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Determining the biodegradation rate of crude oil using some different bacterial species isolated from soil

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ABSTRACT
The current study aimed to determine the biodegradation rate of crude oil using some different bacterial species isolated from soil.100 mg of contaminated and uncontaminated soil samples used in bioremediation experiments were collected from three areas in Tikrit city, including generator soil, refinery soil, and normal uncontaminated soil. The soil samples (weight: 100 g and depth: 3-12 cm) were collected using dark counters and then transferred to the laboratory. Hydrocarbon materials were added with the prepared nutrient agar medium and left to solidify, then both contaminated and normal soils were placed and incubated for 24 hours at 37 °C. The results showed that the most isolated bacteria was Bacillus spp., which amounted to 80.8% compared to the rest of the genera, as the isolation percentage of the genus Enterobacter was one isolate, at a rate of 7.7%, while the isolation percentage of the genus Acinetobacter was two isolates, at a rate of 11.5%. for biodegradation efficacy, Bacillus thuringiensis showed that the highest degradation percentage was 75% for a concentration of 1% crude oil, while, Bacillus subtilis showed that the highest degradation percentage was 67% for a concentration of 1% crude oil. Bacillus firmus showed that the highest decomposition percentage was 74% for a concentration of 1% crude oil. Bacillus tropicus showed that the highest degradation percentage was 62% for a concentration of 1% crude oil. Bacillus taxishowed that the highest degradation percentage reached 42% for a concentration of 1% of crude oil. Bacillus muralis that the highest degradation percentage was77% for a concentration of 1% crude oil. Bacillus megaterium that the highest degradation percentage reached74% for a concentration of 1% crude oil. Enterobacterhormaechei that the highest degradation degradation rate was 61% for a concentration of 1% crude oil. Acinetobacterlwoffii that the highest tion rate was 61% for a concentration of 1% crude oil. The results of the current study showed the ability of the studied bacteria to biodegrade contaminated crude oil in soil. The most efficient bacterial species in biodegrading crude oil was Bacillus muralis.
Keywords: biodegradation, crude oil, Bacillus, E.hormaechei, A.lwoffii.

INTRODUCTION

Crude oil pollution is a well-known problem worldwide, especially in countries that involve crude oil exploration and exploitation. The land, water, and air that receive crude oil pollutants have suffered greatly, and consequently, all that inhabits them. As a result, efforts have been made, such as creating legislations that target environmental pollution management [1,2]. However, this study deals with land pollution, but studies on water pollution by crude oil have been widely reported [3,4]. The majority of crude oil-related water contamination starts on land and travels to the closest body of water, such as a swamp, river, stream, or even the ocean. When this occurs, the crude oil is carried into the water along with a variety of contaminants, including biological, chemical, and particle matter, rendering the water unfit for human consumption and unfit for its residents [5]. The transfer of crude oil qualities to soil, the impacts of crude oil mobility in soil, remediation approaches, factors influencing the remediation process, and many other particular objectives have been researched in relation to soil contamination by crude oil [6].Therefore, the penetration rate and effects of crude oil migration in soil were described by Amro et al. [7]. According to this study, the initial penetration of crude oil in soil is greater, but as time goes on, the transport distance becomes gradual and slow. Different crude oil derivatives in the soil environment exhibit this transport behavior similarly, although generally speaking, the pollutant content in soil drops with increasing depth [8].In order to address the impacts of oil spills, physical and chemical treatments have been used for the most part. But when examined, the methods often leave behind residues that can either bury the oil or just move it to a new area, which could have a long-term impact on the underwater habitat.Therefore, bioremediation provides more effective waste treatment, with optimal cleaning potential. Bioremediation refers to a more economical and effective technology for hydrocarbon degradation, due to ease

of operation and maintenance [9,10]. The main principle of this process is to explore the metabolism of microbes [10]. According to Sudrajat et al. [11], Pseudomonas sp. and Bacillus sp. can break down hydrocarbons in oil-contaminated soils by 67 and 61%, respectively, whereas Vinothini et al. [12] estimated that Pseudomonas putida can break down hydrocarbons in crude oil by 65%. Numerous investigations on bioremediation have already been carried out on soils contaminated by oil.So, the current study aimed to determine the biodegradation rate of crude oil using some different bacterial species isolated from soil.

MATERIALS & METHODS

Soil Sample Collection

100 mg of contaminated and uncontaminated soil samples used in bioremediation experiments were collected from three areas in Tikrit city, including generator soil, refinery soil, and normal uncontaminated soil. The soil samples (weight: 100 g and depth: 3-12 cm) were collected using dark counters and then transferred to the laboratory. Hydrocarbon materials were added with the prepared nutrient agar medium and left to solidify, then both contaminated and normal soils were placed and incubated for 24 hours at 37 °C.

Measuring the percentage of quantitative loss of crude oil

The rate of crude oil degradation was measured using the gravimetric method by measuring the difference between the weight of the amount of crude oil added to the culture medium used before and after the development of the bacterial isolates used, as followed in Wehner and Teschner[13]. The percentage of the rate of crude oil degradation was measured by each isolate separately.

Isolation and identification of bacteria

Isolation

1 g of soil was diluted using test tubes containing 9 ml of distilled water and then 1 ml of the fourth and fifth dilutions were transferred to Petri dishes. After that, nutrient agar containing 1% crude oil was added as a carbon source. Petri dishes were incubated at 37 °C for 24-48 hours [14].

Bacterial Identification

Bacteria were identified based on the following aspects

Pheteromorphic diagnosis and cultural characteristics

Bacterial colonies were identified based on the cultural characteristics of the colonies growing on MacConkey agar and blood agar. The growing colonies were isolated and purified by taking a single colony and re-cultivating it using the Streaking method on MacConkey agar again to ensure the purity of the isolated colonies[15].

Microscopic Examination

The morphological features of the bacterial cells under the microscope were used to identify the bacterial colonies. This was done by looking at how the bacteria reacted with Gum stain, which reveals the sort of reaction as well as the shape and arrangement of the bacterial cells.

Biochemical Tests

The following biochemical tests were conducted as stated in [15, 16].

RESULTS AND DISCUSSION

Isolation and Identification of bacteria

Table (1) shows the bacterial isolates that were isolated and identified from the soil, where it is noted that 86.7% of the bacterial isolates gave positive growth after being cultivated on blood and MacConkey agar media. While 13.3% of the soil samples showed negative bacterial growth when grown on the same media.

Table 1: Number and percentages of bacterial isolates from the soil

Results	No. of isolates	Percentage of isolates
+ve	13	86.7%
-ve	2	13.3%
Total	15	100%

Table (2) shows the number of single isolates and the number of isolates containing more than one type. The percentage of single isolates was 30.8% of the total 13 contaminated soil samples, while the percentage of double isolates was 69.2%, which contained more than one type of bacteria.

Table 2: Number and percentage of single and mixed isolates from soil

Results	No. of isolates	Percentage of isolates
Single	4	30.8%
Mixed	9	69.2%
Total	13	100%

Table (3) shows the number and percentages of each type of bacteria that were isolated from the soils collected from three regions. It is noted that the most isolated bacteria were Bacillus subtilis, which amounted to 23.1%, followed by Bacillus tropicus, which amounted to 15.4%, while the lowest isolation percentage was for Bacillus taxi and Bacillus flexus, which amounted to 3.8% out of a total of 26 isolates. On the other hand, the isolation percentages of the genus Bacillus spp. were the highest, amounting to 80.8% compared to the rest of the genera, as the isolation percentage of the genus Enterobacter was one isolate, at a rate of 7.7%, while the isolation percentage of the genus Acinetobacter was two isolates, at a rate of 11.5%.

Table 3: Number and percentages of each type of bacteria

Results	No. of isolates	Percentage of isolates
Bacillus thuringiensis	2	7.7%
Bacillus subtilis	6	23.1%
Bacillus firmus	3	11.5%
Bacillus tropicus	4	15.4%
Bacillus taxi	1	3.8%
Bacillus muralis	2	7.7%
Bacillus megaterium	2	7.7%
Bacillus flexus	1	3.8%
Enterobacterhormaechei	2	7.7%
Acinetobacterlwoffii	3	11.5%
Total	26	100%

The results of the current study also showed that the highest isolated genera were Bacillus spp. If it reached 80.8%, the species Bacillus subtilis was the highest among the Bacillus spp. genera, where its percentage reached 23.1%. The results of the current study agreed with the study of Ibrahim and Neihaya[17], where they indicated that the isolation percentage of Bacillus spp. reached 73.1% of different soil samples, and they also indicated that the isolation percentage of Bacillus subtilis reached 28.9%, and they indicated that the diagnosis of the bacillus bacteria was done through morphological characteristics, microscopic diagnosis and biochemical tests, and then it was confirmed using the Vitek 2 system. The bacillus bacteria, which is the most abundant genus in soil, is a natural soil bacteria that colonizes plant roots aggressively, and benefits plants by providing growth promotion [18]. The results of the current study also agreed with the study of Allos and Asmaa[19] who indicated that the isolation rate of Bacillus spp. bacteria reached 70.9%, as out of a total of 55 soil samples taken in the study, 39 isolates of Bacillus spp. bacteria were found.

Biodegradation of crude oil

Biodegradation of crude oil was carried out by different types and genera of bacteria according to the ideal conditions for each type.

Bacillus thuringiensis

Table (4) shows the percentages of crude oil degradation at different concentrations (1%, 3%, 5%) by Bacillus thuringiensis. The best degradation percentage was 75% for a concentration of 1% crude oil, while the lowest degradation percentage was for a concentration of 5% crude oil, which reached 27%.

Table 4: Percentage of crude oil degradation by Bacillus thuringiensis

Conc.	Untreated Unanalyzed		Weight of crude oil decomposed	% of degradation of crude oil sample
Control	0.8642	0.8642	0.0	0.0%
1%	0.8642	0.214	0.6503	75%
3%	0.8642	0.3465	0.5177	59%
5%	0.8642	0.63	0.2342	27%

Bacillus subtilis

Table (5) shows the percentages of crude oil degradation at different concentrations (1%, 3%, 5%) by Bacillus subtilis. The best degradation percentage was 67% for a concentration of 1% crude oil, while the lowest degradation percentage was 18% for a concentration of 5% crude oil.

Table 5: Percentage of crude oil degradation by Bacillus subtilis

Conc.	Untreated Unanalyzed		Weight of crude oil decomposed	% of degradation of crude oil sample
Control	0.867	0.867	0.0	0.0%
1%	0.867	0.285	0.582	67%
3%	0.867	0.415	0.452	52%
5%	0.867	0.706	0.161	18%

Bacillus firmus

Table (6) shows the percentages of crude oil degradation at different concentrations (1%, 3%, 5%) by Bacillus firmus. The best degradation percentage was 74% for a concentration of 1% crude oil, while the lowest degradation percentage was 35% for a concentration of 5% crude oil.

Table 6: Percentage of crude oil degradation by Bacillus firmus

Conc.	Untreated Unanalyzed		Weight of crude oil decomposed	% of degradation of crude oil sample
Control	0.832	0.832	0.0	0.0%
1%	0.832	0.21	0.622	74%
3%	0.832	0.331	0.501	60%
5%	0.832	0.54	0.292	35%

Bacillus tropicus

Table (7) shows the percentages of crude oil degradation at different concentrations (1%, 3%, 5%) by Bacillus tropicus. The best degradation percentage was 62% for a concentration of 1% crude oil, while the lowest degradation percentage was 50% for a concentration of 5% crude oil.

Table 7: Percentage of crude oil degradation by Bacillus tropicus

Conc.	Untreated Unanalyzed		Weight of crude oil decomposed	% of degradation of crude oil sample
Control	0.914	0.914	0.0	0.0%
1%	0.914	0.3405	0.5734	62%
3%	0.914	0.395	0.519	56%
5%	0.914	0.4562	0.4578	50%

Bacillus taxi

Table (8) shows the percentages of crude oil degradation at different concentrations (1%, 3%, 5%) by Bacillus taxi. The best degradation percentage reached 42% for a concentration of 1% of crude oil, while the lowest degradation percentage was for a concentration of 5% of crude oil, reaching 32%.

Table 8: Percentage of crude oil degradation by Bacillus taxi

Conc.	Untreated Unanalyzed		Weight of crude oil decomposed	% of degradation of crude oil sample
Control	0.9041	0.9041	0.0	0.0%
1%	0.9041	0.52	0.3846	42%
3%	0.9041	0.5985	0.3061	33%
5%	0.9041	0.613	0.2916	32%

Bacillus muralis

Table (9) shows the percentages of crude oil degradation at different concentrations (1%, 3%, 5%) by Bacillus muralis. The best degradation percentage reached 77% for a concentration of 1% crude oil, while the lowest degradation percentage was for a concentration of 5% crude oil, reaching 12%.

Table 9: Percentage of crude oil degradation by Bacillus muralis

Conc.	Untreated Unanalyzed		Weight of crude oil decomposed	% of degradation of crude oil sample
Control	0.9323	0.9323	0.0	0.0%
1%	0.9323	0.211	0.7212	77%
3%	0.9323	0.3421	0.591	63%
5%	0.9323	0.8132	0.199	12%

Bacillus megaterium

Table (10) shows the percentages of crude oil degradation at different concentrations (1%, 3%, 5%) by Bacillus megaterium. The best degradation percentage was 74% for a concentration of 1% crude oil, while the lowest degradation percentage was 45% for a concentration of 5% crude oil.

Table 9: Percentage of crude oil degradation by Bacillus megaterium

Conc.	Untreated Unanalyzed		Weight of crude oil decomposed	% of degradation of crude oil sample
Control	0.884	0.884	0.0	0.0%
1%	0.884	0.225	0.659	74%
3%	0.884	0.3437	0.5403	61%
5%	0.884	0.482	0.402	45%

Enterobacterhormaechei

Table (10) shows the percentages of crude oil degradation at different concentrations (1%, 3%, 5%) by Enterobacterhormaechei. The best degradation percentage was 62% for a concentration of 1% crude oil, while the lowest degradation percentage was 20% for a concentration of 5% crude oil.

Table 10: Percentage of crude oil degradation by Enterobacterhormaechei

Conc.	Untreated Unanalyzed		Weight of crude oil decomposed	% of degradation of crude oil sample
Control	0.905	0.905	0.0	0.0%
1%	0.905	0.34	0.565	62%
3%	0.905	0.6	0.305	33%
5%	0.905	0.72	0.185	20%

Acinetobacterlwoffii

Table (11) shows the percentages of crude oil degradation at different concentrations (1%, 3%, 5%) by **Acinetobacterlwoffii**. The best degradation rate was 61% for a concentration of 1% crude oil, while the lowest degradation rate was 11% for a concentration of 5% crude oil.

Table 11: Percentage of crude oil degradation by Acinetobacterlwoffii

Conc.	Untreated Unanalyzed		Weight of crude oil decomposed	% of degradation of crude oil sample
Control	0.8349	0.8349	0.0	0.0%
1%	0.8349	0.32	0.5149	61%
3%	0.8349	0.519	0.3159	37%
5%	0.8349	0.739	0.0959	11%

The results showed that the biodegradation rate of crude oil by bacterial isolates after incubation at 37°C for 28 days in the above tables. The results showed that the maximum degradation rate achieved by Bacillus muralis reached (77%) at a concentration of 1%. On the other hand, and with regard to the rest of the studied bacterial genera, the biodegradation rate of crude oil reached 62% by Enterobacterhormaechei, and 61% by Acinetobacterlwoffii at a concentration of 1% of crude oil.The results also showed that Bacillus spp isolates are the most effective in analyzing petroleum derivatives compared to Enterobacterhormaechei and Acinetobacterlwoffii, and this is consistent with many studies that indicated that Bacillus spp isolates are the most effective in terms of their ability to biodegrade petroleum compounds and derivatives due to their ability to degrade hydrocarbon compounds by oxidizing and decomposing these compounds and using the resulting carbon in their growth and building their structures [20,21,22].Al-Rawi and Al-Akeedi[23] indicated that Bacillus bacteria were able to decompose types of Kirkuk medium crude oil, as the types of Bacillus used in this study were close in terms of their ability to decompose crude oil, while the Acinetobacterlwoffii isolate was the

weakest in its ability to decompose crude oil compounds, and this may be related to the nature of pollutants and chemical compounds entering into its composition [24]. The degradation and degradation of crude oil hydrocarbons is of great benefit for their transformation into other types that are less toxic and less dangerous to the environment [25].Our results showed an inverse relationship between the biodegradation rate of hydrocarbons and the concentration of the pollutant, which is in agreement with [26,27,28]. High concentration of pollutants that cause a decrease in the biodegradation rate due to high concentration can be inhibitory to microorganisms through toxic effects [27], for this reason it was stated that bioremediation is a useful method for soil remediation if the concentrations of pollutants are moderate. The results also show differences in the biodegradation rate of hydrocarbons, which is in agreement with the study of Ambrazaitiene et al. [28] who concluded that the biodegradation rate depends on the type and number of microorganisms and the type, structure and level of pollution.

CONCLUSIONS

The results of the current study showed the ability of the studied bacteria to biodegrade contaminated crude oil in soil. The most efficient bacterial species in biodegrading crude oil was Bacillus muralis.

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