International Science and Technology Journal
المجلة الدولية للعلوم والتقنية

Volume 38 العدد
Part 1 المجلد

http://www.doi.org/10.62341/istj-vol38-1-80aw

Received	2026/03/05	تم استلام الورقة العلمية في
Accepted	2026/03/30	تم قبول الورقة العلمية في
Published	2026/03/31	تم نشر الورقة العلمية في

Experimental Study on the Bioremediation of Petroleum Hydrocarbons through Integrated Environmental Optimization and Natural Amendments

Soad Moftah Ahmd Emshher[1], Abdullah Mohamed Abdullah Salem,[1] jamal Ibrahim Elzwai[1], Mustafa Abdalla Gaja[2]

1. Wadi Al-Shati University, Libya
2. Petroleum Research Center - Libya

soad.moftah23@gmail.com

Abstract

One of the most critical environmental concerns is hydrocarbons of petroleum origin, which pose enormous challenges and risks for soil ecology, water resources, and human health, especially for those regions where fuel and oil handling activities are performed. The major aim of this study is to evaluate an integrated bioremediation technique using environmental optimization and natural enhancement materials for the degradation of petroleum hydrocarbons. A series of laboratory-scale batch experiments were carried out using hydrocarbon-degrading microorganisms isolated from hydrocarbon-contaminated soils. The degradation of hydrocarbon compounds was done under varying pH and temperature conditions, as well as varying combinations of amendment materials. The effectiveness of the degradation was monitored by observing the concentration of TPH, microbial density, and health parameters of the contaminated soil. The results revealed that the degradation of petroleum hydrocarbon compounds is possible in a temperature that is mild and in an almost neutral pH environment. The activated charcoal and bile salts work in combination to degrade the TPH compounds as efficiently as possible. The density of microbes was increased through the addition of compost and plant extracts, leading to the efficient degradation of the petroleum hydrocarbon compounds. It should be

http://www.doi.org/10.62341/istj-vol38-1-80aw

noted that the control of petroleum hydrocarbon compounds is possible through the use of the combination of natural substances and environmental control techniques.

Keywords: Bioremediation, Petroleum Hydrocarbons, Soil Contamination, Biosurf

دراسات سابقة للمعالجة الحيوية للهيدروكربونات البترولية من خلال دمج التحكم البيئي والتحسينات الطبيعية

سعاد مفتاح أحمد أمشهر[1]، عبد الله محمد عبد الله سالم[1]، جمال إبراهيم الزواي[1]
مصطفى عبد الله قاجه[2]
1- جامعة وادي الشاطئ، ليبيا
2- مركز بحوث النفط – ليبيا
Soad.moftah@su.edu.ly

الملخص:

تُعدّ الهيدروكربونات البترولية من أهمّ القضايا البيئية، إذ تُشكّل تحديات ومخاطر جسيمة على بيئة التربة، والموارد المائية، وصحة الإنسان، وخاصتا في المناطق التي تجرى فيها أنشطة تداول الوقود والزيوت

ويهدف هذا البحث بشكل أساسي إلى تقييم تقنية المعالجة الحيوية المتكاملة من خلال تحسين الظروف البيئة وأضافه التحسينات الطبيعية لتحليل الهيدروكربونات البترولية. أُجريت سلسلة من التجارب المخبرية على دفعات باستخدام كائنات حية دقيقة محللة للهيدروكربونات معزولة من تربة ملوثة بها. تم تحليل مركبات الهيدروكربونات في ظل ظروف مختلفة من الرقم الهيدروجيني ودرجة الحرارة، بالإضافة إلى تركيبات متنوعة من مواد التحسين. رُصدت فعالية التحليل من خلال مراقبة تركيز الهيدروكربونات البترولية الكلية، وكثافة الكائنات الدقيقة، ومؤشرات صحة التربة الملوثة.

كشفت النتائج أن تحليل مركبات الهيدروكربونات البترولية ممكن في درجة حرارة معتدلة وبيئة ذات رقم هيدروجيني شبه متعادل. يعمل الفحم المنشط وأملاح الصفراء معًا على تحليل مركبات الهيدروكربونات البترولية الكلية بأقصى كفاءة ممكنة. زادت كثافة الكائنات الدقيقة بإضافة السماد العضوي ومستخلصات النباتات، مما أدى إلى تحليل فعال لمركبات

http://www.doi.org/10.62341/istj-vol38-1-80aw

الهيدروكربونات البترولية. تجدر الإشارة إلى أن السيطرة على مركبات الهيدروكربونات البترولية ممكنة باستخدام مزيج من المواد الطبيعية وتقنيات المكافحة البيئية.
الكلمات المفتاحية: المعالجة الحيوية، الهيدروكربونات البترولية، تلوث التربة، المعالجة الحيوية السطحية.

INTRODUCTION

One of the principal environmental problems in the current world is the pollution caused by petroleum hydrocarbons. This pollution is driven by the rising tide of industrialization, urbanization, and the rising tempo of oil exploitation and transport. One of the key challenges of the modern world is environmental pollution due to the presence of hazardous substances and their impacts on the environment and health, which has come to be a major concern in the last few decades (Ahmed et al., 2023; Vadakkan et al., 2024). Polycyclic aromatic hydrocarbons are a set of chemicals extensively widespread in the environment and are a big problem facing the contemporary world. They have been recognized as a set of priority pollutants through the United States Environmental Protection Agency due to their toxicity (Chaudhary & Kim, 2019; Chidewe, 2024). As a result of their stability, PAHs stay in the soil for long periods, thus becoming a threat to the health of human beings.

Some of the major contaminants known to be responsible for causing deterioration of the environment include those of petroleum hydrocarbons such as aliphatic and aromatic types. Oil spills and leaks of stored oil, ruptures of oil pipes, and oil loss during fuel station and refinery activities represent some of the sources of petroleum hydrocarbons entering the environment (Arghiropol et al., 2025). Once they enter the environment, petroleum hydrocarbons carry the danger of poisoning groundwater, infiltrating the soil, and evaporating into the air. There are various health hazards associated with petroleum hydrocarbons, which include the risk of human cancers, genotoxicity, mutagenicity, immunosuppression, and various other kinds of human hazards associated with it.

Human hazards related to the exposure of petroleum hydrocarbons are related to cardiovascular and respiratory-related human hazards, as mentioned in Shetty et al. (2023) and Alaidaroos

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(2023). Petroleum hydrocarbons are also known to be responsible for various health hazards, which include changes in the microbiota that are found in the soil, which are integral in maintaining the healthy ecosystem, changes in the structure of the soil, and reduction in the fertility of the soil. The effects of petroleum pollution do not only end at the environmental and health aspects but also encompass severe socio-economic aspects. Oil spills may end up contaminating agricultural land and water sources, thereby affecting agricultural productivity and food security.

Various studies have demonstrated that hydrocarbon pollution may lead to a substantial reduction in the nutritional content of agricultural products and affect the livelihood of the community based on agricultural and fishing activities (Udensi et al., 2026). Globally, hydrocarbon pollution has been identified as a widespread phenomenon. It is believed that millions of tons of petroleum are discharged into the environment annually due to both natural and human activities such as industrial emissions, accidents during transportation, and fallout from the atmosphere (Kianfar, 2025). For the remediation of sites contaminated by hydrocarbons, the conventional practices of mechanical removal, chemical dispersion, incineration, and solidification and stabilization have been employed.

It is found that the conventional practices have been successful in reducing the concentration levels of the pollutants, but they were often expensive, energetically intensive, and environmentally hazardous (Petrillo et al., 2025). The mechanical remediation techniques are highly site-dependent, and the chemical remediation techniques can lead to the introduction of secondary pollutants into the environment (Michael-Igolima et al., 2022). Although the process of incineration is highly effective in the remediation of contaminated sites, it also contributes to the generation of greenhouse gases and toxic chemicals, which is considered to be a disadvantage of the traditional remediation processes (Hamidinasab & Nabavi-Pelesaraei, 2025). As a result, there is now a greater interest in the development of alternative techniques that are ecologically acceptable and effective.

Utilizing the metabolic abilities of this process, the bioremediation of petroleum hydrocarbons was proven to be an efficient and environmentally friendly way of dealing with

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pollutants. Petroleum hydrocarbons can be used by microorganisms such as bacteria and fungi as a supply of carbon and energy for their growth. Many researchers have proven the capability of the microorganisms to transform hazardous and sophisticated petroleum-based pollutants into harmless and simple ones (Al Hoqani et al., 2025). The most efficient green technology that has widely been employed in the decontamination of petroleum hydrocarbons in polluted environments and sites is bioremediation, as several studies have proved the degradability of petroleum hydrocarbons through biodegradation, as discussed in the research studies of Lin et al. (2022), and Orchid et al., (2023). Some of the already established organisms employed in the decontamination of petroleum hydrocarbons are Pseudomonas, Acinetobacter, Alcanivorax, and Rhodococcus.

Nevertheless, a number of biological variables and physicochemical aspects have limited the effectiveness of the bioremediation process in the environment. The availability of appropriate environmental variables such as pH, temperature, oxygen, moisture, and nutrients has limited the effectiveness of microbial decomposition. In most cases, the environmental conditions in the area of contamination may not be optimal, which may limit the growth of the microorganisms (Kuppan et al., 2024; Armanu et al., 2025). In addition, the high molecular weight hydrocarbons and PAHs have low solubility and high adsorption capacity, which may limit their bioavailability and increase their resistance to microbial degradation (Colmo et al., 2025). All these limitations suggest that the remediation of such sites using only the natural microbial population may not be very effective.

In order to address the above challenges, recent research has emphasized the use of an integrated approach to bioremediation, which involves the use of environmental control and enhancement techniques in addition to microbial techniques. For example, it has been shown that the rate of biodegradability of hydrocarbons can be enhanced through techniques of biostimulation, where they are subjected to the addition of nutrients and oxygen, and bioaugmentation, which involves the introduction of particular microbes into the ecosystem (Lakhani et al., 2022). Furthermore, it has been shown that natural materials, such as compost, plants, charcoal, and those that are similar in nature to biosurfactants, are

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effective in the bioremediation of hydrocarbons. This is particularly because they are effective in the biodegradability of hydrocarbons, for example, when it comes to their availability and the creation of an environment to support biodegradability. This can be attributed to the ability of the aforementioned compounds to adsorb hydrocarbons, their availability to support nutrient usage, and their solubility in hydrocarbons, thus enhancing biodegradability (Sah et al., 2022; Elayaperumal et al., 2025).

Despite the rising interest in the application of these methods, there is still much to be studied concerning the appropriate combination of these environmental control factors and natural enhancement materials for large-scale application. Most literature focuses either on the microbial isolation process carried out in the lab or engineering-based interventions without considering their combined potential (Sharma et al., 2024; Hossain, 2024). This is where extensive research is required to assess the potential of these controlled environmental factors such as pH and temperature, combined with natural materials such as bile salts, charcoal, plant materials, and compost, for maximum biodegradation efficiency.

Unlike other studies that only focused on either microbial degradation or individual enhancement strategies, the current study is characterized by a holistic experimental approach that encompasses both environmental optimization, as well as a range of natural amendments, which include activated charcoal, bile salts, compost, and plant extracts. The current study is also original in that it is the first of its kind to investigate the combined effects of the variables on hydrocarbon degradation efficiency. Additionally, the current study is also original in providing a comparative understanding of the combined effects of the variables on hydrocarbon bioavailability.

In locations such as Sirte, Libya, which is affected by oil spillage in fuel stations leading to pollution of the environment, the importance of biodegradation techniques cannot be overstressed. Therefore, the need for an efficacious bioremediation technique that considers the environment conditions has been identified as a potential solution for bioremediation. Therefore, the main aim of this research is to evaluate and determine the effect of environment conditions and enhancements in the biodegradation of hydrocarbons

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for the purpose of developing a bioremediation technique for petroleum hydrocarbons.

LITERATURE REVIEW

Hydrocarbon Pollution

Owing to its toxicity and its effect on living organisms and the environment, hydrocarbon pollution has been a notable area of concern within this last five decades of research on environmental studies and science. The group of hydrocarbons, including petroleum crude fractions, polycyclic aromatic compounds, volatile organic compounds, and BTEX compounds, including benzene, toluene, ethyl benzene, and xylene, have been considered as areas of concern owing to their effects, which include mutagenic and carcinogenic effects on living organisms (Patel et al., 2020). In addition, various studies have asserted that, owing to its hydrophobic properties, hydrocarbon pollution displays persistence within its toxicity, which leads to an accumulation within the environment (Briones-Venegas et al., 2023). It is this persistence of hydrocarbon pollution in the environment that has brought it out as a separate entity from the other pollutants, with emphasis given to the preventive and innovative aspects (Devendrapandi et al., 2024).

Environmental and Health Impacts of Hydrocarbon Contamination

Evidence of interdependent, multifaceted effects on various ecosystems, such as terrestrial, aquatic, and atmospheric ecosystems, has, in most cases, validated the negative effects of hydrocarbon pollutants on the environment. If hydrocarbons are introduced into the environment, the hydrocarbons will distribute unevenly into unique individual fractions depending on their solubility and volatility level, affecting the ecosystem differently, as experimented. After the experiment, hydrocarbons, including crude oil and its products, were identified as the major contributors to long-lasting pollution on the surface of the earth, as the hydrophobic properties of hydrocarbons result in poor porosity, loss of the nutritional cycle, and lack of microbiological diversity, as observed by Sattar et al. (2022). Hydrocarbons are easily absorbed into the soil, thus leaving behind pollutants with lasting impacts, which lower agricultural productivity and inhibit the rehabilitation of the

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affected lands (Yu et al., 2023). In addition, hydrocarbon body water pollution has caused tremendous damage to the ecosystem. In this case, the dissolved hydrocarbons, such as BTEX, penetrate the groundwater, while the light hydrocarbons result in sheen on the surface of water, which does not allow light penetration, thereby interfering with photosynthesis in aquatic plants (Rodrigo-Ilarri et al., 2023).

Conventional Approaches to Hydrocarbon Remediation

Environments that are hydrocarbon-contaminated have over the years been remediated using various methods. The traditional methods have been employed extensively in the remediation of oil spill sites, industrial sites, and soil remediation. These methods have been effective in the quick remediation and containment of hydrocarbon contaminants (Ambaye et al., 2022). However, literature has shown that there is sufficient evidence that points to the limitations of the traditional methods in the remediation of hydrocarbon contaminants. These limitations include the expensiveness of the methods, the degradation efficiency of the methods in the removal of hydrocarbon contaminants, and the possibility of pollution in the environment, which questions the sustainability of the methods. The use of physical methods in the remediation of hydrocarbon contaminants is one of the oldest and most common methods. The use of physical methods involves the encapsulation and separation of hydrocarbons.

Bioremediation as an Eco-Friendly Alternative

Due to the economical and green benefits that result from the process of bioremediation, on one hand, it was also recognized as one of the most promising alternatives to traditional remediation techniques. The process of employing living organisms, especially microbiota, for the detoxification and/or degradation of environmental pollutants into less harmful compounds, such as carbon dioxide, water, and biomass, could be described as the process of bioremediation in the broadest definition of the terms (Perez-Vazquez et al., 2024). Unlike other techniques of bioremediation, which involve physics and chemistry and offer no environmental restoration toward sustainability, bioremediation primarily focuses on environmental restoration and plays no

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significant role in secondary contamination. It is based on the metabolic diversity of microorganisms that have the ability to utilize hydrocarbons as a source of carbon and nutrients for growth, leading to the simultaneous degradation of contaminants and the growth of microorganisms (Elshafei & Mansour, 2024).

Influence of pH on Microbial Hydrocarbon Degradation

Review of the literature will indicate that temperature and pH are critical in regulating microbial biodegradation of hydrocarbons. Bile salts and activated charcoal are important in the promotion of microbial growth and bioavailability of hydrocarbons. Compost and plant extracts are necessary for the promotion of successful biodegradation of hydrocarbons. The pH of the soil, be it basic, neutral, or acidic, affects the biodegradation process and proliferation of bacteria. All literature review estimates have shown that the success of hydrocarbon contamination biodegradation is not affected by pH variations (lmron et al., 2020).

Effect of temperature on bioremediation efficiency

One of the factors related to the growth of bacteria includes temperature. In the biological treatment, it is very vital, and the effect of temperature on the efficiency of the removal of hydrocarbon contaminants is quite large. It was discovered from the experiment that the range of the best temperature for the decomposition of hydrocarbons in polluted soils, which occur when the microorganisms interact, lies within 30 to 40 degrees Celsius. Thus, it could be noted that the temperature plays a vital role during the interaction of the microorganisms. According to the experiment, it was discovered that as the temperature decreases, the microorganisms' ability to decompose hydrocarbons lowers due to the viscosity of oil and decreases in enzymatic activity (Nengzi et al., 2023).

Role of biochar and activated charcoal in bioremediation

This is a denser state of carbon and is formed through the thermal degradation of biomass with fewer oxygen atoms. According to current research, the addition of activated charcoal to the mixture can be used to enhance the biodegradation of the contaminants. The level of microbial activities within the soil was upregulated. This resulted in an acceleration of the process of microbial degradation of petroleum hydrocarbon contaminants within the soil. This was a study conducted to test the efficacy of activated charcoal as a tool to enhance the biodegradation of the contaminants within petroleum hydrocarbon-contaminated soil (Chaudhary et al., 2021). Another study traced the interaction effect of using biochar with bacteria present in petroleum hydrocarbon-contaminated soils and came to the conclusion that biochar can be employed to enhance bacterial activities for the better efficiency of bacteria (Guo et al., 2022).

Role of bile salts in enhancing microbial activity

Bile salts is a product that is produced in the liver and stores the gall bladder and such products have the ability to emulsify fats and hence can be used for the purpose of improving biodegradation so as to reduce carbon (Ahmed, 2022). In a research study done by Franziska Maria., et al, the researchers aimed at evaluating the efficacy of Bacillus and Pseudomonas strains for hydrocarbon degradation in the presence of bile salts. The scientists noted that the addition of bile salts enhanced the growth of the bacteria and the degradation of the hydrocarbons and therefore can be used as biocatalysts. In another study, the role of bile salts in enhancing the biodegradation of diesel oil was proved, and it was proved that the addition of bile salts enhances the rate of degradation of hydrocarbons and that the addition of bile salts enhances the activity and biofilm on the surface of polluted sites, hence enhancing the rate of degradation of hydrocarbons (Elumalai et al., 2021).

Fertilization as a Biostimulation Strategy

Fertilization may raise the percentage of hydrocarbon decomposition in soil. Fertilization should be researched and analyzed extensively (Henderson et al., 2023). A researched study for oily sludge contaminated soil biological treatment, the study had two phases, first, natural bacteria found in the locality were used to begin the hydrocarbon decomposition, second phase, additional nutrients were added and there was an improvement in bacterial activity and growth, which led to better decomposition processes. When fruit-based fertilizer is added in fertilizing the contaminated soil for oil, the result showed that the rate of hydrocarbon decomposition was increased by about 6-6.5 times higher than the non-fertilized contaminated soil, in addition, the bacterial community analysis showed a clear difference in the bacteria

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population between the fertilized and fertilized group (Yasir et al., 2025).

Enhancements to Bioremediation Efficiency

Although bioremediation is a green technology compared to other remediation technologies, it is assumed that the effectiveness of bioremediation technology in the real world will be influenced by environmental factors and hydrocarbon availability. Hydrocarbon compounds are hydrophobic, less soluble, and have the ability to adsorb on soil particles, making it difficult for microbes to degrade hydrocarbons (Hadibarata et al., 2025). In addition, there may be a possibility of nutrient deficiency, pH, and temperature that may influence the microbial degradation of pollutants in the systems. To address these challenges, scientists have recently started to explore the list of improvement strategies, such as nutrients, biochar, biosurfactants, bile salts, and plant residues (Bala et al., 2022).

METHODOLOGY

The approach to be employed in this study in assessing the effectiveness of an integrated bioremediation technique in the remediation of petroleum hydrocarbon-contaminated soils will be an experimental approach. The approach will be guided by the following steps: the assessment of the potential of natural enhancement materials such as bile salts, activated charcoal, plant extracts, compost, and environmental control parameters such as pH and temperature in enhancing the biodegradation of petroleum hydrocarbons by microorganisms. Microorganisms with hydrocarbon degradation potential will be isolated from petroleum hydrocarbon-contaminated soils collected from oil-impacted sites (Ajona & Vasanthi, 2020). The soil samples will be enriched with a medium consisting of a mineral salt solution with petroleum hydrocarbons as the carbon and energy source for hydrocarbon-degrading microorganisms. The isolated microbial colonies will be purified and assessed for hydrocarbon degradation potential through gravimetric analysis and/or spectrophotometric and chromatographic techniques. The microorganisms with high hydrocarbon degradation efficiency will be characterized on the basis of their morphological, biochemical, and molecular properties for accurate identification and reproducibility (Zaman et al., 2025).

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To determine the best environmental factors for biodegradation, controlled batch experiments will be conducted. The microbiological matter will then come into contact with the flasks containing the hydrocarbon-containing fluids or polluted soil. It will be kept in an environment that has the desired temperature, which is close to that of the natural environment, while the pH will be maintained in the range of acidic to slightly alkaline. At this time, the amount of TPH will be gauged in order to determine the rate of degrading the hydrocarbons. To establish the relationship between environmental factors and the efficiency of biodegradation, the growth potential will be measured (Gu et al., 2023).

The use of bile salts and activated charcoal as natural enhancers would be investigated through a series of factorial experiments, where bile salts would be applied in varying concentrations to investigate their use as biosurfactants, which are known to enhance the solubility of hydrocarbons. Activated charcoal would be employed in the treatment systems to act as an adsorbent for the hydrocarbon compounds, as well as to act as a medium to support microbial colonization. Different treatment scenarios would be investigated, including the use of bile salts individually or in combination with charcoal to investigate their synergistic or antagonist effect on the biodegradation of hydrocarbon compounds. The effect on the composition of hydrocarbon compounds would be investigated using appropriate methods, while the microbial populations would be investigated to assess their role in enhancing microbial activity with the use of these enhancers (Conlon et al., 2025).

To further enhance the efficiency of microbes, plant extracts and compost will be employed in the remediation process. Eucalyptus leaf extracts were employed in this research due to its active features. In this research, compost was used as a treatment for the soil, which improves the texture, water-holding capacity, and value of the soil for microorganism growth (Ayadi et al., 2025).

However, in the determination of the effect of the process in the ecosystem as a whole, the health of the soil will also be determined in the process. The factors in the soil that will be measured include pH level, moisture content, organics, and nutrients such as nitrogen and phosphorus. The rate of hydrocarbon degradation will also be determined in accordance with the degradation of total petroleum

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hydrocarbon content and other hydrocarbon compounds as well. The experiments will be conducted in triplicate to ensure that they can be statistically analyzed. The results will then be analyzed to establish the optimal conditions and natural amendments using tools such as analysis of variance and other multivariate techniques to compare the results and establish the optimal conditions for a scientifically viable and applicable approach to the integration of bioremediation, which can then be applied in the field to clean hydrocarbon-contaminated environments (Pishini et al., 2025).

RESULT

The findings of the study make it abundantly clear that environmental factors had a definite impact on the degradation of petroleum hydrocarbons by microbes. The mode of degrading TPH was affected by temperature and pH.

Table1. Temperature And Ph Affect The Degradation Of Total Petroleum Hydrocarbons (TPH).

pH Level	Temperature (°C)	TPH Initial (mg/kg)	TPH Final (mg/kg)	Degradation Efficiency (%)
5.5	25	10,000	7,800	22%
6.5	30	10,000	5,400	46%
7.0	30	10,000	3,200	68%
7.5	35	10,000	2,900	71%
8.5	35	10,000	4,600	54%
Optimal condition: pH 7.5 and 35°C				

As indicated in Table 1, the degradation efficiency increased with the increase in pH and temperature up to the point of optimality. The lowest degradation efficiency was obtained at a pH of 5.5 and a temperature of 25°C, while the highest degradation efficiency was obtained at a pH of 7.5 and a temperature of 35°C. There was a decrease in degradation efficiency at a pH of 8.5, indicating a decrease in performance from the point of optimality.

Table 2. Effect of Bile Salts on Hydrocarbon Degradation

Bile Salt Concentration (%)	TPH Initial (mg/kg)	TPH Final (mg/kg)	Degradation Efficiency (%)	Microbial Growth (CFU/g $\times 10^6$)
0 (Control)	10,000	4,800	52%	3.2
0.1	10,000	3,600	64%	4.5
0.2	10,000	2,700	73%	5.8

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Bile Salt Concentration (%)	TPH Initial (mg/kg)	TPH Final (mg/kg)	Degradation Efficiency (%)	Microbial Growth (CFU/g $\times10^6$)
0.3	10,000	2,400	76%	6.0
0.5	10,000	3,900	61%	4.1
Best performance: 0.3% bile salts				

This is further supported by Table 2, where increasing bile salt concentration from 0% to 0.3% resulted in increasing degradation efficiency from 52% to 76%, while microbial growth increased from 3.2 to 6.0 CFU/g $\times10^6$. However, increasing bile salt concentration to 0.5% resulted in decreasing degradation efficiency to 61%, accompanied by reduced microbial.

Table 3: Combined Effect of Bile Salts and Activated Charcoal

Treatment	TPH Initial (mg/kg)	TPH Final (mg/kg)	Degradation Efficiency (%)
Control (No amendment)	10,000	4,800	52%
Charcoal only	10,000	3,500	65%
Bile salts only (0.3%)	10,000	2,400	76%
Charcoal + Bile salts (0.3%)	10,000	1,600	84%

As shown in Table 3, both individual and combined treatments were found to increase degradation efficiency compared to the control, which was 52%. Activated charcoal, when used alone, recorded 65%, while bile salts, when used alone (0.3%), recorded 76%. However, the combined treatment recorded the highest degradation efficiency, which was 84%.

Table 4. Effect of Plant Extract and Compost on Soil Health and Degradation

Treatment	Microbial Count (CFU/g $\times10^6$)	Soil Organic Matter (%)	TPH Degradation (%)
Control	3.2	1.8	52%
Eucalyptus Extract	4.9	1.9	66%
Compost	6.1	3.4	74%
Extract + Compost	7.3	3.6	82%

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As shown in Table 4, organic amendments were found to increase microbial counts, organic matter, and degradation efficiency. The combined treatment, which included both extract and compost, recorded the highest microbial counts, which were 7.3 CFU/g $\times 10^6$, and degradation efficiency, which was 82%, compared to the control, which was 52%.

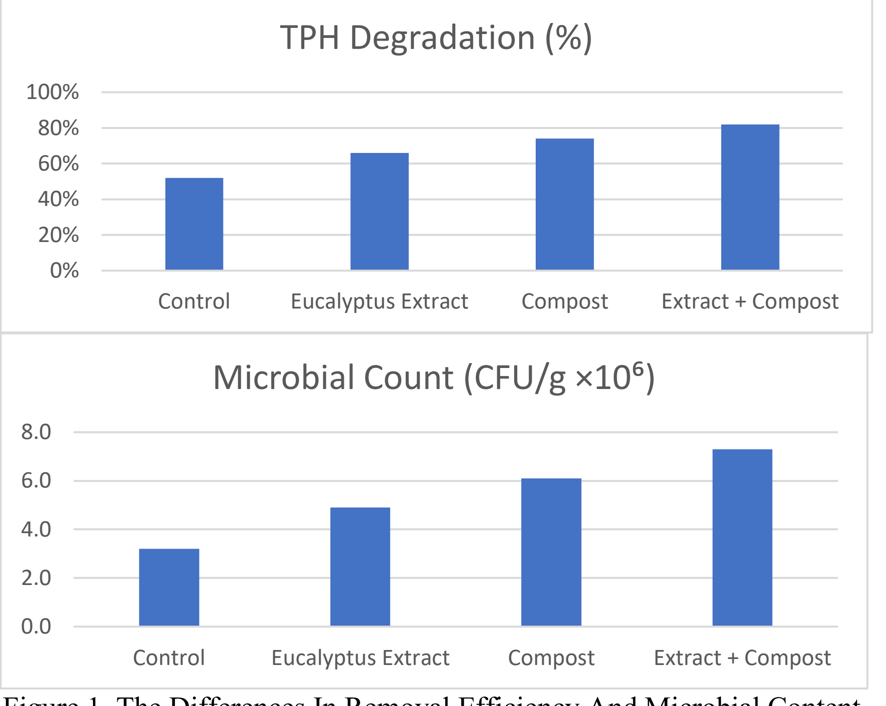

Figure 1. The Differences In Removal Efficiency And Microbial Content

From the results, it is clear that as the microbial count increases, the efficiency of degradation also increases. The lowest values were recorded in the control, i.e., 3.2 CFU/g $\times 10^6$ and 52% (figure 1). The eucalyptus extract and compost treatments increased the values of microbial growth and degradation efficiency. The combined treatment resulted in the highest value for microbial growth and degradation efficiency. This is an indication that the two treatments have a synergistic effect in enhancing degradation efficiency.

DISCUSSION

The result of this study clearly indicates and demonstrates the effectiveness and efficiency of integrating environmental control

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with natural enhancement materials in the bioremediation of petroleum hydrocarbon-contaminated soils. The result of this study has shown that the findings of this study are in line with the increasing recognition and acknowledgement of the fact that microbial degradation of hydrocarbon contaminants, without the optimization of the environmental conditions, is characterized by limited efficiency in the bioremediation of hydrocarbon-contaminated soils (Mekonnen et al., 2024). The examination of the impacts of pH, temperature, bile salts, activated charcoal, plant extracts, and compost in this study clearly indicates the efficiency and effectiveness of the synergistic interactions in the biodegradation of hydrocarbon contaminants.

It was also shown that microbial processes and the rate of hydrocarbon decomposition are affected by various environmental factors, in particular pH and temperature. It was found that the highest rate of hydrocarbon removal was obtained when the pH was in the slightly alkaline range (pH 7.0-7.5) and the temperature was in the range of 30-35 C, which was close to optimal conditions for the growth of most bacteria used for hydrocarbon decomposition. The rate of hydrocarbon decomposition decreased when the pH was decreased to the acidic zone; this may be explained by the inhibition of enzyme and microbe metabolism (Das et al., 2023). In a similar manner, sub-optimal temperatures were also shown to influence the growth of microorganisms and the enzymatic reactions that are significant in the biodegradation of hydrocarbons. This further underlines the significance of the biodegradation process and the need for the provision of optimal environmental conditions for the process to occur. In areas such as Sirte, the natural temperature of the environment may already be conducive for the growth of mesophilic microorganisms, and the modification of the pH of the soil may be the most significant factor for the improvement of the process (Rekadwad et al., 2023).

The positive effect of bile salts on the rates of hydrocarbon degradation, as observed in this study, therefore emphasizes the need to concentrate on hydrocarbon bioavailability. Petroleum hydrocarbons, particularly hydrocarbons of higher molecular weight, have hydrophobic properties and tend to adhere to the particles of the soil, thus becoming unavailable for microbial degradation. However, the addition of bile salts results in an

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enhanced rate of hydrocarbon degradation, particularly at moderate concentrations (Abbot et al., 2022). This suggests that bile salts have enhanced hydrocarbon solubilization, thus making them hydrocarbon availability for microbial degradation. However, the low efficiency of microbial growth and degradation efficiency at higher bile salt concentrations suggests that care should be taken when applying bile salts as a surfactant. This is because bile salts, when used at higher concentrations, have the potential to become toxic to microbial cells, which is also the case for synthetic surfactant (Maher et al., 2023).

The complementary but distinct role of activated charcoal in the biodegradation process was also observed. As noted by Chew et al. (2023), activated charcoal, on account of its extremely porous texture, is able to avail considerable surface area for the potential adsorption of hydrocarbons, in addition to providing a microhabitat for the development of microorganisms. In this research, the treatment containing charcoal degraded faster than the control treatment, indicating that apart from making hydrocarbons immobile, adsorption also helped bring microorganisms closer to the contaminants. The complementary role of charcoal as a sorbent and a microhabitat for microorganisms may have contributed to the increased efficiency of biodegradation. Activated charcoal can also serve as a buffer against environmental changes and help to protect microorganisms from toxic levels of hydrocarbons, thereby increasing their survival and activity (Bolan et al., 2023).

It has also been established that the combined use of bile salts and activated charcoal showed the highest efficiency in the degradation of hydrocarbons. Thus, it becomes evident that the synergistic effect has occurred (Valencia-Luna et al., 2025). Bile salts increased the availability of hydrocarbon in the aqueous phase, and the charcoal covered the microbes with concentrated contaminants and stable colonization sites. The synergistic effect therefore proves that the use of a combination of treatments is more effective compared to the use of a single treatment. The use of a combination of treatments is effective in overcoming several limiting factors. The synergistic effect has therefore been statistically significant, proving that the use of a combination of treatments is more effective (Morales-Durán et al., 2024).

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Moreover, other amendments such as compost and plant extracts have been reported to increase the efficiency of the process of biodegradation. The application of compost to the contaminated soil was reported to significantly increase the content of organic matter, nutrient availability, and microbial densities in the soil (Lhaj et al., 2025). This is all beneficial to the microbial populations because they will have an environment conducive to the process of biodegradation. Nutrient availability is usually a limiting factor in hydrocarbon-contaminated soils because of the high amounts of carbon in the petroleum products, which disrupt the normal balance of carbon and nitrogen required by the microbes in order for their processes to continue (Siddique et al., 2024).

The application of Eucalyptus plant extract was also observed to increase microbial activity and efficiency of degradation. Although they are known for their antimicrobial activities, they can contain other bioactive compounds in small amounts, which can stimulate microbial metabolism or be mild surfactants (Salvatori et al., 2023). Among the various amendments applied in the present study, it was noticed that the combined treatment of plant extracts and compost showed maximal microbial populations and hydrocarbon degradation. This suggests that plant-derived materials can increase microbial resilience when applied at controlled levels. The combination of compost and plant extract also supports the notion that natural substances can be applied in bioremediation (Vaou et al., 2022).

In addition to the degradation of contaminants, the enhancement of the health of the soil, which was noted in the study, indicates that bioremediation has the potential to bring about ecological restoration. Quantities of organic matter, pH, and diversity of microorganisms in the soil can be considered as a measure of health of the soil. As such, these are of crucial importance to the functionality of the soil. The diversity of microbes present in the soil is of great importance to the production of the soil. Hence, the benefit of the integrated strategy can be seen as the restoration of soil productivity together with the degradation of pollutants (Chen et al., 2024).

The results obtained in this research are very important for oil-impacted sites like the Sirte region in Libya. In this region, hydrocarbon pollution is an issue due to the presence of fuel stations

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and storage facilities. In this region, conventional bioremediation techniques may be very costly and/or logistically difficult. In contrast, the bioremediation technique employed in this research is based on natural phenomena and the use of available materials (Fei-Baffoe et al., 2024). The adjustment of pH levels in the soil and the use of organic matter and other easily accessible materials are techniques that are easily applicable in this region. However, some aspects need consideration before the application of this technique. In this case, the situation is more complex in the field compared to the lab. Soils of different types and climatic conditions are some of the challenges that need consideration (Ţopa et al., 2025).

However, long-term research has been needed to ensure that the soil ecology was fully restored and that these degradation byproducts do not accumulate in the soil. In addition, the optimum amount of natural amendments required for various types of soil would be critical to avoid any inhibitory effects. The present study would bridge a wide knowledge gap by demonstrating how these materials for environmental control and natural enhancements could be integrated into a unified approach to bioremediation (Oyebiyi et al., 2026). The present study has confirmed that the optimum pH and temperature, bile salts for hydrocarbon bioavailability, charcoal for microbial habitats, and compost and plant extracts for soil fertility enhancement are all critical to ensure better remediation results. This is a viable approach to managing petroleum hydrocarbon pollution in a sustainable manner (Tang, 2025).

CONCLUSION

This study revealed the possibility of enhancing the biodegradation of petroleum hydrocarbon–contaminated soils through the integration of environmental control factors and natural enhancement materials. This study proved the possibility of enhancing the biodegradation of total petroleum hydrocarbons (TPH) through the optimization of pH and temperature to create a favorable condition for the growth of microorganisms. A near-neutral to slightly alkaline pH and moderate temperatures were found to be optimal conditions to maximize the efficiency of biodegradation. Bile salts increased the bioavailability of hydrocarbons through dispersion and solubilization of hydrophobic

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compounds, but high concentrations of bile salts showed inhibitory properties.

Activated charcoal also had a part in the adsorption of hydrocarbons, as it provided adsorption sites as well as habitats for microorganisms. The mixture of bile salts and charcoal had the best degradation rate, indicating the synergy between the two approaches of enhancing degradation. Moreover, the incorporation of compost and plant extracts helped in improving the soil as well as microbial development. Although the use of plant extracts was beneficial in promoting microbial growth and resistance, compost was found beneficial in the development of soil and microorganisms. The consortia of organic amendments facilitated in the ecological restoration of the polluted site and in the degradation of hydrocarbons. To conclude, the method of bioremediation was found to be efficient and environmentally friendly and could be beneficial in controlling petroleum hydrocarbon pollution.

It has been recommended that the future remediation processes should be made to rest on the foundation of the application of an integrated approach to bioremediation instead of individual practices, based on the implications of the study. To further enhance the pH, temperature, and concentration levels of the amendment, field-scale work needs to be conducted. To make the system economical and a sustainable process, the application of natural materials such as compost, plants, and charcoal needs to be encouraged. The levels of bile salt compounds need to be monitored to prevent any toxicity. To ensure total recovery of the health of the affected soil, ecological work needs to be conducted. Further, it is advised that additional studies should be conducted in order to establish whether the use of bacteria, other plant-based biosurfactants, and monitors improves the degrading process. For the process to be sustainable while affecting the least amount of ecosystem, policymakers in areas that have been affected by oil spillage have to understand the possibility of monetizing on natural resources.

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