



Original Research Article

Role of Microorganisms for Removing Oil Pollution from Water

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ABSTRACT

This study investigates the role of microorganisms in removing oil pollution from water. The fight against oil pollution since the emergence of this black and precious substance, has been part of scientific research which had been attracted the attention of experts. For nearly 50 years, microbiologists have been researching removal of heterocyclic compounds, including sulfur heteroatoms, from petroleum fractions. The first patent for this was in Zobel's research in the early 1950s. Research in this area continued until 1989, when the 4S pathway for the decomposition of sulfur compounds was identified by Dr. Kilbane at the I.G.T. It should be noted that in this pathway, the bacteria separate and release sulfur from the model compound, di benzothiophene in a completely specific manner without damaging the carbon skeleton. The final product of this reaction is mono hydro xybiphenyl which is dissolved in oil and organic cut, and as a result, while maintaining the calorific value of the fuel, the separation step is easier in the reactor.

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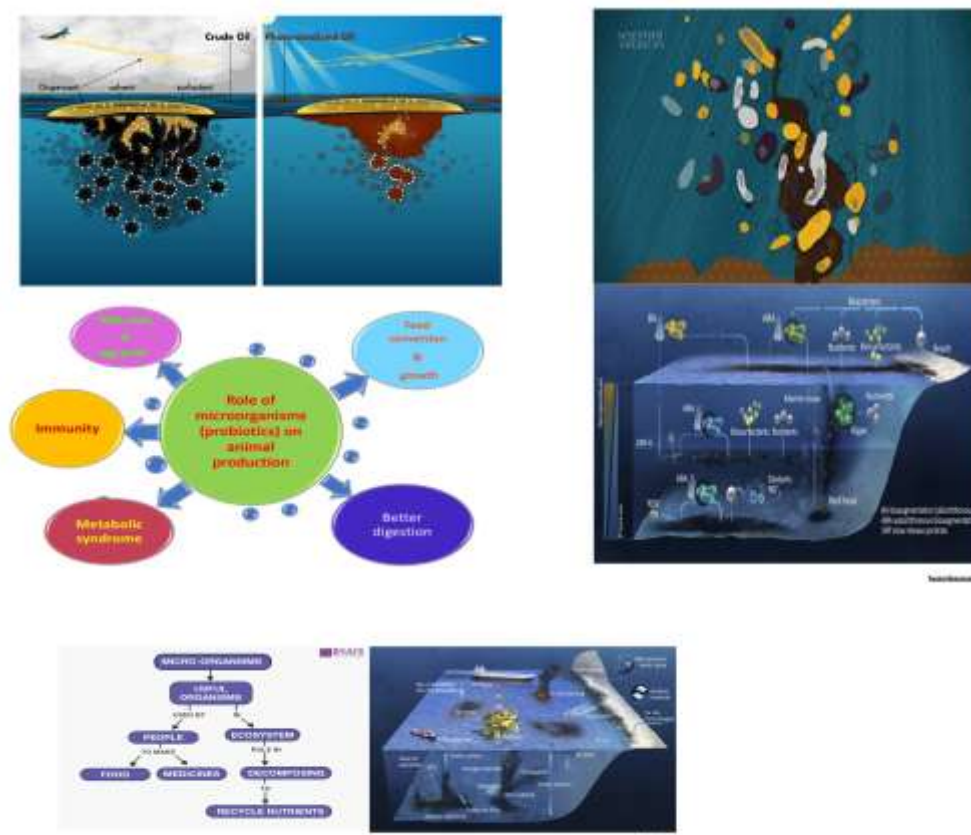
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GRAPHICAL ABSTRACT



Introduction

With more than 340 products, crude oil is one of the main sources of energy and driving force of the global economy, while Iran has 9% of the world's total oil reserves. Crude oil is a complex of hundreds of different compounds, including hydrocarbons, nitrogen, sulfur and vanadium. The hydrocarbons are aromatic, aliphatic and asphaltene [1-3]. Oil pollution is almost an inevitable consequence of rapid population growth and energy consumption based on oil technology. Over the years, all the attention of experts has been focused on ocean oil pollution caused by tanker accidents, the largest of which occurred in 1967 in British waters. In 1975, about 1,000 gallons of crude oil leaked into

seawater from a crude oil reservoir in South Carolina [4-6].

Also in 1991, more than 5 tons of crude oil was spilled in the waters of the Persian Gulf, which caused destruction of plants and coastal ecosystems in the Persian Gulf. The Persian Gulf is home to about 160,000 tons of oil and petroleum products annually and is known as one of the most polluted seas in the world due to the movement of oil tankers, drilling several wells and extracting oil in it.

In general, the events mentioned in some of them caused more attention to be paid to the construction and invention of various methods to eliminate oil pollution in the environment, the

most important of which are manual collection of oil pollution from the water surface, enclosing oil pollution by physical means and use of materials such as feathers and straw that absorb oil particles. Also fire production, using bipolar solvents and finally, bioremediation [7-9].

Purification or Biodegradation

Twenty-five percent of the oil released into the water is lost through evaporation, and the rest is broken down by light oxidation and oxidation of microorganisms, which is called bioremediation. The presence of microorganisms that decompose carbohydrates in seawater and soils has led to decomposition of one of the most effective methods of removing oil pollution and bioremediation accelerates the use of biological systems to destroy or reduce the concentration of toxic substances such as petroleum hydrocarbons [10-12]. As a result this accelerates the presence of oxygen and nutrients, especially nitrogen and phosphorus. Biodegradation products are usually low in CO₂ and small organic matter with very low toxicity. The foregoing methods are commonly used in industrialized countries such as the United States, Japan, Germany, United Kingdom, South Korea and Russia, and are being researched in other countries, especially in the Persian Gulf. One of the limitations of these methods is the limitation in determining the amount of oil extracted from well. While it contains a large amount of sulfur compounds [13].

There are also numerous studies showing that different bacterial strains are not only resistant to oil pollution, but some of them are able to use these compounds as a source of carbon and nutrients [14].

So presence of these compounds not only doesn't prevents bacteria to grow, but rather causes compatible bacteria to grow faster and faster. In a study, Kairimura et al. succeeded in isolating *Sphingomonas elodea* strain from contaminated soils that were able to use carbazol and other

petroleum compounds, and their results showed that the rate of decomposition of petroleum products increases with increasing number and growth of bacteria while increasing turbidity of culture medium. Emtiazi et al. also evaluated degradation of various petroleum compounds by a strain of *Pseudomonas* for 9 days by measuring the bacterial growth curve at 600 nm and evaluated its efficiency in the degradation of petroleum compounds as positive [15].

In a study on the potential for oil removal by *Bacillus* bacteria isolated from oil-contaminated soils in India, Khan et al. found that these bacteria degraded and biodegraded at least 27% of soil oil contamination after 7 days of heating. In a study in the South African region, Ojo found that soil bacteria were able to biodegrade decay-contaminated soils. In another study, 368 strains belonging to the *Bacillus* genus were reported from soil samples collected from deserts contaminated with petroleum compounds. According to other studies, the efficiency of inoculation method of native bacteria isolated from the same contaminated environment and its application in the field of crude oil-soil bioremediation has been proven, due to the increase in fuel content and genetic compatibility. Microbial populations in their own environment have been reported to play an important role in the bioremediation success of contaminated environments [16].

Due to the fact that the microbial flora of contaminated soils is different in various climatic and biological conditions the need for this research is felt in the weather conditions of Tabriz. In addition while various factors such as pollutant concentration and growth temperature can affect the microbial decomposition performance of the contaminant, and due to the lack of conducting research similar to cold weather conditions, this research plan was organized [17].

Desulfurization operations and biological methods

Traditionally, desulfurization operations were performed using chemical methods. Chemical desulfurization imposes heavy costs on the oil industry [18].

Advances in biosulfurization

Biocatalysts are now being developed that can completely separate sulfur from petroleum compounds in a short period of time. This work is currently being done by the American company "Biosystem Energy" and has made many advances in this field. The biocatalyst used is a bacterium called *Rhodococcus IGTS8*. Most research has focused on this process so that they

can prepare the petroleum material as a culture medium for this microorganism and thus avoid spending a lot of money [19-21].

It should be said that in the current situation, using only biological methods for desulfurization is not cost effective. Since we can extract a large percentage of sulfur with the help of chemical methods that are more cost-effective, it is wiser to use the biological method as a complement to the chemical method. If these two methods are used as a complement operating costs are significantly reduced [22-24].

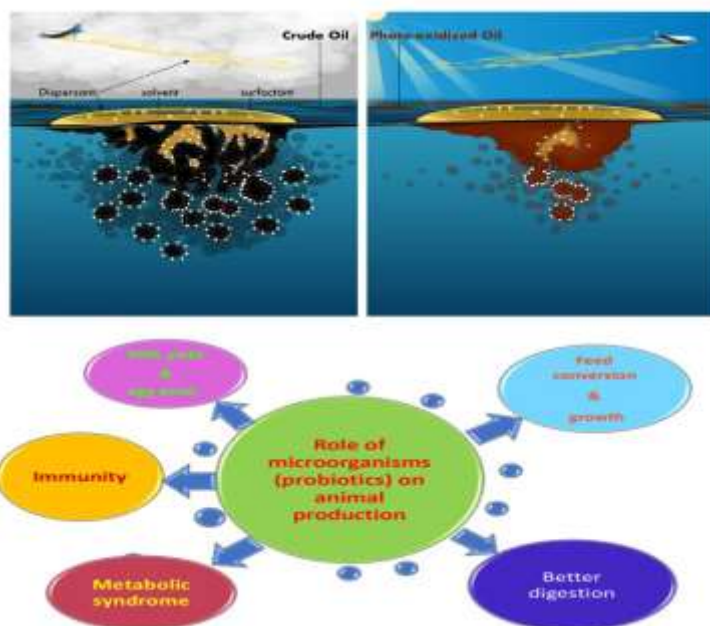


Figure 1. Effective Role of Microorganisms in Livestock Development

Use of sewage sludge on bioremediation of crude oil contaminated soils

Iran is one of the largest oil producers in the world and accounts for about 9% of the world's oil. The current production of Iranian oil is about 4 million barrels per day [25].

Increasing growth of industrial activities on the one hand and non-compliance with

environmental requirements on the other hand, has caused large amounts of hydrocarbon pollutants [26].

It emerged due to factors such as improper disposal of wastewater and industrial waste such as emissions from power plants, leakage pollutants from underground oil tanks and refueling stations; and tanker accidents, etc. On the other hand, due to limited soil and

groundwater resources, soil pollution is one of the most important environmental problems in the country. For this purpose, methods such as direct engineering methods or natural cleaning, i.e. without human intervention in modifying this control, reduce or eliminate pollution from the environment by increasing biological activities of the environment. Bioremediation is a useful method for remediation of oil-contaminated soils [27-29].

In this method, microscopic organisms use carbon dioxide as a source of food and energy and convert it into simpler and more informal substances such as water and carbon dioxide. The result of this process is reduction of total petroleum hydrocarbons in the soil. In fact, bioremediation is an interface between engineering methods and natural methods which includes human interventions such as agricultural technologies [30].

As a result processes such as plowing and fertilizing can improve the biological conditions for soil microscopic organisms and thus increase decomposition situation and reduce pollutant content [31].

In addition, it can use methods that accelerate decomposition of pollutants, such as use of nitrogenous and phosphorous organic matter while microbial activity is accelerated. It should be noted that bacteria and fungi are the only biological species with metabolic ability to use petroleum junctions in their cellular synthesis. In 2000, in the bioremediation of diesel-contaminated soils, Namkung et al. found that mixing contaminated soil with sewage sludge at a ratio of 0.5:1 showed the highest pollutant decomposition. In 2003, Gogi et al. in the bioremediation of crude oil-contaminated soils at the oil spill found that aeration, application of

nitrogen and phosphorus organic matter, and microbial inoculation degraded 75% of the contaminants [32-34].

The role of microorganisms in the removal of oil pollution

In general, the events mentioned in some of them caused more attention to be paid to the invention of various methods in order to deal with pollution of seas and coastal areas. The degradability of petroleum compounds is basically as follows:

Alkane < Alkene = Alkene < Aromatic
Hydrocarbons < PolyAromatic Hydrocarbons

Among the above microorganisms, *Pseudomonas* are more important because despite their presence of multiple plasmids, they are able to produce multiple enzymes and break down oil. *Pseudomonas* decompose both aliphatic and aromatic compounds in petroleum, but the effect of these microorganisms on n-alkanes is far beyond conceivable, so that with the P-450 complex cytochrome and rubredoxin, they are able to oxidize and break them down [35]. The latter is carried out by the enzyme monooxygenase, but in aromatic compounds, *Pseudomonas* invade the ring by one of the "ortho" or "meta" routes, in which the enzyme dioxygenase participates. Mucoic acid is produced through ortho and aldehyde hydroxy mucoic acid through Meta [36].

Pseudomonas are the only bacteria that are able to break down branched hydrocarbons. Basically, microorganisms are able to break down petroleum hydrocarbons with the help of the following three main products:

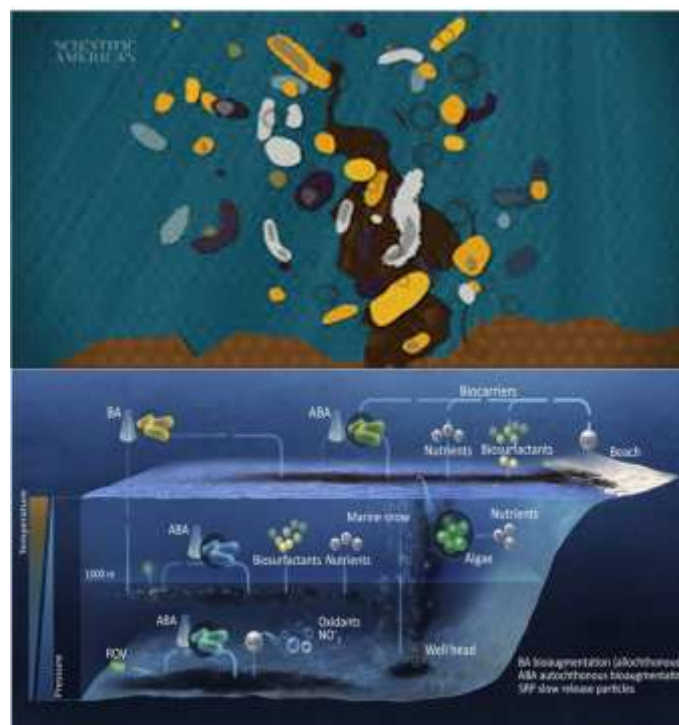


Figure 2. Biotechnologies for Marine Oil Spill Cleanup

1. *Enzymes:* As explained above, monooxygenase and dioxygenase enzymes are the most important enzymes involved in the breakdown of petroleum hydrocarbons, and products of the activity of these enzymes are alcohols [37].
2. *Biosurfactants:* Biological substances have hydrophilic and hydrophobic groups on the cell surface and are produced by a large number of microorganisms. Based on their chemical structure, they are classified into glycolipid groups, phospholipids, fatty acids and leukopolysaccharides. Biosurfactants increase the aqueous concentration of hydrophobic compounds by emulsifying and releasing hydrocarbons adsorbed into the soil material, thereby increasing the rate of mass transfer can help to accelerate

biodegradation, greatly. Biosurfactants are used to clean oil storage tanks, treat oil wastewater, and biodegrade oil contaminants on land and at sea [38].

3. *Acids and Solvents:* Many microorganisms are able to use hydrocarbons as a source of carbon and energy to produce various acids and solvents such as acetone, ether, benzene and oxaloacetic acid, which dissolve petroleum hydrocarbons.

It is need to mention that no microorganism alone can completely decompose petroleum hydrocarbons into CO₂ and water as the final solution. Basically, fungi are used more in the soil environment and bacteria are more commonly used in the aquatic environment [39].

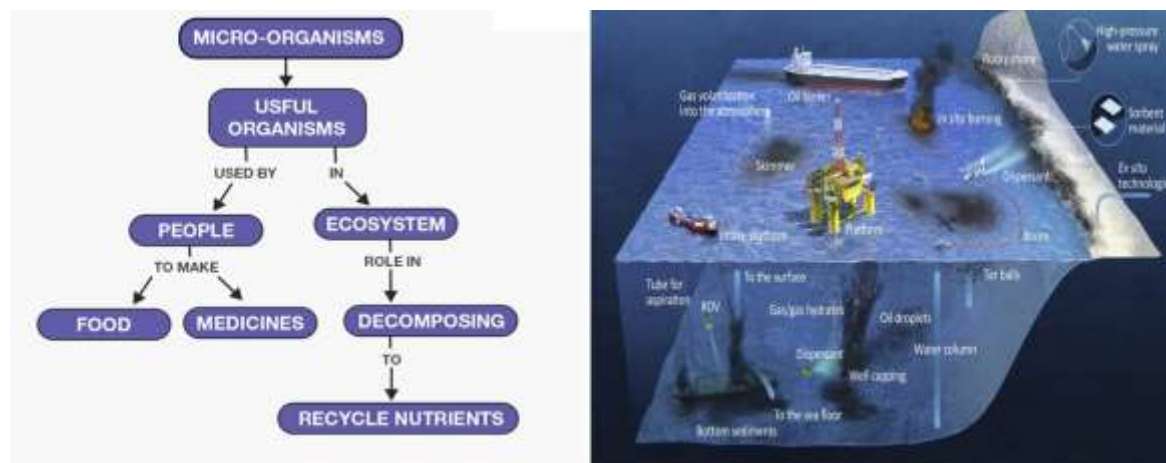


Figure 3. Useful Microorganisms

Desulfurization operations and biological methods
From ancient times, desulfurization operations were performed with chemical shredders. Chemical desulfurization imposes heavy costs on the oil industry [40-42].

Advances in biosulfurization

Biocatalysts have now been developed that can completely separate sulfur from petroleum compounds in a short time [43].

This work has already been done by the American company "Biosystems" and has made a lot of progress in this field. The biocatalyst used is a bacterium called *Rhodococcus IGTS8* and most research has focused on this in order to prepare petroleum material as a culture medium for this microorganism and thus avoid spending a lot of money. It should be noted that in the current situation, the use of biological methods alone for desulfurization is not cost effective [44].

Using microbes to produce oil cuts

In this article, microorganisms are used in the production of oil slices from renewable sources. For example, special microscopic algae have been discovered that produce oil cuts using light and CO_2 [45].

They have found algae that make up 60-70% of their cell mass, or they have found algae that

produce sediment in their culture medium. Therefore, in this way, firstly, clean fuel can be produced that does not need to be refined so much and therefore does not incur high costs. Secondly, oil can be produced from renewable sources that are more environmentally friendly and less polluting, and finally the most applicable and specific production can be produce. In this regard, genetic engineering has a critical role [46-48].

The difference between bio cracking and production of pure incisions by microbes

In bio-cracking, larger compounds, especially paraffin compounds, are broken down into smaller compounds, resulting in a change in the molecular weight of the precipitate and reduction of the inertia, which is accounts for better fuel combustion [49-50].

Conclusion

Crude oil is a complex natural mixture of hydrocarbon and non-hydrocarbon compounds. Oil-based products are a major source of energy for industry and everyday life. However, today, environmental pollution by petroleum hydrocarbons and its adverse effects are significant among many environmental problems. The effect of oil on the microbial population depends on the chemical composition

of the oil and species of microorganisms present and increases the population of some microbes. However, many microorganisms consume petroleum hydrocarbons as nutrients. The bioremediation process is defined as the use of microorganisms to remove contaminants due to their diverse metabolic capabilities, which is an evolving method for removing and decomposing many environmental contaminants, including products of the oil industry. Biodegradation by natural populations of microorganisms is one of the main mechanisms by which oil and other hydrocarbon pollutants can be removed from the environment and is cheaper than other regeneration technologies. Petroleum products are one of the most widely used chemicals. They are in today's world and are considered a serious threat to the environment. These materials contaminate the soil during production, transportation, improper use and leakage. Among petroleum compounds, polycyclic aromatic hydrocarbons are considered due to their high stability in the environment and their harmful effects on human health. Polycyclic aromatic hydrocarbons are a large group of organic compounds with two or more aromatic rings. These relatively neutral compounds with low solubility in water, are lipophilic and many of them evaporate at low pressure. These compounds reduce fertility, permeability, water holding capacity and soil binding capacity, so that unusable soil may be used.

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