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Exploring Oil Contaminated Environments for Bacteria with Bioremediation Potential

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Abstract
Oil spills wreak havoc on both land and water, poisoning soil, rivers and oceans. Bioremediation is a promising option which uses nature's ability to break down pollutants. Oil-eating bacteria and other specialized microorganisms are used to break down oil molecules into harmless byproducts. This natural decomposition process reduces the long-term environmental damage caused by oil pollution. The main objective of this study was to isolate oil degrading bacteria from local environment to study their oil degradation potential under laboratory conditions. Bacterial isolates were obtained from two soil samples collected from mechanic shops from Lahore, Pakistan. These isolates were characterized morphologically, physiologically and biochemically and further studied to evaluate their ability to break down burnt engine oil. Bacteria were cultivated in minimal salt medium (MSM) supplemented with burnt engine oil as the main carbon source. Bacterial growth was monitored by measuring optical density at 600 nm, which increased over time and the corresponding oil degradation potential was calculated. For UMT LS1 the percentage degradation was found to be 77% after seven days of incubation whereas for UMT LS2 the percentage degradation was found to be 81% after seven days of incubation. Biochemical analysis showed that UMT LS1 belonged to genus *Corynebacterium* while UMT LS2 belonged to the genus *Mycobacterium*. These bacterial isolates can be used as potential candidates for degradation of oil contamination.

Introduction

Microorganisms live in diverse habitats, including air, water, soil, and organisms. They have a diverse set of metabolic capabilities. The microbial community contains a diverse array of organisms, including bacteria, fungi, and viruses, all of which play an important role in sustaining life on Earth. They contribute significantly to ecological processes such as biochemical cycles and food chains, as well as important interactions with other organisms [1, 2].

Compared to any other site on earth, soil is home to the most diverse microbial population. Without bacteria, activities such as mineralization, nitrogen fixation of

legumes, and conversion of ammonia to nitrates that will be used by plants would not be possible. Additionally, bacteria play an important role in sustainable, closed-cycle organic waste treatment systems as well as cleaning landfills [3, 4]. Bacteria have great nutritional flexibility and environmental tolerance. Furthermore, because they contain an abundance of intracellular and extracellular enzymes, they can efficiently convert complex pollutants into carbon and energy sources [5].

Pollution loads are increasing rapidly due to industrialization and population growth. The extensive use and production of chemicals for modern technologies has led to the accumulation of pollutants such as xenobiotics, hydrocarbons, heavy metals and other harmful substances. Similarly, Crude oil is also a major pollutant composed of volatile liquid hydrocarbons, nitrogen, oxygen, and sulfur, creating diverse and complex molecular structures, some of which are difficult to identify [6]. Despite variations in composition, crude oil typically contains about 12-15% hydrogen with burnt engine oil.

Materials and Methods

Sample Collection

Two soil samples were collected for this study, first soil sample was collected from an automobile workshop near PIA road, Lahore. Other soil sample was collected from another automobile workshop near University of Management and Technology (UMT), Lahore. The soil samples were collected to the depth of 10 cm and kept in sterilized plastic bags. Samples were air-dried, ground and homogenized. Both these soils were contaminated with crude oil due to routine automobile oil change occurring at these locations. The physicochemical characteristics of these samples were noted at the time of sample collection.

by weight and 82-87% carbon by weight. The interaction of these elements produces a number of PAHs (polycyclic aromatic hydrocarbons), which act as pollutants when released into the environment [7].

Bioremediation shows great promise as a method to detoxify environmental pollutants. Microorganisms, including fungi, yeasts, and bacteria, have emerged as exceptionally effective agents in detoxifying pollutants [8] Due to their prevalent and robust nature, crude oil has been of great concern for the ecosystems and must be addressed in a wise and practical approach [9]. The efficacy of this bioremediation process is based on the presence of certain factors like nutrients, oxygen, and temperature, composition of the oil and most importantly the availability of the hydrocarbon-degrading microbes [10, 11].

The main objective of this study was to isolate hydrocarbon degrading bacteria from soil, characterize them and check their potential for hydrocarbon degradation in MSM supplemented

Enrichment of the samples and isolation of oil degrading bacteria

Oil degrading bacteria were isolated in minimal salt medium (MSM) following the procedure by Hossain *et al.,* [12], where, the sole carbon source used was the burnt engine oil. A very minimal quantity (0.005% v/v) of tween 20 was used to solubilize the oil in the water [13]. Briefly, 1 gram of soil was added 100 ml sterile distilled water in 250 ml conical flask, mixed very well and waited for sedimentation. Then, 1 ml of supernatant was added in MSM (K_2HPO_4, 2.0gm;

$(NH_4)_2SO_4$, 0.5gm; KH_2PO_4, 0.02gm; $MgSO_4$, 0.05gm; $FeSO_4.4H_2O$, 400 mg; $MnSO_4.4H_2O$, 400 mg; $ZnSO_4.7H_2O$, 200 mg; $CuSO_4.7H_2O$, 40 mg; KI, 300 mg; $Na_2MoO_4.2H2O$, 50 mg; $CoCl_2.6H_2O$, 40 mg and distilled water 1L) supplemented with burnt engine oil (0.5%). The pH of the medium was adjusted to 7.0. The flasks were then incubated at 37°C for 7 days (180 rpm). Obtained enrichment cultures were spread on MSM agar plates supplemented with burnt engine oil (0.5%). Then morphologically distinct colonies were identified and cultured in MSM with burnt engine oil were finally considered as a pure cultures.

Morphological and Biochemical Characterization of the bacterial isolates

The isolated oil degrading bacteria were characterized morphologically, physiologically and biochemically following the procedures described in [14].

Crude Oil Degradation

The isolated bacteria were checked for their oil degradation potential using different concentration of burnt engine oil in MSM following the procedure described by Koolivand *et al.,* [15]. Briefly, MSM was prepared and added in flasks and autoclaved (100 ml per flask). In the next step, added burnt engine oil in the flasks to (1%). Overnight bacterial cultures of the selected isolates were inoculated in the flasks. Negative controls of MSM medium without adding bacterial isolates were also prepared. The experiment was performed in triplicates.

Visible bacterial growth was observed in each flask of the experimental group which showed the oil degrading abilities of the bacteria. Optical density was measured at 600nm for the experimental group flasks every day for 7 days during their incubation period. After measuring the optical density, we made growth curves for each of the flasks over the course of one week. For the calculation of oil degradation percentage, following formula was used:

Percentage of Degradation (%) = [(initial weight of the oil inoculated - residual Weight after degradation) / initial weight of the oil inoculated] ×100

Physicochemical characteristics of the soil samples measured after their collection have been given in Table 1.

Results

Physicochemical characteristics of soil samples

Table 1: Physicochemical characteristics of soil samples

Soil Samples	PIA road Lahore	UMT Lahore
Moisture Content	20%	22%
Temperature	30°C	32°C
pH	7.5	7.5

Isolation and characterization of oil degrading bacteria

After an incubation period of four days on agar medium two morphologically distinct colonies were obtained (one from each sample) as potential candidates to be used for bioremediation potential of oil. These bacterial isolates were given the codes of UMT LS1 and UMT LS2 These colonies were further purified and studied for their colony characteristics which have been given in table 2. The biochemical (Table 2)

and physiological characteristics (Figure 1) were also studied for the selected isolates. The optimum pH and temperature for the oil degrading bacterial isolates were found to be 7 and 37°C for both the isolates.

Table 2: Morphological and biochemical characteristics

Bacterial Isolates	**Shape**	**Edge**	**Texture**	**Color**	**Size**	**Opacity**	**Gram staining**	**Spore Staining**
UMT LS1	irregular	irregular	rough	grayish white	small	opaque	+ve / rods	-ve
UMT LS2	circular	entire	smooth	creamy white	small	opaque	+ve / rods	-ve
Biochemical characteristics								
Bacterial Isolates	**Catalase Test**	**Oxidase Test**	**Urease Test**	**Citrate Test**	**H_2S production**	**Indole Test**	**Methyl Red-Voges Proskauer (MR/VP)**	**Oxidation fermentation (OF) test**
UMT LS1	+ve	-ve	-ve	-ve	+ve	-ve	MR +ve / VP-ve	Fermentative
UMT LS2	+ve	-ve	+ve	+ve	-ve	-ve	MR –ve / VP -ve	Oxidative

Identification of bacterial isolates

Based on the biochemical analysis UMT LS1 were found to be closely related to genus *Corynebacterium* while UMT LS2 were found to be closely related to the genus *Mycobacterium*.

Oil degradation

The bacterial isolates UMT LS1 (*Corynebacterium* sp.) and UMT LS2 (*Mycobacterium* sp.) were able to grow in MSM while utilizing burnt engine oil as its sole carbon source, therefore degrading the oil in the process. The increase in optical density in both the cultures was observed with the passage of time and so the percentage degradation. In case of UMT LS1 the percentage degradation was found to be 15%, 28%, 45%, 58%, 67%, 73%, 77% with increasing the time of incubation at 24, 48, 96, 120, 144, 168, 192 hours respectively. Similarly, for UMT LS2 the percentage degradation was found to be 18%, 32%, 48%, 63%, 72%, 78%, 81% with increasing the time of incubation at 24, 48, 96, 120, 144, 168, 192 hours respectively (Figure 2).

Discussion

Microbial hydrocarbon degradation, also known as crude oil degradation by microbes, is an ecological process in which microbes such as bacteria and fungi break down complex hydrocarbons contained in crude oil into simpler molecules [16, 17, and 18]. This mechanism is an important part of the innate carbon cycling system of earth and serves an important role in minimizing the negative ecological effects of oil spills. Microbial decomposition of petroleum products is extremely useful to the environment, particularly in times of oil spills. It helps to mitigate the effects of oil pollution by progressively breaking down spilled oil and turning it into less hazardous chemicals [19].

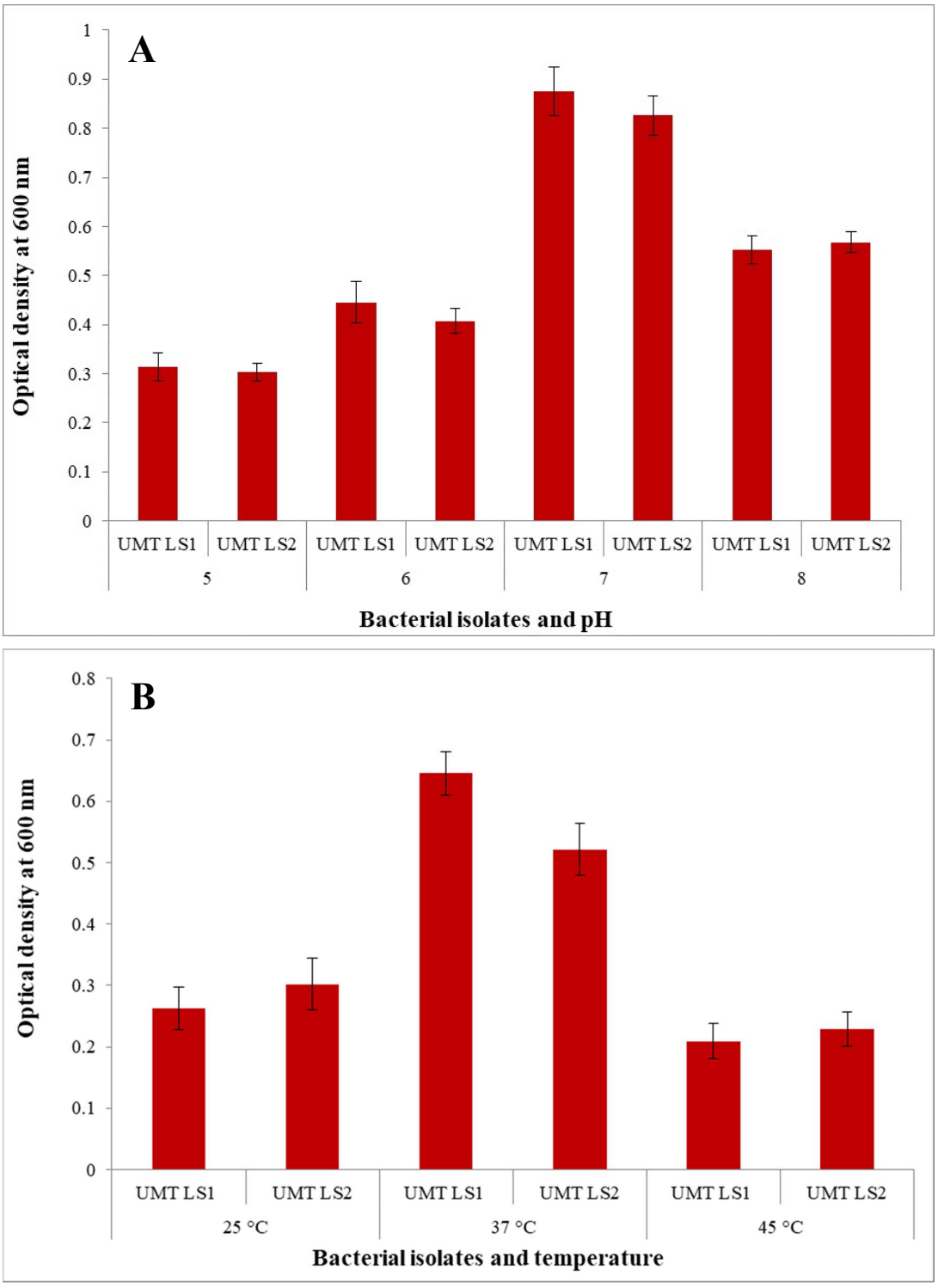
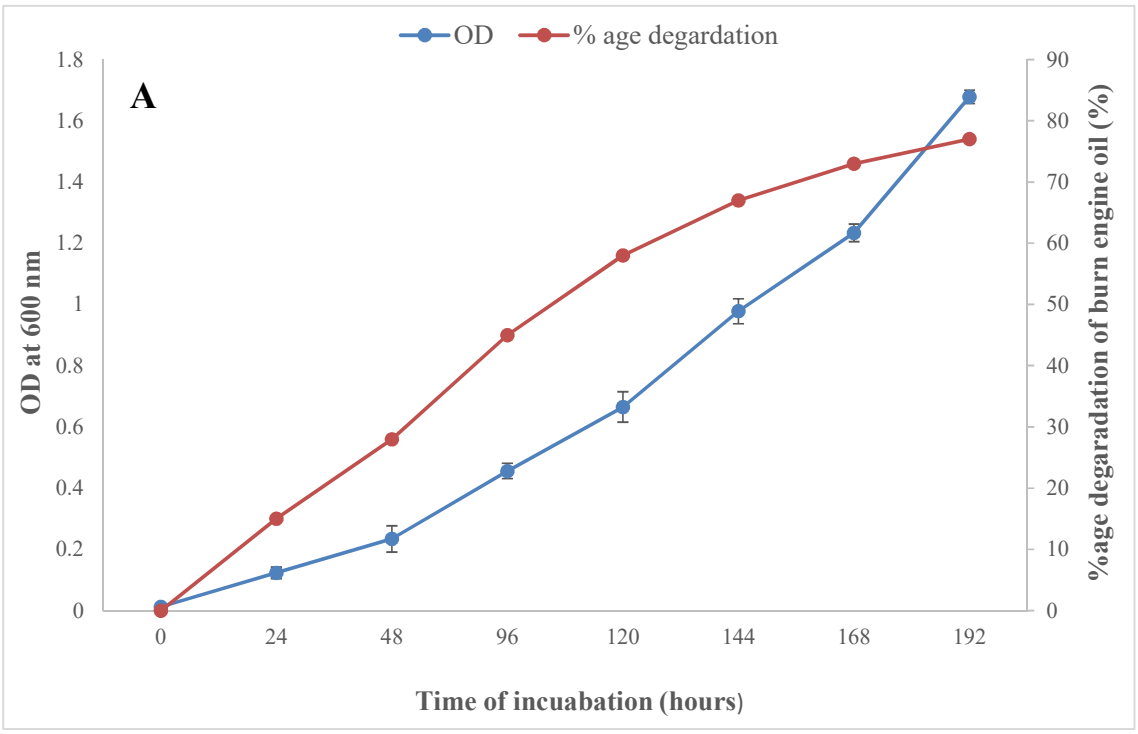


Figure 1: Physiological characterization of bacterial isolates, optimum pH for the bacterial isolates (A) and optimum temperature for bacterial isolates (B)



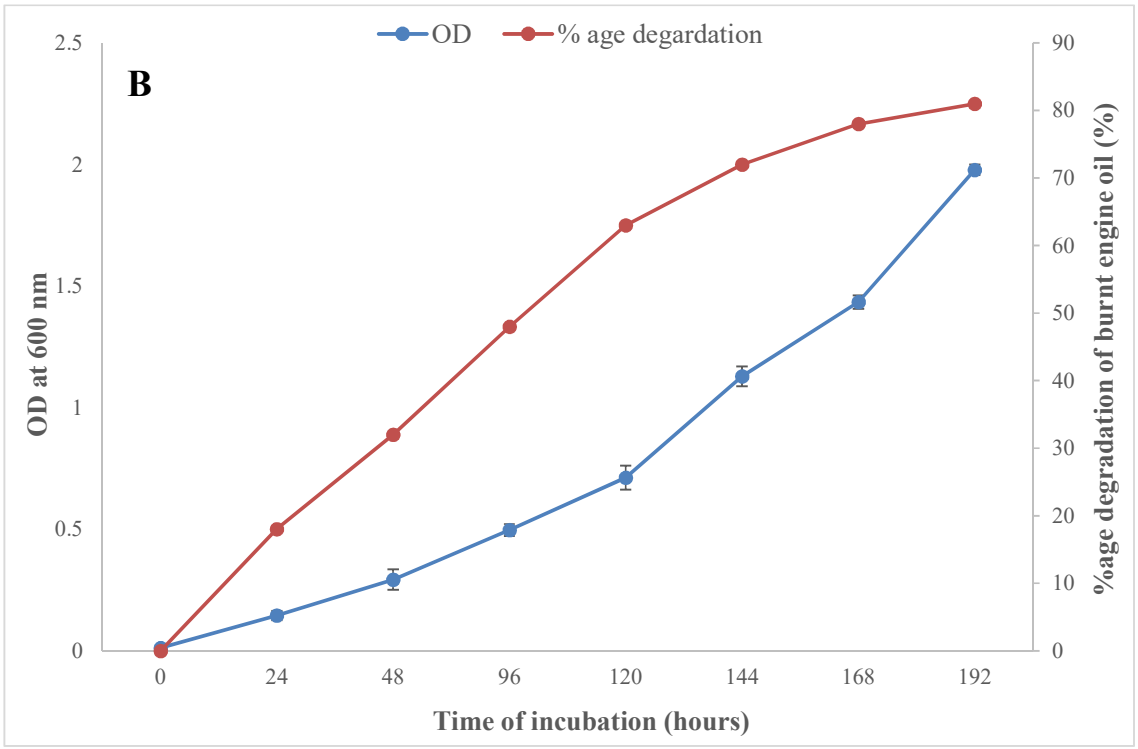


Figure 2: Degradation %age of burnt engine oil for UMT LS1 (A) and UMT LS2 (B)

However, numerous factors can alter the pace and effectiveness of microbial degradation: environmental conditions, microbial community, oxygen and nutrient availability and oil composition.

After an incubation period of four days on agar medium two morphologically distinct colonies were obtained (one from each sample) as potential candidates to be used for bioremediation potential of oil. These bacterial isolates were given the codes of UMT LS1 and UMT LS2 These colonies were further purified and studied for their morphological, biochemical and physiological characteristics. The optimum pH and temperature for the oil degrading bacterial isolates were found to be 7 and 37℃ for both the isolates.

The bacterial isolates UMT LS1 and UMT LS2 were able to grow in MSM while utilizing burnt engine oil and effectively degrading the oil in the process. The increase in optical density in both the cultures was observed with the passage of time and so the percentage degradation also increased with the increasing incubation time. In case of UMT LS1 the percentage degradation was found to be 15%, 28%, 45%, 58%, 67%, 73%, 77% with increasing the time of incubation at 24, 48, 96, 120, 144, 168, 192 hours respectively. For UMT LS2 the percentage degradation was found to be 18%, 32%, 48%, 63%, 72%, 78%, 81% with increasing the time of incubation at 24, 48, 96, 120, 144, 168, 192 hours respectively. Among these two bacterial isolates, UMT LS2 was found to be the best candidate for the degradation of oil as it showed 81% degradation after seven days compared to the UMT LS1 which showed 77% degradation after 7 days of incubation. Moreover the percentage degradation of the UMT LS2 was higher on each incubation day compared to the UMT LS1 as shown in the figure 2.

Other researchers have also isolated the oil degrading bacteria with different percentages of oil degradation. These bacteria belong to different genera [20, 21, and 22]. So in addition to these two bacterial isolates, other genera can also effectively degrade the different fractions of hydrocarbons [23, 24].

Based on the biochemical analysis UMT LS1 were found to be closely related to genus *Corynebacterium* while UMT LS2 were found to be closely related to the genus *Mycobacterium*.

Conclusion

The study highlighted the important role of oil-degrading bacteria in bioremediation. The results of our study provide a comprehensive strategy to address the pressing issue of oil contamination by oil spills. This work serves as a foundation for further investigation and application in the fields of agricultural and environmental science. *Corynebacterium* and *Mycobacterium* sp. should be investigated further for their crude oil degrading capabilities and its role in bioremediation should be further analyzed.

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Author Contribution

Sara Afzal, Zoha Yasin and Noor-ul-Ain designed and performed the experimental Work of this project and also prepared the manuscript for publication, whereas, Maryam Tahir and Khadija Khan helped in the research work and the preparation of this manuscript.

Conflict of Interest

Authors declare no conflict of interest.

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