Phytoremediation of Petroleum Hydrocarbon by Micro Green Algae: A Review

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ABSTRACT

Background: The Industrial Revolution included processes of extracting, refining, and transporting oil and fossil fuels, as well as the increased demand for liquid oil worldwide, which has led to unprecedented pollution processes and the significant spread of pollutants. This led us to stop and think about the methods of treating pollutants that affect all forms of life, whether human, animal, or plant. Treatment must be at the lowest cost and environmentally friendly, such as the use of green technology by using many living organisms, including green algae, which participate indirectly in the breakdown of hydrocarbon compounds by activating mechanisms ranging from attack through the release of free radicals or the production of enzymes capable of decomposing hydrocarbons. Or by using organic carbon derived from hydrocarbon compounds as a food source for algae. Pollutants can also be removed by bioaccumulation outside or inside the algae cell body. The following review presents an analysis of published works available in the last ten years related to the ability of green microalgae to remove hydrocarbons with the aim of identifying alternative technology in these microorganisms for the use of bacteria and fungi. **Aim of the study**: The use of microorganisms in treating oil pollutants. **Results**: The best types of bacteria used in treatment are *arinobacterM, Acinetobacter*, *Bacillus*, *Pseudomonas*, *Neptunomonas*, *Alcanivorax*, *Cycloclasticus*, and *Oleiphilus*, while the most efficient microalgae species are *Chlorella vulgaris* and *Scenedesmus spp*. **Conclusion**: There is the possibility of using bacteria, fungi, and algae in bioremediation, but the best is algae because it is environmentally friendly and inexpensive.

Keywords: Oil, contamination, Green Technology, Algae.

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Introduction

Fossil fuels are considered the global economy's source of energy, represented by coal and oil, and this led to an increase in oil consumption by 0.9 million barrels per day in 2020, while the daily demand for liquid fuels reached approximately 100 million barrels per day, as oil is considered the main driver of the markets. Because of this, crude oil extraction, refining, and transportation remain continuous and intense global activities. The great interest in extracting, refining, and transporting crude oil and its derivatives causes many problems, leading to soil and water environmental contamination. Oil spills significantly affect the health of plants and animals in aquatic environments. For this reason, in recent years, there have been active searches for a solution that allows the removal and decomposition of oil more effectively and less expensive and tries to avoid using chemical treatments that are considered toxic. With increasing awareness of the importance of protecting and preserving the environment and keeping pace with technological and economic developments, a modern concept was represented by green treatment as one of the new solutions to environmental problems accompanying economic development that relies on fossil fuels as its energy source. (1)

The long-term presence of crude oil in the biosphere affects the quality of its components by changing their physical, chemical, and biological properties, in addition to its impact on biodiversity, as crude oil waste and its derivatives affect living organisms in water or soil, by increasing the levels of toxic elements and reducing Absorption of oxygen, which leads to the death of organisms or their slowdown in growth rates and consequently disruption to the ecosystem sustainability (2). Recently, there has been an increasing interest in bioremediation, as it is the most sought-after technology, which uses phytoremediation plants to remove environmental pollutants or detoxify them to make them harmless.

Many researchers have described methods for phytoremediation of soils, rivers, and lakes contaminated with petroleum pollutants (3-5). In many ancient and modern sources, research into the bioaccumulation of organic xenobiotics/biodegradation in green algae is of great importance from an environmental point of view because the widespread distribution of these compounds in agricultural areas has become one of the problems, Major in the aquatic ecosystem (6). Algae have proven their effectiveness in accumulating heavy metals and decomposing foreign substances in the environment. Recently, researchers have turned to using microalgae in wastewater treatment because of their main role in fixing carbon dioxide. In addition, the algae generated are used as raw materials for fuel production (7). Other studies showed the benefits of microalgae bioremediation capabilities for environmental sustainability (8-9).

Microalgae are mostly single-celled photosynthetic organisms that form phytoplankton in the environment. In the last century, microalgae and their pharmaceutical, food, and industrial applications have been studied because they produce various metabolites such as lipids, carotenoids, and antioxidants (10). Most local studies are related to environmental studies of inland waters and soil, with a taxonomic study of algae and determination of water quality. Some research has indicated the species that are most resistant to various pollutants (11-18). Some of these pollutants, especially hydrocarbon compounds found in river water within cities, were extensively studied concerning their concentration and types (19-22). In this review, information was collected regarding the potential of microalgae, with special attention to green algae, to remove or mitigate pollutants derived from pure crude oil locally and globally. Microalgae could represent a good solution due to their ability to metabolize various pollutants and be used in photosynthesis as carbon sources and oxygen production in the atmosphere (13).

The oil or petroleum industry is defined as a combination of complex, interconnected processes based on exploiting oil and transforming it from its crude form into a product capable of satisfying a specific need using complex techniques and means. The oil refining industry is a stage of the oil industry that consists of processing the crude oil extracted from the ground to extract various petroleum products that are capable of final consumption, such as diesel fuel, kerosene, gasoline, and others. The oil refining industry takes place inside industrial facility Oil, which are huge industrial complexes made up of complex equipment, means, and units which include distillation, hydrocracking units, and contaminated water treatment units that use different technologies to process and convert crude oil into petroleum products (1).

Water represents a raw material and an essential element in the extraction, refining, and transformation of oil and its derivatives, as water is used to generate steam in the distillation stages and generate vacuum pressure in insulation towers. It is also used to operate steam-powered engines and is involved in cooling processes (23). Large quantities of water laden with industrial waste and oil waste come out (from the various stages of oil refining inside the refineries, and the polluted water is released after passing through stages and treatment basins into the river (24). The water excreted from the treatment basins of the refineries is rich in oil pollutants, representing the most complex mixture of hydrocarbon compounds, which causes a global contamination problem that affects water and may penetrate into groundwater and reduce large numbers of recyclable organisms of elements, thus disrupting food chains and hindering the flow of energy. Refined oil may be more toxic to microorganisms than crude oil (25).

Hydrocarbons are organic compounds consisting of carbon and hydrogen. They are the most widespread and diverse compounds on planet Earth. They are composed of straight, branched, or ring chains (26). Compounds that contain only carbon and hydrogen are called parent compounds, while compounds in which a carbon group or hydrogen derivative is replaced by another are called hydrocarbon derivatives (27).

Crude oil is a liquid mixture containing hundreds of thousands of different hydrocarbon compounds, formed naturally by animals, plants, and bacteria in conjunction with changing environmental conditions such as pressure and temperature under the soil surface. Therefore, crude oil's chemical composition and physical properties differ significantly depending on the location, conditions of origin, and distinctive characteristics. Every hydrocarbon compound affects the properties of the general petroleum mixture and is characterized by being non-polar compounds, so they cannot dissolve in water (28). The number of hydrocarbon compounds in petroleum derivatives is estimated at three million compounds, which can increase annually. This is due to the ability of carbon atoms to bind to each other. Or with anything else, and the formation of long, branched, and unbranched chains, in addition to the strength of the C-C bond, which does not weaken along the length of the chain, as well as the circular shapes that the chains may form, which often include 5-7 atoms, with the ability to bond with other rare elements such as nickel, cobalt, cadmium, chromium, etc. It negatively affects living organisms, as these elements are linked to carbon in organic compounds by covalent bonds, and each element is linked by a number of bonds equal to its valence, so carbon, which has a tetravalency, is always linked in organic compounds with four covalent bonds.

The petroleum hydrocarbon compounds that make up crude oil can be divided depending on their composition into four sections: 1- Asphaltenes - Alkenes (Olefins), 2- Aromatic Compounds, 3- Paraffin or Alkane Compounds, and 4- Naphthenes.(26) The state of linear hydrocarbons varies based on their number of carbon atoms (29). Alkanes that contain less than five carbon atoms are in the gaseous state, while compounds containing more than five carbon atoms are in the liquid state (30). On the other hand, cycloalkanes are formed starting from compounds such as cyclopropane, from which, in rare cases, cyclopentane and cyclohexane are the lightest hydrocarbons in natural conditions, and cyclobutene arises. In the gaseous state, propane methane, ethane (31).

Crude oil hydrocarbons contain polycyclic naphthenes (32). Among hydrocarbons, there are aromatic compounds present in the liquid state, and the most toxic ones are BTEX compounds: benzene, toluene, ethylbenzene, and xylene, which constitute up to 60% of the light fraction of petroleum. Polycyclic aromatic hydrocarbons are formed from multiple aromatic anthracenes and phenanthrene rings fused and divided into soluble resin-like and non-resin atoms. Insoluble asphaltenes (33) or in the form of homogeneous asphaltenes and pyrene in crude oil, which constitute less than 1% of the total composition, are mainly oxygen and sulfur. Phenols, carboxylic acids, alcohols, esters, and ketones also contain oxygen (34). Carboxylic acids also contain fatty acids and naphthalene, which weigh about 1000 Da (35). The amount of sulfur present affects the properties of crude oil; it can be more or less acidic, depending on the amount. It is common to find ionic compounds such as sodium chlorite or metallic porphyrins such as nickel or vanadium in petroleum (36).

1- Current Bioprocessing Techniques:

There are currently several techniques for oil extraction that vary according to the material to be purified. Typically, multi-step protocols involve the use of chemical agents and the action of microorganisms such as bacteria and microalgae (37). Some organisms can use oil and its components as a source of food and energy and grow in the presence of hydrocarbons. There are 175 bacterial genera, many microorganisms, and some eukaryotic microorganisms (38). However, bioremediation carried out by microorganisms is a complex mechanism that requires many steps and cooperation between different species capable of acting on hydrocarbons synergistically. Moreover, it must be considered that many factors, such as temperature and nutrient concentration, play an essential role in the treatment process (39).

Bioremediation generally begins with some bacterial genera capable of attacking straight- and branched-chain alkanes present in large quantities. Bacterial bioremediation of crude oil exploits the ability of various species of bacteria and archaea to metabolize organic carbon and produce surfactants that improve waste oil's chemical and physical properties. The most commonly used bacteria are *Clostridium*, *Zymomonas*, *Klebsiella*, and *Enterobacter* (40-41).

2- Biological methods for removing oil pollutants from various environments:

Bioremediation is the process of using living organisms, such as bacteria, fungi, and algae, to convert organic or inorganic pollutants in the environment into harmless or less harmful substances. Biology and living organisms are used to remove pollutants from soil, water, and air. This process includes many different techniques, such as biodegradation, fermentation, and bioprecipitation, or has various types of enzymes to decompose pollutants and use them in metabolic and other processes and is generally used in treating environmental contamination and rehabilitating contaminated sites, This treatment method is characterized by being low-cost, highly efficient and environmentally friendly (42-43).

Biological treatment of hydrocarbon pollutants may be in the presence of oxygen (aerobic fermentation), which is considered faster and easier, and the end products are often less environmentally harmful. On the other hand, in the absence of oxygen (anaerobic fermentation), the rate of biological treatment is lower and slower than in aerobic fermentation, and this may lead to the production of by-products such as methane. Most types of bacteria, algae, and fungi have the ability to decompose under aerobic conditions (44). The presence of oxygen in polluted environments increases the rate of biodegradation several times over what occurs naturally. In aerobic environments, microbes usually break down hydrocarbon pollutants into alcohol by adding an unsaturated ring of polycyclic aromatic hydrocarbons or hydroxyl group to the end of alkanes. Thus, the resulting compounds are easily soluble in water (45).

Bacteria represent the first group of microorganisms responsible for the first breakdown of petroleum hydrocarbons and generate intermediate compounds used by various other microorganisms (46). In the marine environment, there are more than 200 species and 100 genera of microorganisms that degrade petroleum hydrocarbons; most of them are bacteria, including several strains of oil-decomposing bacteria, such as *Alcanivorax*, *Cycloclasticus*, *Oleiphilus*, ,*Neptunomonas* ,*Marinobacter*, *Acinetobacter*, *Bacillus*, *Pseudomonas spp.*, *Actinomycetes* (47-48). Among the first organisms used in the biological treatment of hydrocarbon compounds were bacteria, as the researcher (49) indicated that enzymes and genetic factors for hydrocarbon decomposition are in bacteria. These genes are usually carried on the plasmid in *Pseudomonas spp*. That is, the ability to decompose hydrocarbons is of genetic origin. Fungi play an essential role in the biodegradation of petroleum hydrocarbons within the polluted aquatic environment in addition to sediments, as carbon is derived from hydrocarbons.Fungi have the ability to decompose the total content of petroleum hydrocarbons with other microbial organisms, such as bacteria (50).

3- **Microalgae and petroleum bioremediation:**

Algae can be used in the bioremediation of petroleum hydrocarbons inspite of the source of these hydrocarbons from contaminated soil, wastewater, or even the air. However, the use of microalgae in the bioremediation of petroleum hydrocarbons remains a major area of research. Some studies have shown that some algae, including green, red, and brown algae, have the ability to decompose some hydrocarbons into compounds that are less harmful to the environment (51).

Since Algae are low-cost absorbents for oil removal and can affect spilled oil and its various compounds, it constitutes an essential component in the treatment of water contaminated with crude oil and hydrocarbons. (52)Some studies evaluate the ability of some microalgae grown on biofilms to remove contaminants of petroleum origin, including polycyclic aromatic hydrocarbons and total petroleum hydrocarbons (TPH) (53). Many studies have shown that algae eliminate nutrients such as nitrogen and phosphorus, heavy metals, toxic hydrocarbons, inorganic toxins, and pesticides (53-56). Various algae are used to biologically process phenols for their ability to bioaccumulate, biodegrade, photodegrade, and adsorption (57-58).

A study by Beyer *et al*. (2016) (59) indicates that there is cooperation in the biological activities of various types of microorganisms that work synergistically in polluted water, causing the decomposition of hydrocarbons and often forming agglomerations that settle at the bottom of the water body

The microbial community in the vicinity of oil spills increases the expression of genes that participate in the biodegradation process, as it improves the motility of bacteria and the production of enzymes involved in aliphatic decomposition (60). The presence of water-driven currents helps bacterial reproduction and accelerates their metabolism. Moreover, in order to metabolize alkanes, mainly aerobic microorganisms intervene in the degradation process, which contain many enzymes of rubredoxin monooxygenase and rubredoxin reductase to convert alkanes into simpler form by increasing the expression of several alkaline genes (61).

The results of a study on the use of *Chlorella algae* showed a decrease in the concentration of nutrients from the water polluted with it, such as sulphates - 17.5%, chloride 14.65%, nitrates - 33%, total suspended solids 26TS, dissolved solids TDS 7.9, chemical oxygen demand (COD) decreased, and the demand for biochemical oxygenates (BODs) were 8% and 16.7%, respectively. Although not in high percentages, the removal of TPH was equal to 15% after 14 days (62). On the other hand, some researchers add the algae *Chlorella spp*. to crude oil, using the mechanical action of sea waves in nature as a catalyst, which helps form an emulsion of crude oil and water, making the pollutants more available to algae. Their results show that after an initial adaptation period, after 5 days, the Chlorella can emulsify 80% of oil (63).

The effect of mixing water and using a variety of algae together and individually was studied for its effect on oil contaminants. The results showed that mixing the water column containing crude oil does not significantly affect the concentration of total petroleum hydrocarbons (TPH). Still, it increases the concentration of some alkanes and PAHs and causes the formation of colloidal fine particles (1-70 micrometers), improving hydrocarbons' degradation (64). *Chlorella spp*. is one of the most widely used types of green algae in biological purification processes due to its ability to survive in contaminated media that treat petroleum effluent (PE) . When this species was used for hydrocarbon degradation, phosphorus was utterly removed after 13 days, nitrogen was reduced by 78%, and COD was reduced from 504 mg/L to 144 mg/L. With *Chlorella vulgares*, initially, the biomass increased, but in the long term, it began to become toxic and inhibit cell growth (65). The nature and concentration of crude oil and its components greatly affect the growth of *Chlorella*, as it has been shown that the use of water derived from diesel is more effective than the oil itself. The reason for this is that diesel water contains many low molecular-weight hydrocarbons that cause damage and affect cell membranes (66).

The best removal of low molecular weight (LW) hydrocarbons equal to 100% was achieved when using 10 g/L for 14 days, while at higher concentrations (20 g/L) after 14 days, Encouraging results were obtained. LW was reduced by 82%. The removal of heavy molecular weight hydrocarbons (HW) followed the same trend as light hydrocarbons, reaching higher values during 14-day periods at a concentration of 78% equal to approximately 10 g/L HW (67). Tested the growth of *Chlorella* in mixed conditions using 1% crude oil, and the results showed that the concentrations of hydrocarbons present after 30 days of incubation showed that aliphatic compounds were the most significant compound removal compared to control (68). Another study showed that polycyclic aromatic hydrocarbons were reduced more efficiently at different nutrient conditions using *Chlorella vulgaris* and *Scenedesmus obliquus* (69). This supports the hypothesis that eukaryotic microalgae, such as *Chlorella*, utilize the organic carbon in the solution, improving their growth range and biomass by using heterogeneous metabolism that allows it to utilize, break down, and/or convert into intermediate metabolites. This hypothesis is also confirmed by the study (70), which showed how *Chlorella* reached the highest yield of hydrocarbon biomass (1.72 g/L) under mixed feed conditions with the addition of pre-treated produced water (PPW) of petroleum origin; it removed 92% of total nitrogen (TN) and 73% of TOC.

4- Mechanism of Action

The green microalgae, Chlorophyceae, are excellent candidates for removing crude oil pollutants. The mechanism of action by which oil pollutants are withdrawn is not entirely known. Still, there are two main hypotheses: either they use organic carbon derived from hydrocarbons, or they treat the pollutants as real pollutants and accumulate indoors through the implementation of a defense mechanism (62). After analyzing the two hypotheses, they found that in microalgae, there is an increase in saponins after treatment of oil pollutants. Usually, saponins play a protective role thanks to their terpene nature. They form solutions indicated that they are colloidal and foamy, lowering surface tension and increasing alkaloids, flavonoids, and carotenoids within

Table (1): Microalgae and Principal enzymes involved in the process of bioremediation (5)

Microalgae	Pollutants Degraded	Enzyme
Chlorophyceae	Total Crude oil	Saponine
	PAHs	Lypoxygenase Hydroperoxidase
	THC	ROS production
Chlorella spp.	THC	Extracellular polymeric substances
Haematococcuspluvialis	PAHs	Cytochrome P450

After treatment of oil pollutants, algae indicate that reactive oxygen species produced by microalgae have led to the decomposition of hydrocarbons and protected them from their toxic effect. Moreover, scanning electron microscope images show how the oil affected the surface shape of the microalgae. If the cell surface was rough before treatment, it became polished and smooths afterward (71).

Some algae produce multi-polymer materials that they remove from the cell. They accumulate elements such as silicon, aluminum, and iron and bind to them due to the presence of functional groups such as C = O OH CO. This is what was observed in the algae *Chlorella spp*., where it forms a layer of sugary substances outside the cell wall (72).

Haritash *et al*. (2020) (73) focused on the genetic nature behind the bioremediation mechanism. They suggested that PAHs could source alkoxyls (RO) and hydroxyls (OH) inside cells. Based on the information available on blue-green algae, they hypothesized that green algae could have dioxygenase enzymes which are used to hydrolyze and remove polycyclic aromatic hydrocarbons, with special emphasis on lipoxygenases (LOXs), which oxidize polycyclic aromatic hydrocarbons through the introduction of oxygen and rupture of the aromatic ring, and this was confirmed by Radice *et al*. (2023) (5). The mechanism of PAH degradation in microalgae could be similar to that carried out by prokaryotes based on the idea that higher plants and animals share enzymatic and genetic pathways in removing exogenous substances. In the hydroacre form, the addition of oxygen to the hydrocarbon skeleton results in hydroperoxidase production that is activated to become oxylipins. The molecular mechanism has not yet been studied in microalgae. Still, it has been shown that exposure to 1% crude oil for 21 days stimulated the expression of LOX genes, leading to the degradation of hydrocarbons and the production of hydroperoxy acids. Lipids and oxylipins, which are beneficial for algae to grow and survive (74).

There is an antioxidant mechanism to decompose petroleum pollutants, especially hydrocarbons, and it works in a double capacity, as it removes the toxic substance and produces nutrients beneficial for cell growth. Ancient sources mentioned the hypothesis that low doses of pollutants can activate mechanisms to repair not only the damage caused by the toxin but also other damage previously accumulated by the cell; according to the hormone hypothesis, the catabolic hypothesis claims that an organism responds adaptively to small doses of stress to survive (75). However, some studies show that the ability of microalgae to remove contaminants persists even after cell death, as the microalgae can absorb tiny drops of crude oil on its surface, thus removing them from the solution, as explained (76).

Conclusion:

Pollution to the environment is a result of oil extraction and refinement. Since oil is extracted and refined using water, the water becomes contaminated with hydrocarbons, which are thought to number in the three million ranges. Using living organisms like bacteria, fungi, and algae to transform pollutants into less harmful or harmless compounds is known as bioremediation. The bacteria *Clostridium*, *Zymomonas*, and *Klebsiella* are most frequently used. Numerous techniques, including biodegradation, fermentation, bioprecipitation, and enzymes, are used in treatment. Algae, including red, brown, and green algae, are crucial to the treatment of water tainted with hydrocarbons and crude oil. There are primarily two theories. Regarding removing algae: They either consume hydrocarbon-derived organic carbon as food or accumulate inside algae.

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المعالجة الحيوية للهيدروكاربونات النفطية باستخدام الطحالب الدقيقة : مراجعة

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الخلاصة

خلفية عن الموضوع: ان الثورة الصناعية وعمليات استخراج النفط وتكريره و نقله و الوقود الاحفوري وزيادة الطلب على النفط السائل في العالم ادت الى عمليات تلوث غير مسبوقة وانتشار الملوثات بشكل ملحوظ وهنا يجب التوقف والتفكير في عمليات معالجة الملوثات المؤثرة على جميع اشكال الحياة سواء كان انسان او حيوان او نبات كما يجب ان تكون المعالجة باقل كلفة وصديقة للبيئة مثل استخدام التكنلوجيا الخضراء عن طريق استخدام كائنات حية عديده منها الطحالب الخضر التي تشارك بشكل غير مباشر في تحطيم المركبات الهيدروكربونية عن طريق تفعيل آليات تتراوح بين الهجوم من خلال تحرير الجذور الحرة او إنتاج الإنزيمات القادرة على تحلل الهيدروكربونات، او عن طريق استخدام الكاربون العضوي المشتق من المركبات الهيدروكربونية كمصدر غذاء للطحالب كما يمكن ازالة الملوثات بالتراكم الحيوي خارج او داخل جسم الخلية الطحلبية . تبين المراجعة التالية تحليل الأعمال المنشورة المتوفرة في السنوات العشر الماضية والتي تتعلق بقدرة الطحالب الخضر الدقيقة على إزالة الهيدروكربونات بهدف تحديد تكنولوجيا بديلة في هذه الكائنات الحية الدقيقة لاستخدام البكتيريا و الفطريات. **الهدف من الدراسة:** استخدام الاحياء الدقيقة في معالجة الملوثات النفطية ،**النتائج**: افضل أنواع البكتريا المستخدمة في المعالجة هي، *Alcanivorax* ، و*Cycloclasticus* ، و *Oleiphilus* ، و *Neptunomonas* ، و *Marinobacter*، ، *Acinetobacter*، *Bacillus*، *Pseudomonas* ، واكفاءه أنواع الطحالب الدقيقة هي Chlorellaو Scenedesmus **الاستنتاجات:** إمكانية استخدام البكتريا و الفطريات والطحالب في المعالجة الحيوية ولكن افضلها الطحالب لأنها صديقة للبيئة وغير مكلفة.

الكلمات المفتاحية: النفط ، التكنلوجيا الخضراء، التلوث، الطحالب.