plant species (a process called *phytoremediation)* and fungal species (a process called *mycoremediation*) [1, 9, 10].

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. [1, 9].

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Mycoremediation is a type of Bioremediation that uses fungi to degrade or remove toxicants from the environment. Fungi are important decomposers in the natural environment. They create enzymes to degrade the plant polymers cellulose and lignin, two very durable compounds that give plants their structure. Using similar mechanisms, fungi can also break down certain toxic substances [11].

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Penicillium chrysogenum:

Penicillium chrysogenum and other fungi normally oxidize aromatics hydrocarbons (e.g. benzene, toluene, ethyl benzene and xylene; BTEX), using mono-oxygenases, forming a trans-diol. The low molecular weight aromatics are more soluble at a higher temperature, higher temperature, and 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 [13, 14].

Benzene, toluene, ethyl benzene and xylene (BTEX)

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 [1, 13, 14].

Advantages of bioremediation: Bioremediation has many advantages over Traditional (Physical) and Chemical 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 unlike chemical methods, no foreign or toxic chemicals are added to the site.

It is also 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 [1, 5].

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 [1, 5].

CONCLUSION

Microorganisms, most especially bacteria and fungi have the capacity of bioremediating and biodegrading 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. Bacteria and fungi are the key agents of biodegradation, with bacteria assuming the dominant role in marine ecosystems and fungi becoming more important in freshwater and terrestrial environments. Bioremediation is an advantageous due to its time and cost saving than physical method. Also unlike chemical method, no foreign or toxic chemicals are added to the site.

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