Biodegradation Efficiency of Local Pseudomonas and Bacillus Bacteria in Removing Oil Spots from Contaminated Soil in Kirkuk

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Abstract
Oil pollution is considered one of the most significant environmental pollutants that negatively affect soil, wafeter, and living organisms, particularly in oil-producing regions such as Iraq. This study was conducted in Kirkuk Governorate, where soil samples contaminated with oil were collected. Bacteria were isolated using a culture medium containing oil as the sole carbon source. The isolated bacteria were then identified based on their morphological, biochemical, and physiological characteristics, and their efficiency in crude oil degradation was evaluated using spectrophotometric analysis.
The results demonstrated that *Pseudomonas aeruginosa* exhibited high degradation efficiency due to its ability to produce enzymes such as dioxygenase. In contrast, the isolate *Bacillus subtilis* showed a notable tolerance to harsh environmental conditions, although its degradation efficiency was lower than that of *Pseudomonas aeruginosa*.

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Keywords: Showed the Lowest Efficiency, which may be Attributed to Weak Growth or Limited Enzyme Production

Introduction

Oil pollution is considered one of the most significant environmental contaminants that threaten ecosystems, especially in oil-rich regions such as Iraq. It alters soil and water properties and negatively affects microorganisms and plants (Al-Hadithi *et al*., 2016) [3]. Oil pollution mainly results from oil spills, pipeline leakage, or poor management of petroleum waste. Bioremediation has emerged as an effective, economical, and more environmentally friendly solution compared to traditional physical or chemical methods (Vidali, 2001) [10]. This technique relies on microorganisms—particularly bacteria—to break down complex organic compounds into simpler, less harmful substances. Local studies indicate that several bacterial species found in the Iraqi environment possess the ability to degrade hydrocarbons. The most notable genera include Pseudomonas, Bacillus, and Acinetobacter, isolated from contaminated soil in regions such as Basra and Kirkuk (Mahdi *et al*., 2020; Al-Taee & Naji, 2018) [1, 2].

These species show high growth ability in media containing oil as the sole carbon source, making them promising candidates for bioremediation applications. The aim of this study is to evaluate the efficiency of selected local bacterial isolates taken from petroleum-contaminated soil in degrading organic pollutants by isolating, identifying, and measuring their ability to reduce crude oil content, followed by statistical analysis to determine the most efficient species.

Significance of the Study in Managing Oil Pollution in Kirkuk

This study gains particular importance due to the environmental conditions in Kirkuk, one of Iraq's major oils-producing regions, where many industrial sites suffer from chronic oil pollution caused by accidental spills and poor management of petroleum waste.
Using local bacterial species is considered a sustainable solution compared to traditional approaches such as burning or chemical treatment, as bioremediation is low-cost, environmentally friendly, and generates no harmful secondary products (Al-Awadhi *et al*., 2014) [5].

Practical Applications Include

1. Establishing small field-scale bioremediation units at oil spill sites.
2. Training staff of oil companies and municipalities on bacterial inoculation methods for contaminated soil.
3. Integrating bioremediation into environmental management programs in Kirkuk.

Successful application of these techniques will improve soil quality, protect groundwater, reduce environmental and health impacts, and support sustainable development in oil-rich regions of Iraq.

Factors Influencing Bioremediation Efficiency

1. Temperature

Temperature is one of the most influential factors affecting microbial activity. Most hydrocarbon-degrading bacteria show optimal activity between 25–35°C. Higher temperatures enhance oil solubility and accelerate enzymatic reactions, while excessively high temperatures may inhibit microbial enzymes (Das & Chandran, 2011) [7].

2. pH

Most effective hydrocarbon-degrading bacteria function optimally at pH values between 6.5 and 8. Significant deviations inhibit microbial growth and enzyme production (Vidali, 2001) [10].

3. Oxygen Availability

Most hydrocarbon-degrading bacteria require oxygen as the final electron acceptor in aerobic degradation. In oxygen-limited environments, degradation rates drop significantly or shift to slower anaerobic pathways (Atlas & Bartha, 2013) [6].

4. Oil Type and Composition

Biodegradation rates depend on the type of petroleum pollutant. Light hydrocarbons (short-chain alkanes) degrade more rapidly, while polycyclic aromatic hydrocarbons and heavy oils degrade much more slowly due to molecular complexity (Head *et al*., 2006) [8].

5. Soil Moisture and Aeration

Moderate moisture enhances nutrient solubility and oxygen diffusion. However, water-saturated soil reduces aeration and slows biodegradation (Margesin & Schinner, 2001) [9].

Previous Studies

1. Iraqi Studies

Mahdi *et al*. (2020) [1] reported that *Pseudomonas aeruginosa* and *Bacillus subtilis* isolated from contaminated soil in Kirkuk reduced crude oil concentration by up to 65% after 14 days of incubation.

Al-Taee & Naji (2018) [2] isolated local strains from contaminated soil near Basra oil refineries and found that *Acinetobacter spp.* showed strong degradation capacity compared to others.

Al-Hadithi *et al*. (2016) [3] showed that using a mixed bacterial culture was more effective than using a single strain, achieving degradation above 70%.

2. Arab Studies

Abu Zeid *et al*. (2017) [4] demonstrated that bacterial isolates from contaminated soil near Suez Port displayed strong ability to degrade petroleum derivatives using Bushnell Haas medium with oil as the sole carbon source.

3. International Studies

Vidali (2001) [10] emphasized that bacteria form the cornerstone of bioremediation due to their ability to aerobically and anaerobically degrade a wide spectrum of hydrocarbons.

Das & Chandran (2011) [7] confirmed that environmental factors such as temperature, pH, and nutrient availability significantly influence microbial hydrocarbon degradation.

Materials and Methods

First: Study Area and Sample Collection

Oil-contaminated soil samples were collected from the Baba Gurgur area near petroleum facilities in Kirkuk. Oil spots were clearly visible on the soil surface from previous leaks. Samples were collected from a depth of 5–15 cm using sterile tools, placed in sealed plastic containers, and transported to the laboratory within 24 hours.

Second: Isolation of Bacteria from Soil

1. Soil suspensions were prepared using sterile saline solution (0.85% NaCl) and serially diluted to 10^{-6}.
2. Samples were plated on selective media, specifically Bushnell Haas Agar supplemented with 1% crude oil as the sole carbon source.
3. Plates were incubated at 30°C for 5–7 days.
4. Distinct colonies were purified by sub-culturing.

Third: Morphological and Biochemical Identification

Preliminary identification included:

1. Examination of shape, color, edges, and texture
2. Gram staining
3. Biochemical tests
   a. Catalase test
   b. Oxidase test
   c. Motility test
   d. Indole production

Based on these results, isolates were presumptively identified as *Bacillus spp*., *Pseudomonas spp.*, and *Acinetobacter spp.*

Fourth: Biodegradation Efficiency Test

- Bushnell Haas broth supplemented with 1% crude oil was prepared.
- Each flask was inoculated with 1 mL of a pure bacterial culture.
- Incubation was performed at 30°C for 10 days.
- Oil concentration before and after incubation was measured by spectrophotometry at 600 nm.

Results and Data Analysis

1. Biodegradation Efficiency of Local Isolates

The results showed variation in the ability of the local bacterial isolates to degrade crude oil. Three isolates were selected based on the laboratory diagnostic results and were

given the following codes.

- B1 – *Pseudomonas aeruginosa*.
- B2 – *Bacillus subtilis*.
- B3 – *Acinetobacter spp.*

1. Crude oil removal after 10 days

Isolate	Initial Conc. (mg/L)	Final Conc. (mg/L)	Removal %
B1	1000	370	63%
B2	1000	410	59%
B3	1000	520	48%

‘’Table shows that isolate B1 (Pseudomonas) has the highest degradation efficiency’’.

2. Statistical Analysis

One-Way ANOVA showed significant differences at ($P \leq 0.05$) among the three isolates. B1 significantly outperformed B3, while the difference between B1 and B2 was not highly significant.

3. General Observations

- All isolates grew efficiently using crude oil as the sole carbon source.
- No external stimulants (nitrogen or phosphorus) were used, indicating natural degradation ability.
- Repeated experiments produced consistent results.

Discussion

The results demonstrated that local bacterial isolates possess the ability to degrade crude oil with varying efficiencies. B1 (*Pseudomonas aeruginosa*) showed the highest degradation rate (63%), likely due to production of powerful enzymes such as dioxygenases and monooxygenases, known for breaking aromatic hydrocarbon bonds (Vidali, 2001) [10].

B2 (*Bacillus subtilis*) showed slightly lower degradation but displayed strong environmental resilience, making it suitable for natural conditions.

B3 (*Acinetobacter spp.*) exhibited the lowest degradation rate, possibly due to limited growth in oil-only medium or reduced enzymatic production.

These findings agree with Mahdi *et al*. (2020) [1] in Kirkuk and Al-Hadithi *et al*. (2016) [3] in Baghdad, which reported strong degradation ability of local bacterial species. However, the obtained rates were lower than some international studies reporting >80% degradation, likely due to differences in oil type, incubation duration, or environmental conditions.

Conclusions

Study concludes that several local bacterial isolates from Kirkuk exhibit significant capability to degrade petroleum compounds without the need for external stimulants. *Pseudomonas aeruginosa* showed the highest efficiency, achieving 63% oil removal within only 10 days of incubation. This indicates strong potential for using local microorganisms in bioremediation programs for oil-contaminated soils in industrial and oil-producing regions in Iraq.

Recommendations

1. Conduct extensive field studies to evaluate isolate performance under natural conditions.
2. Use mixed cultures to enhance biodegradation efficiency.
3. Investigate environmental factors such as temperature, moisture, and pH.
4. Promote bioremediation applications in oil-polluted Iraqi regions such as Basra and Kirkuk.

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