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Aspergillus Flavus, Aspergillus Niger, and Aspergillus Terreus are Three Distinct Types of Fungi Used Utilized in Crude Oil Biodegradation

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Abstract. The biodegradation process is one of the best ways to remove organic pollutants with different organic concentrations that have a very dangerous impact on the ecosystem. Soil samples contaminated with crude oil were collected from eight oil producing sites of Basrah Governorate. In the present study three types of fungi species were isolated from the soil contaminated with crude oil in the producing fileds .The fungi species were diagnosis of in the laboratories of the College of Science / University of Basrah to identify them accurately. as Aspergillus flavus , Aspergilus niger and Aspergillus terreus ltheir ability to biodegrade crude oil was tested of the mineral salts medium. As single isolated for 10 , 20 and 30 days incubation. results showed that the Aspergillus flavus the best with biodegradation ability at different incubation periods.

Highlights:

1. Biodegradation effectively removes harmful organic pollutants from ecosystems.
2. Crude-oil-contaminated soils in Basrah revealed three fungi species: Aspergillus flavus, niger, terreus.
3. Aspergillus flavus showed superior crude oil degradation over 10–30 days incubation..

Keywords: crude oil concentrations, fungi and biodegradation.

Introduction

The dangers that various pollutants, especially oil pollutants, pose to the ecosystem. These pollutants cause an imbalance because of numerous accidents, such as the frequent unintentional spills and leaks of crude oil that occur during the exploration, manufacturing, and other industrial processes of petroleum products. One of the main causes of yearly contamination of water and soil is the accidental or intentional leakage of petroleum hydrocarbons into the environment . Which seriously harms a variety of ecosystems, including those of humans, animals, and plants. High

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levels of petroleum hydrocarbon pollutants can cause several harmful illnesses and cancers because of the toxicity of oil and the solubility of certain of its constituents, including benzene, toluene, xylene, and naphthalene '' [1, 2] .

Numerous techniques are being researched to restore petroleum hydrocarbon-contaminated areas to their former state or less hazardous locations because of the negative environmental effects of oil pollution. Many efforts have been made to treat oil pollution using natural processes like bioremediation and phytoremediation because the physical and chemical methods currently utilized to clean up oil spills are costly and harmful to the environment [3] .

Microorganisms transform environmental contaminants into innocuous end products for carbon and energy sources in a process known as bioremediation. It is an inexpensive, large-scale, straightforward technique that eliminates petroleum hydrocarbon contaminants. This technique converts crude oil spilled in terrestrial and marine settings into innocuous molecules by using the metabolic capacity of microorganisms like bacteria, fungi, and a few protozoa. One benefit of bioremediation is that it maintains the soil's texture, physical characteristics, and chemical makeup, including its ion-exchange capacity, water-holding capacity, and aeration pH [4,5 ,6].

Environmental factors including pH, temperature, nutrition, and aeration have less of an impact on the growth of fungi because they may extend through their hyphae. Penicillium,

Pleurotus, Polyporus, Rhizopus, Cladosporium, Alternaria, Aspergillus, Candida, Cephalosporium, Mucoar, Paecilomyces, Fusarium, Geotrichum, Gliochadium, and Rhodoto are a few of the fungi that are employed as hydrocarbon decomposers. In contrast, the proliferation of bacteria is influenced by their surroundings [7, 8] .

Depending on the depth of the soil and the nutrients present, fungi make up the majority of soil bacteria and are a significant part of microorganisms in the majority of soils. Because they have adapted to conditions that require biological treatments for the majority of contaminants, fungi play a very complicated role in soil and are vital to the ecosystems in the soils in which they reside [9].

Through their metabolic activities, microorganisms can adapt to various hydrocarbon molecules in a variety of ways Microorganisms can break down hydrocarbon

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compounds in a variety of ways by breaking down organic and inorganic contaminants with the help of enzymes. Other microbes facilitate this process by secreting surfactants to boost the degradability of crude oil or by releasing glucose to encourage the growth of species that break down hydrocarbons [10, 11] .

However, only a small number of microbes, including fungi and bacteria, are capable of fully mineralizing complex substances, including asphaltenes and resins, from their organic to inorganic condition. The availability of a broad set of metabolic genes in a bacterial consortium is one advantage of microbial communities. These genes work in concert to achieve promising purification of numerous contaminants [12] .

Methods

Gathering of samples Petroleum hydrocarbon-contaminated soil samples were taken from eight different oil field locations, For each site, the contaminated soil was put in sterile polyethylene bags Following the scraping of the top layer of soil to a depth of 5 to 15 cm. The soil samples were then transported to the laboratory and stored at 4 ° C in a refrigerator until they were required [13].

''Isolation and Identification of Fungi''

To separate fungus from crude oil contaminated soil, [14] approach was employed. To make a 1 x 10-3 dilution, 10 g of each sample was weighed and combined with 90 ml of sterile distilled water in a glass flask. After vigorously shaking the solution with an electric shaker for 10 minutes, one milliliter was removed using a sterile pipette and placed on a sterile Petri plate. Before it solidified, an oil agar medium was added. The medium was mixed using a millimeter motion to guarantee uniformity. After allowing the plates to solidify, they were incubated at 25°C. Three duplicates of each sample were made.

Identification of fungal colonies in the laboratory

Five days later, the fungal plates were inspected to determine which fungus was growing on the culture material. Using a microscope for dissection, the morphological characteristics of the growing fungi were recognized. Following their isolation on potato dextrose agar medium and pure cultures, the fungus was created into glass slides. They were then stained with Lactophenol cotton blue dye, and they were inspected and

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diagnosed in the College of Science/University of Basra labs to ensure an accurate identification.

After that, the fungal isolates were placed in test tubes with PDA tilted media and refrigerated at 4 °C until they were needed for biodegradation.

The ability of Fungi species to biodegradation oil

The capacity of previously reported fungus species to digest crude oil was estimated using the [15] approach. The potential of the fungal species to biodegrade crude oil as single isolates was assessed using those that received a score of (+++) in the first test. In the lab, the fungal isolates that were employed in the experiment were active. Fill each of the 250 ml glass beakers with 100 ml of the salt medium. MSM mineral following pH adjustment to 4.5 and sterilization of all used flasks. Fill each flask with 1 milliliter of laboratory crude oil after sterilization. Two fragments of fungal colonies were added to the used flasks at the pace of two individual replicates.

''Crude oil extraction''

Following the previously stated procedure, 1 ml of HCl of 1N was added to the liquid media to extract petroleum hydrocarbons, which terminated the Fungal activity after the incubation period. According to [16] , 80 milliliters of a 1:1 ether petroleum and acetone mixture were added to the separating funnel along with the liquid growth media, and the funnel was quickly shaken several times. The material was then allowed to settle and separate into two layers. Its purpose is to remove any remaining water and impurities from the sample and to retrieve the descending solution from the separator column in the glass flask. Anhydrous sodium sulfate is layered on top of glass wool at the bottom of the separation column. While the lower layer (water + acetone) ignored the lower layer and passed the upper layer over it, the top layer contains petroleum hydrocarbons. Following vaporization of the solvent, the crude oil was measured gravimetrically by [17].

$$\text{Degradation}\% = \frac{mg\ of\ \ crud\ oil\ control - mg\ of\ crud\ oil\ test}{mg\ of\ \ crud\ oil\ control} * 100$$

''Statistical Analysis''

A Complete Randomized Design was used to examine the study data (CDR). Using the pre-made statistical tool Genstat, the least significant difference (LSD) test was used to analyze the significance of differences between the analyzed averages at

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the level of significance ($p<0.05$) based on a factorial experiment with three components and their interactions.

Result and Discussion

Identifying the types Fungi

The ability of majority of fungi isolated from petroleum hydrocarbon-contaminated soils to biodegrade petroleum hydrocarbons varies. When evaluated in a culture media containing crude oil, the fungal isolates Aspergillus flavus, Aspergillus niger, and Aspergillus terreus demonstrated their capacity to biodegrade petroleum hydrocarbons. Its capacity to biodegrade crude oil in liquid media is used in laboratories. At a temperature of 30 °C for 10, 20, and 30 days of incubation.

Evaluation of fungi's capacity to biodegrade ‘’crude oil in liquid media’’

Table (1) and the results of the statistical analysis showed The fungal species that were biodegrading petroleum hydrocarbons in liquid culture conditions and over 10, 20, and

30 days of incubation differed significantly ($P < 0.05$). As the petroleum hydrocarbons become more biodegradable throughout the incubation time, the biomass of the fungal species grows. It was shown that the isolate Aspergillus flavus performed better than other species in terms of clearance rates, despite Aspergillus niger and Aspergillus terreus being less successful in terms of biodegradation .This demonstrates that the various incubation times and the impact of part in the fungal species played a unique.

Table (1) shows the average total amounts of leftover petroleum hydrocarbons in media containing fungi at various incubation periods (µg/L).

Time effect rate	*A.terreus*	*A.niger*	*A.flavus*	Control	Treatmen Tim
35.425	27.144^{c}	26.096^{c}	24.724^{c}	63.737	10
28.229	21.537^{b}	19.144^{b}	15.923^{b}	56.313	20
19.742	13.175^{a}	12.433^{a}	9.026^{a}	44.335	30

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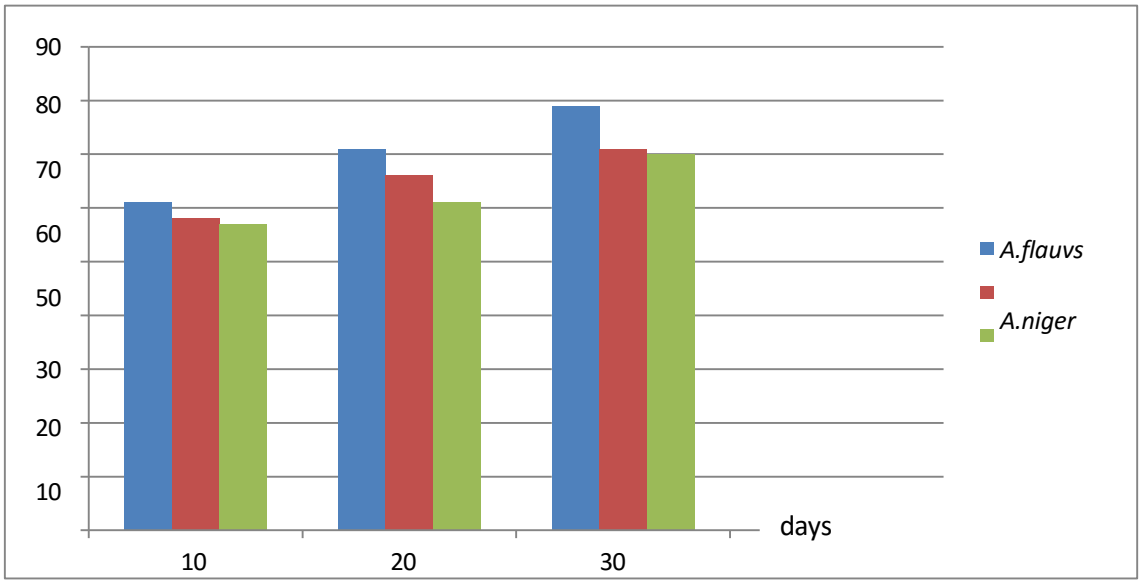
	20.619	19.224	16.558	54.795	Fungi effect rate

Time = 0.144, total overlap = 0.289%, and coefficient = 0.169% are the LSDs.



The percentages of biodegradation fungal species in vitro and the incubation duration (days) are shown in Shape (1).

''Discussion''

Certain compounds in petroleum can be biodegraded by using microorganisms such as fungi in the bioremediation process. Some fungal species that have these characteristics are Aspergillus flavus, Aspergillus niger, and Aspergillus terreus [18]

According to the findings of the statistical analysis in Figure 1, A. flavus outperformed the other two isolates, A. niger, in the biodegradation process of crude oil in terms of its capacity to biodegradation petroleum hydrocarbons at varying incubation durations .The fungus A.flavus showed significantly better performance than the A.niger and A.terreus fungi in terms of biodegradation rates of hydrocarbon compounds at an incubation period of 30 day. the highest percentage was 9.026 mg/L-1. However, In terms of overall concentration, After 30 days of incubation, A. niger came in second with 12.433 mg/L-1. while A. terreus ranked lowest with 13.175 mg/L-1 during the same incubation period. According to these findings, the biodegradation of petroleum compounds improves with an extended incubation period [19].

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In terms of biodegradation, Figure 1 further demonstrates the superiority of isolation A. flavus over the other isolates, as it recorded 79% 30 days, 71% 20 days, and 61% at 10 days of incubation. Fungi A. niger followed, recording 58% at 10 days and 66% at 20 days. At 30 days,

It 's maximum rate of biodegradation is 70%. The fungus A. terreus, on the other hand, showed less biodegradation, recording 57% at 10 days, 61% at 20 days, and 70% at 30 days. This demonstrates that the largest biodegradation occurred throughout the extended incubation period. This research can be used by [9].

The capacity of other microorganisms to express hydroxylase enzymes, specifically hydrocarbon oxidizing enzymes like alcohol dehydrogenase and alkane hydroxylase, which contribute to the biodegradation of hydrocarbons, makes the biodegradation process superior in terms of removal [20].

Conclusion

This study showed that laboratory-selected fungal isolates could effectively remove petroleum hydrocarbons because of their unique enzymes that biodegradation petroleum hydrocarbons. This allowed the isolates to successfully biodegrade and remove these harmful substances from soil that had been contaminated

Acknowledgement

By supporting microorganisms in all types of pollution, this method demonstrates that biological remediation is the most effective in terms of biodegradation, lower costs, and less environmental harm

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