Annals of
Biotechnology and Biomedical Science

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Sustainable Bioremediation of Marine Oil Contaminants by Novel Strains of *Bacillus subtilis* and *Staphylococcus aureus*

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Submission: June 14, 2025; Published: June 26, 2025

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ABSTRACT

Urban environmental pollution caused by the indiscriminate disposal of used engine oil poses a significant public health threat worldwide due to its persistent toxicity. This study evaluates the biodegradation potential of selected bacterial isolates against used engine oil and mineral oil contaminants in marine water environments. The agar well diffusion method was employed to assess the hydrocarbon-degrading capabilities of four bacterial strains.

Among the tested isolates, *Bacillus subtilis* showed notable biodegradation, producing clear zones of 48 ± 0.5 mm and 35 ± 0.2 mm for used engine oil and mineral oil, respectively. Likewise, Staphylococcus aureus exhibited strong degradation activity with zones measuring 52 ± 0.2 mm for used engine oil and 47 ± 0.2 mm for mineral oil. Conversely, *Salmonella enterica* and *Escherichia coli* displayed minimal degradation under the same conditions. Ciprofloxacin and sterile water were used as positive and negative controls, respectively.

These findings highlight the significant biodegradative potential of *S. aureus* and *B. subtilis*, supporting their application in bioremediation strategies aimed at restoring hydrocarbon-contaminated aquatic ecosystems. The study emphasizes the role of microbial agents in promoting environmental sustainability and reducing the ecological and health risks associated with petroleum pollutants.

KEYWORDS

Biodegradation, Used engine oil, environment sustainability, microorganisms, bioremediation, *Bacillus subtilis*

Citation: Vibha Bhardwaj (2025) Sustainable Bioremediation of Marine Oil Contaminants by Novel Strains of Bacillus Subtilis and Staphylococcus Aureus. Ann Biotech & Biomed Sci 1(1): 1-10.

ABBREVIATIONS

SD: Standard Deviation; ATCC: American Type Culture Collection; MHA: Muller Hinton Agar; UEO: Used Engine oil; C: Ciprofloxacin; NA: Nutrient Agar

INTRODUCTION

The rapid advancement of technology has greatly enhanced human convenience and productivity. However, this development has often come at the expense of environmental health, which has frequently been neglected in the pursuit of industrial and economic growth. One major contributor to environmental degradation is the widespread use of automobiles powered by petroleum-based fuels such as gasoline and diesel. Although these vehicles have significantly improved transportation efficiency, they have also led to severe environmental consequences through the emission of harmful gases and the generation of hydrocarbon-based waste products [1,2].

Among these pollutants, used engine oil—applied for lubricating internal combustion engines—represents a significant environmental hazard. In many regions, waste oil from auto-mechanic workshops is improperly discarded, often through incineration or direct dumping into soil and water bodies. An estimated one billion gallons of spent lubricating oil are generated annually, with only a small fraction being recycled [3]. This waste oil contains high levels of toxic substances, including heavy metals and polycyclic aromatic hydrocarbons (PAHs), posing serious risks to human health, fauna, and flora [4].

Conventional methods used to remediate petroleum-contaminated environments—such as physicochemical treatments—are often costly, inefficient, and environmentally damaging, as they may merely shift the contamination from one medium to another. In contrast, microbial degradation offers a cost-effective and eco-friendly alternative that can result in the complete breakdown of hydrocarbons into harmless end-products. Several microorganisms, including bacteria, fungi, yeasts, and algae, have demonstrated the ability to degrade hydrocarbons via enzymatic and metabolic processes [5].

Numerous studies have documented the ability of microbes to utilize petroleum hydrocarbons as their sole carbon and energy source. The effectiveness of microbial degradation depends on both the chemical composition of the oil and environmental factors such as temperature, pH, and nutrient availability [6,7]. Specific bacterial genera such as *Pseudomonas, Bacillus, Micrococcus, Acinetobacter, and Enterobacter* have been identified as potent hydrocarbon degraders in oil-contaminated soils [8,9]. For instance, *Pseudomonas aeruginosa* SU-1 has been shown to effectively degrade motor oil when used as the sole carbon source [1]. Similarly, bacterial consortia including *Stenotrophomonas maltophilia*, *Bacillus pumilus*, and *B. cereus* have demonstrated efficient biodegradation of diesel and engine oils [10-12].

Bioremediation utilizing native microorganisms has emerged as a promising strategy due to its adaptability, environmental safety, and low operational cost. Microbes can detoxify organic pollutants through mineralization, polymerization, or transformation processes [13,14].

The primary objective of this study is to investigate the role of bacteria in the biodegradation of petroleum-based contaminants. By isolating and assessing bacterial strains capable of utilizing hydrocarbons as their sole carbon and energy source, this research aims to explore their potential for use in sustainable and efficient bioremediation of oil-contaminated environments.

MATERIALS AND METHODS

Collection of Used Engine oil (UEO) and Mineral oil samples

Two samples of used engine oil (UEO), each of 100 mL volume, were collected from an ADNOC petrol station located in Khuzam, Ras Al Khaimah, United Arab Emirates. The samples were collected aseptically in sterile 500-mL screw-cap glass bottles to prevent contamination. Following collection, the samples were transported to the laboratory under controlled conditions and stored at 4°C until further analysis. The collected UEO samples exhibited a blackish-brown coloration, indicative of extensive use and contamination. In addition, mineral oil (Figure 1b) was procured from LTA Srl, Italy, for comparative analysis (Figure 1).

Microbial Strains and Culture Conditions

The present study investigated the biodegradation potential of used engine oil (UEO) and mineral oil using four selected bacterial strains: *Bacillus subtilis, Escherichia coli, Salmonella enterica,* and Staphylococcus aureus. All strains were procured from the American Type Culture Collection (ATCC) and supplied by LTA S.r.l., Italy.

Pure cultures of each bacterial strain were maintained on nutrient agar slants at 4 °C for long-term preservation. Prior to experimentation, subcultures were freshly prepared in nutrient broth and incubated under optimal growth conditions [15]. All experimental assays were conducted in triplicates to ensure reproducibility and statistical reliability of the results.

Methodology for the Oil Degradation Potential of Selected Bacteria

Inocula Preparation: Bacterial isolates were initially cultured in 5 mL of sterile Nutrient Broth and incubated at 37 °C for 18 hours to obtain actively growing log-phase cultures. These overnight cultures served as inocula for subsequent experimental assays, including antimicrobial and biodegradation evaluations.

Assessment of Degradation Activity by Agar Well Diffusion Method: The degradation potential of the selected bacterial strains was evaluated using the agar well diffusion method, following the protocol described by Bhardwaj [16], with slight modifications. Nutrient Agar (NA) and Mueller-Hinton Agar (MHA) were used as culture media. Standardized bacterial suspensions were uniformly inoculated onto the surface of MHA plates.

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Wells of 6 mm diameter were aseptically punched into the agar, and 30 µL of each oil sample—used engine oil (UEO) and mineral oil—was dispensed into the wells.

The plates were incubated at 37 °C for 24–48 hours. Zones of degradation around the wells were measured in millimeters to assess the efficacy of bacterial degradation activity. Ciprofloxacin served as a positive control, while sterile distilled water was used as a negative control. All experiments were conducted in triplicate, and the mean zone diameters were recorded. The entire procedure was performed under strict aseptic conditions to prevent microbial contamination.

RESULTS

The application of microorganisms for the remediation of oil-contaminated environments has gained increasing attention due to the limitations of conventional methods such as surfactant washing and incineration, which often lead to the formation of more toxic byproducts and are economically unfeasible [17]. Bioremediation, particularly using hydrocarbon-degrading bacteria, is considered a more sustainable and environmentally friendly alternative.

Numerous bacterial species capable of hydrocarbon degradation have been reported, and these organisms are widely distributed in diverse environments, including soil, marine, and freshwater ecosystems [18,19]. Previous studies have established that bacteria isolated from oil-contaminated sites tend to possess greater degradative potential [20].

In the present study, the ability of four bacterial strains—*Bacillus subtilis, Escherichia coli, Salmonella enterica, and Staphylococcus aureus*—to degrade hydrocarbon pollutants was evaluated. The agar well diffusion method was employed to assess the degradation potential of used engine oil (UEO) and mineral oil.

Zones of degradation observed around the wells indicated the enzymatic activity of the bacteria in breaking down the oil components. The diameter of these zones, measured in millimeters, provided a comparative measure of the degradation efficiency of each strain. The presence of clear zones confirmed the utilization of oil as a carbon source by the respective bacteria, thereby validating their bioremediation potential. The use of ciprofloxacin as a positive control ensured the validity of the assay, while sterile distilled water served as the negative control.

All assays were conducted in triplicate to ensure statistical reliability, and the mean values were recorded. The results demonstrated variability in degradation efficacy among the tested strains, suggesting species-specific differences in enzymatic capacity and hydrocarbon metabolism. These findings support the potential use of indigenous bacterial strains in the development of eco-friendly and cost-effective strategies for the bioremediation of petroleum-contaminated environments.

Biodegradation of Used and Mineral Oil by Bacterial Strains

The biodegradation potential of the four selected bacterial strains was assessed against two types of used engine oil (UEO I and UEO II) and mineral oil using the agar well diffusion method. Among all tested combinations, *Staphylococcus aureus* exhibited the highest degradation activity against UEO II, producing a clear zone of 52 ± 0.6 mm on Nutrient Agar (NA). Mineral oil also showed significant susceptibility, with a maximum degradation zone of 47 ± 0.2 mm by *S. aureus* on NA (Table 1).

Staphylococcus aureus **diameter of zone (cm)**						
		Water	**Mineral oil**	**Antibiotic**	**Used oil I**	**Used oil II**
MHA	Plate 1				4.0± 0.6	5.1± 0.3
	Plate 2		3.8± 0.6			4.3± 0.1
NA	Plate 1				3.0± 0.5	5.2± 0.2
	Plate 2	No zone				4.9± 0.5
	Plate 3		4.7± 0.2	4.8± 0.4		

Note: Values represent the mean ± standard deviation of triplicate experiments.

Table 1: Results of degradation zone of used engine oil and mineral oil against test bacterial strain Staphylococcus

For UEO I, *S. aureus* produced a degradation zone of 40 ± 0.6 mm on Mueller-Hinton Agar (MHA) and 30 ± 0.6 mm on NA (Figure 3). Similarly, *Bacillus subtilis* demonstrated strong degradation activity, with the maximum zone of 48 ± 0.5 mm on NA against UEO II (Table 2). In contrast, mineral oil showed the lowest degradation zone when tested on MHA (Figure 2).

Bacillus subtilis **diameter of zone (cm)**						
		Water	**Mineral oil**	**Antibiotic**	**Used oil I**	**Used oil II**
MHA	Plate 1		3.4± 0.1	3.5± 0.4		
	Plate 2			4.1± 0.6	2.8± 0.2	
NA	Plate 1				3.4± 0.1	4.0± 0.6
	Plate 2		3.5± 0.2		4.0± 0.4	
	Plate 3	No zone				4.8± 0.5

Note: Values represent the mean ± standard deviation of triplicate experiments.

Table 2: Results of degradation zone of Used Engine oil and Mineral oil against test bacterial strain Staphylococcus

The high degradation activity observed for *Bacillus subtilis* supports previous reports that members of the *Bacillus genus* are efficient hydrocarbon degraders [21]. Furthermore, numerous studies have also identified Pseudomonas species as potent degraders of polycyclic aromatic hydrocarbons [22], highlighting the broader relevance of microbial bioremediation.

These findings are in agreement with those reported by Kumar [23], who demonstrated that *Bacillus subtilis* PD6 and *Enterobacter sp.* PD11 effectively degraded used engine oil, although their efficiency was lower compared to *Bacillus sp.* PD9 and *Bacillus sp.* PD14.

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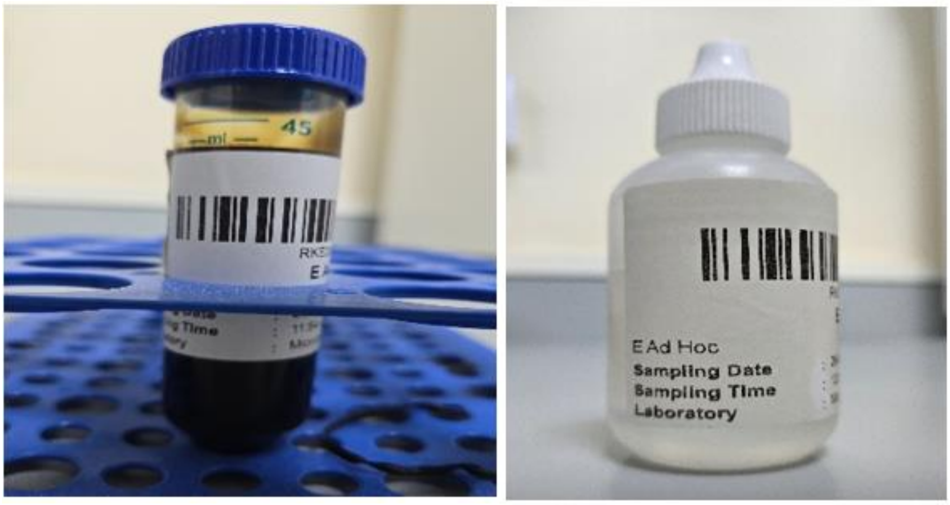
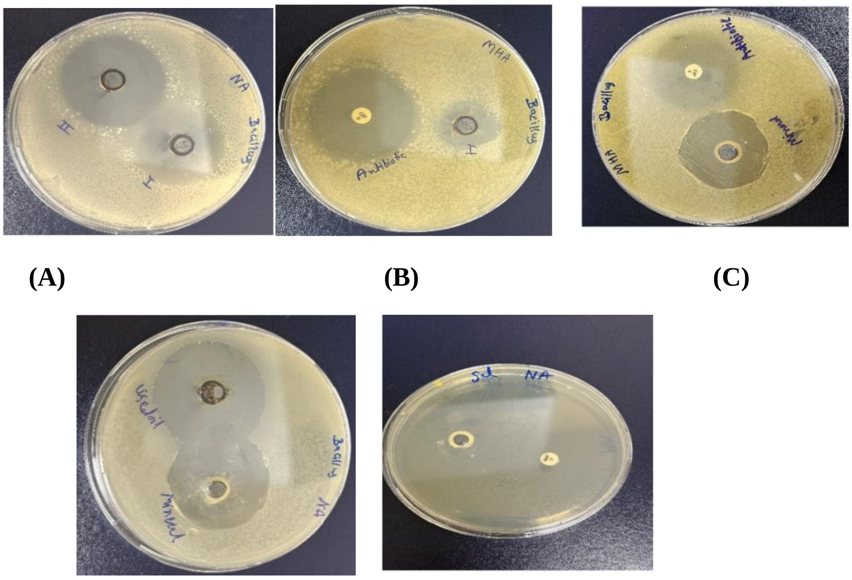
The observed differences in degradation zones among bacterial strains and oil types indicate species-specific and substrate-specific variability in biodegradation efficiency. This suggests that the selection of appropriate microbial strains is critical for the successful application of bioremediation strategies in oil-contaminated environments (Table 1, Table 2, Figure 2, Figure 3).



(a) **(b)**

Figure 1: (a) Used Engine Oil, (b) Mineral oil



(A) **(B)** **(C)**

(D) **(E)**

(A) *Bacillus subtilis* with UEO 1 and UEO II

(B) *Bacillus subtilis* with UEO1 and antibiotic (control)

(C) *Bacillus subtilis* with Mineral oil and Antibiotic

(D) *Bacillus subtilis* with Mineral oil and UEO 1

(E) No degradation zone by *Salmonella enterica*

Figure 2: Visualization of oil degradation zones by tested bacterial strains, demonstrating their comparative degradation efficiency.

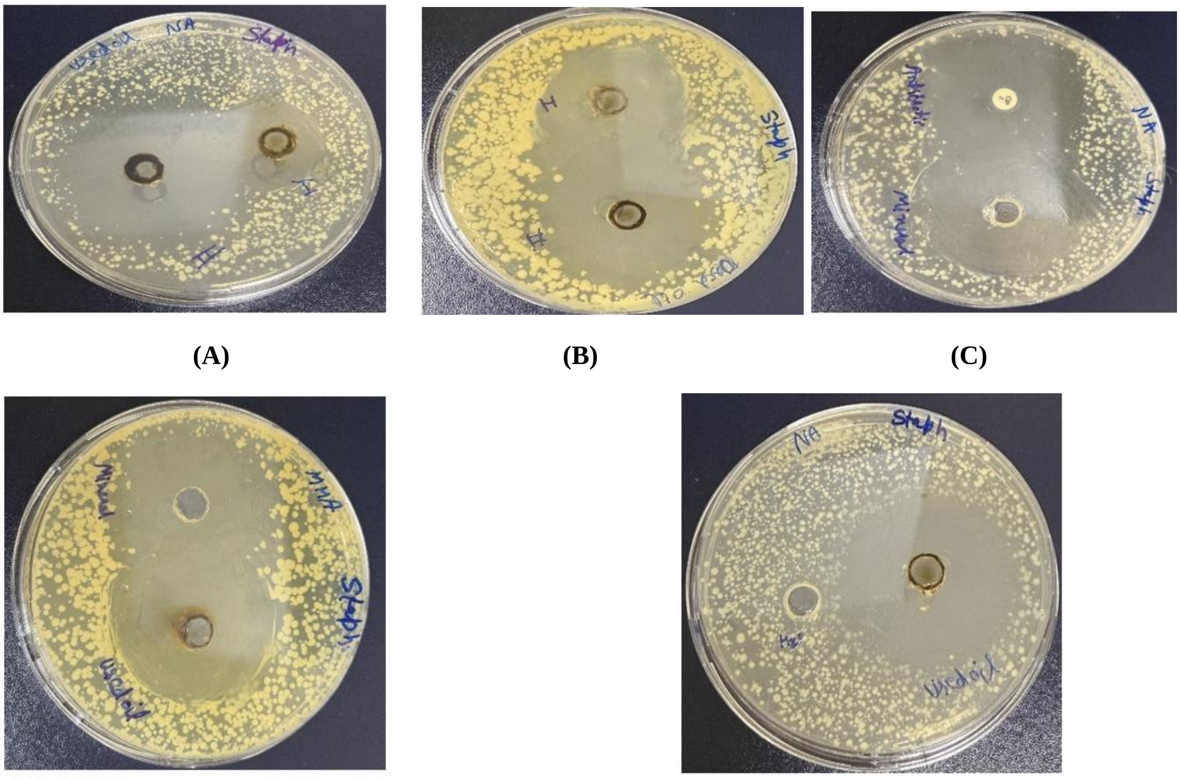
(A) Degradation zones formed by Bacillus subtilis in the presence of Used Engine Oil I (UEO I) and Used Engine Oil II (UEO II).

(B) Degradation zone formed by Bacillus subtilis with UEO I and an antibiotic control.

(C) Degradation zone produced by Bacillus subtilis with mineral oil and antibiotic control.

(D) Degradation zones formed by Bacillus subtilis in the presence of mineral oil and UEO I.

(E) Absence of degradation zone by Salmonella enterica, indicating no oil degradation activity.



(A) **(B)** **(C)**

(D) **(E)**

(A) *Staphylococcus aureus* with UEO 1 and UEO II on NA
(B) *Staphylococcus aureus* with UEO 1 and UEO II on MHA
(C) *Staphylococcus aureus* with Mineral oil and Antibiotic
(D) *Staphylococcus aureus* with Mineral oil and UEO 1
(E) *Staphylococcus aureus* with water and UEO 1

Figure 3: Zones of oil degradation by *Staphylococcus aureus*, illustrating the comparative degradation potential under different conditions.

(A) Degradation zones formed by *S. aureus* with Used Engine Oil I (UEO I) and Used Engine Oil II (UEO II) on Nutrient Agar (NA).

(B) Degradation zones formed by *S. aureus* with UEO I and UEO II on Mueller-Hinton Agar (MHA).

(C) Degradation zone produced by *S. aureus* with mineral oil and antibiotic (positive control).

(D) Degradation zones formed by *S. aureus* in the presence of mineral oil and UEO I.

(E) *S. aureus* with water (negative control) and UEO I, showing no degradation activity.

Microorganisms possess specialized metabolic pathways that enable them to utilize petroleum hydrocarbons as their sole source of carbon and energy [24]. Consistent with our findings, *Bacillus subtilis* demonstrated remarkable oil degradation activity, highlighting its potential in bioremediation applications. Similar results were reported by [25], who identified *Bacillus species* among the most efficient degraders of crude oil and gasoline. These observations reinforce the significance of *Bacillus spp.* as promising candidates for the biodegradation of petroleum-based pollutants in contaminated environments.

These results confirm the ability of the selected bacterial strains to degrade petroleum-based pollutants, indicating their significant bioremediation potential. The catabolic efficiency of bacterial cultures in breaking down various components of crude oil is largely dependent on the presence of specific catabolic pathways encoded within their genomes [26]. The metabolic versatility of these microorganisms enables them to target a broad range of hydrocarbon compounds. Previous studies have extensively documented the catabolic capabilities of soil bacterial isolates toward various aromatic compounds [27,28] as well as polycyclic aromatic and aliphatic hydrocarbons [29,30]. These findings support the role of microbial metabolism in the effective degradation and detoxification of complex hydrocarbon pollutants in contaminated environments.

CONCLUSION

The present study successfully demonstrated the biodegradation potential of four bacterial strains against used engine oil and mineral oil. Among the tested strains, *Bacillus subtilis* and *Staphylococcus aureus* exhibited significant degradation activity, effectively reducing oil pollutants within 24–48 hours. In contrast, *Salmonella enterica* and *Escherichia coli* did not show appreciable biodegradation capabilities under the tested conditions.

These findings highlight the strong catabolic potential of *B. subtilis* and *S. aureus*, suggesting their suitability for application in the bioremediation of oil-contaminated wastewater. The ability of these strains to efficiently degrade petroleum-based hydrocarbons reinforces their potential as eco-friendly and cost-effective agents for the treatment of environments affected by oil spills or industrial discharges containing hydrocarbon pollutants.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The relevant data and materials are available in the present study.

COMPETING INTERESTS

The authors declare that they have no competing interests. All procedures followed were in accordance with the ethical standards (institutional and national).

FUNDING

Not applicable.

ACKNOWLEDGEMENTS

Authors would like to thank all individuals who provided their efforts for this research especially Aaesha Ahmed Alzaabi and Nizamuddin for their assistance during research work.

AUTHORS' CONTRIBUTIONS

VB performed all the experiments. VB analysed the data and wrote the manuscript.

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