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The Effectiveness of *Aspergillus fumigatus* and *A. niger* in Biodegrading Petroleum Derivatives in Contaminated Water

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Abstract

Fungi have attracted increasing attention in biodegradation due to their ability to degrade a wide range of substances through a process known as mycodegradation. This study evaluated that *Aspergillus niger* has a significant effect in biodegrading contaminated water samples. TPH concentrations in water samples before and after treatment with *A. nigar* ranged from 1,850 to 1,023 µg/l. TPH removal efficiency reached 44.7% with *A. niger* and 55.05% with *A. fumigatus* (from1.059 to 0.476 µg/l). The remaining PAHs were analyzed by GC-MS. The 10 not substituted PAHs on the list of important contaminants (EPA 10 PAHs) were the most frequently analyzed PAHs, with concentrations varying from oil to oil. *A. fumigatus* showed a higher degree of degradation of PAHs compared to *A. niger*. Treatment unit containing *Aspergillus* indicates to be effective in removing heavy metals and improving most physical and chemical properties and nutrients in industrial waste waters analyzed.

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1. Introduction

Biodegradation represents an economical, efficient and eco-friendly approach for removing petroleum contaminants from polluted environments and avoiding the harmful impacts of oil spills, it offers a sustainable alternative to conventional physical and chemical methods such as photolysis, combustion, landfilling, ultrasonic breakdown, and the use of dispersants [1]. In recent years, fungi have gained increasing interest in biodegradation due to their ability to break down a wide range of substances through a process called mycodegradation [2], this ability is due to the enzymatic machinery that enables them to utilize a wide variety of both natural and synthetic materials by producing extra cellular enzymes to their environment, thus enabling it to break down complex compounds in to simple ingredients, which can be taken up and metabolized within their cells [3] In the bioremediation processes, fungi performance as a bioreactor by breaking down or are bio-sorbent by accumulating organic pollutants [4]. Certain species of the genus *Aspergillus* recognized as opportunistic pathogens, have been isolated from diverse substrates throughout significant biomes, including soil and water, and play a crucial role in the breakdown of organic matter within ecosystems [5]. With recent technological improvements, there has been an increased need for oil derivatives, resulting in a significant surge in global consumption of these products [6]. Essentially Oil is extracted from the earth, within a periodic course of stages, from extraction and transportation. However during the production processes, accidents and errors may occur, resulting in environmental consequences [7, 8]. Crude oil is often a complex and complicated incorporation of several hydrocarbon molecules and various non-hydrocarbon substances, comprising diverse chemical elements [9]. Petroleum hydrocarbons (PHCs) in aquatic water and residue surroundings can damage the environment and threaten human and animal health [10]. PHCs will pollute aquatic systems in high quantities with alkanes, olefins, and aromatics, which make up most organic molecules and byproducts [11]. The alkane component includes n-alkanes, isomeric alkanes, cycloalkanes, and a difficult-to-classify combination [12]. PHCs are less soluble in water

and rapidly adsorbent onto particulate matter and settle in bottom sediments, making them persistent and recalcitrant in aquatic environments. PHCs with different hydrocarbon kinds and concentrations have different microbial breakdown rates. As PHC molecular weight grows, biodegradation rate decreases [13]. Aromatic hydrocarbons biodegrade less than aliphatic ones [14]. PHC monitoring assesses aquatic system contamination and identifies sources. However, PHC extraction methods vary in scope, solvents, and calibration standards, making comparisons difficult [15]. As extracellular enzymes degrade hydrocarbons as their primary carbon source, fungi have been increasingly studied for bioremediation of crude oil-contaminated soils. Fungi grow and multiply rapidly under harsh environments [16]. Kota *et al.* [17] investigated *Aspergillus* sp., and *Penicillium* sp. for the cleanup of hydrocarbon-contaminated water. His research demonstrated that fungi metabolize hydrocarbons [18]. Gas chromatography is a common petroleum hydrocarbon detection method. It uses a chromatographic column to separate petroleum hydrocarbon components, which a detector quantifies [19]. The primary aim of this research, however was the isolation and identification of *Aspergillus* spp. and the remediation of water contaminated with crude oil, while proposing bioremediation as an innovative treatment method through the utilization of suitable fungal species for the decomposition process.

2. Materials and Methods

2.1. The media used in the study

All of the culture media included in this research were prepared according to the instructions of the producing company installed on each package, and were sterilized by oxidizer at a temperature of 121°C and 1 atmospheric pressure for 20 minutes.

2.2. Isolation of Fungi

Fungi were isolated from the waste water collected in the study areas using the serial dilution method 0.5 of the 3-10 dilution was taken and placed in Petri dishes containing potato dextrose agar medium with the antibiotic chloramphenicol added to prevent bacterial growth [20]. The sterilized dishes were incubated at 25 ± 2 °C for 5-7 days, after which the fungi were purified and diagnosed [21].

2.2.1. Purification and preservation of isolates

Fungal isolates were grown on solid potato dextrose medium containing the antibiotic chloramphenicol and incubated at 25 ± 2 °C for one week, with growth monitored until satisfactory results were achieved. The isolates were then refrigerated at 4°C and the sequence repeated every four weeks.

2.2.2. Fungal Identification

Macroscopic identification was performed by observing colony growth on solid potato dextrose medium, and by observing morphological characteristics such as colony color, colony shape, colony texture, and fungal colony size, similarly the colony shape was again observed from the opposite side of the plate. The colonies were diagnosed under microscope respectively

2.3. Water collection

Water samples were collected from gas stations, local power generators and oil changing garages in Diyala, Iraq in December 2024. The samples were collected in dark glass bottles, placed in ice packs, brought to the laboratory and refrigerated in storage for analysis.

2.3.1. Biological treatment of industrial wastewater using *Aspergillus* fungi

Two of the most frequent *Aspergillus* species were individually selected. From the following.

A. They were cultured in solid PDA medium and grown. The most frequent fungi during the study period were then selected and transferred to liquid PDB medium.
B. The fungi were grown on PDB medium. 250 ml of the medium and 10 ml of contaminated water were added to the medium in a 500 ml transparent glass bottle. The culture was incubated for five days at 28-25°C.
C. A sample of the fungi grown in the above paragraph (B) was taken and cultured in PDB medium. 250 ml of the medium and 20 ml of contaminated water were added to the culture in a 500 ml transparent glass bottle. The culture was incubated for five days at 28-25°C. The A sample of the growing fungi in the above paragraph was taken and cultivated in 500 ml transparent glass bottles, then 250 ml of industrial waste water was added to it, and 10 ml of PDB medium equally added and incubated for five days at a temperature of 28-25 degrees Celsius, then filtered with 0.45 micrometer filter paper where physical, chemical and heavy elements tests were conducted on it.

This process was repeated on the most frequent fungal species used in the experiment.

2.3.2. Physical and chemical tests of water samples contaminated with crude oil

The water samples polluted with crude oil undergo physical and chemical tests, including pH, electrical conductivity, bicarbonate, calcium, magnesium, and field capacity, as detailed in [22].

2.3.3. Extraction of hydrocarbon compounds from samples

Hydrocarbons extracted from the water samples adopted the United Nations Environment Programme method [23].

2.3.4. Poly aromatic hydrocarbons (PAHs) sources

The origin of PAHs is assessed based on the following ratios:

A. Low molecular weight to high molecular weight ratio (LPAH/HPAH). Values below 1 indicate thermal origin, values above 1 indicate petrochemical origin from crude oil and its derivatives [24].
B. Phenanthrene/anthracene ratio (Phe/Ant). Values above 1 indicate petrogenic origin of hydrocarbons, values below 1 indicate igneous origin of hydrocarbons [25].
C. Fluoranthene/Pyr ratio (Flu/Pyr). Values below 1 are attributed to petrogenic origin, values above 1 are attributed to thermal origin [26].

2.4. Analysis of samples

Total petroleum hydrocarbons (TPHs) concentrations in samples was determined by using Spectrofluorometric, whereas PAHs concentrations in samples were determined by using Chromatography instrument type Shimadzu (GC 2010) at department of biology, Tikrit University. Chromatography measures contaminants, the carrier gas was Helium at flow rate 1 mL/min and fused silica capillary column of 30 m length, 0.32 mm internal diameter and 0.25 µm film thickness was employed, readings determines the fungus' purifying efficiency according to the procedure of Barnes *et al.* [27].

2.5. Estimating the efficiency of the treatment unit

The percentage of removal was calculated according to the following equation:

2.6. Percentage of removal (PoR)

$$PoR\ \% = \frac{\rho_{before} - \rho_{after}}{\rho_{before}} \times 100 \quad \ldots (1)$$

where ρ_{before} and ρ_{after} are the pollution concentration before and after treatment respectively.

3. Results and Discussion

3.1. Fungal isolation and identification

The morphological diagnosis of the fungus grown on PDA medium shows two types of *Aspergillus* (*niger* and *fumigatus*). *A. fumigatus* was characterized by the appearance of light green colonies on the surface. Under the microscope, the conidia appeared blue and were characterized by their distinctive broom-like shape (figure 1).

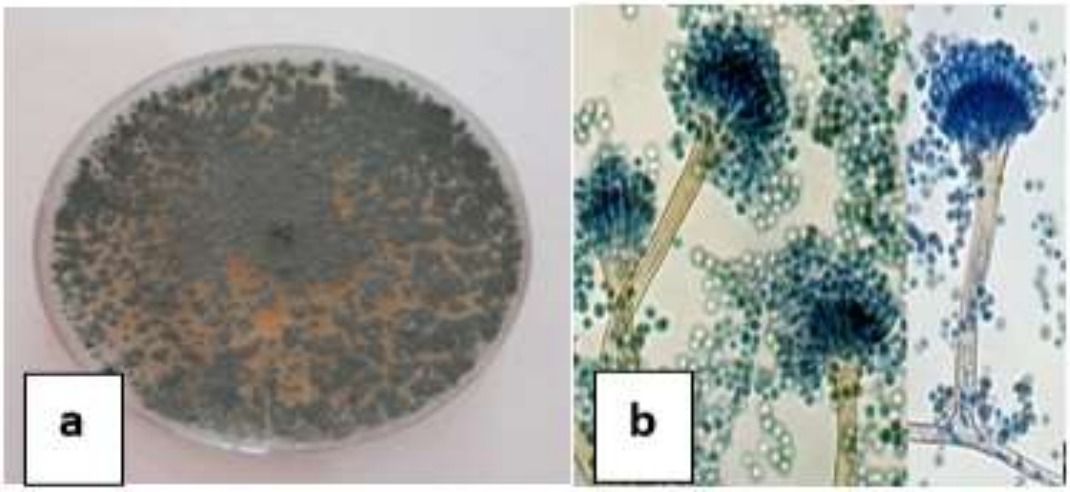

Figure 1: *Aspergillus fumigatus*. (a) conidia under 40X microscopic examination with lactophenol cotton blue, (b) on PDA agar after incubation at 25C for 3-5 days

spergillus niger grown on PDA medium shows that the colony appears black and homogeneous on the culture media. Similarly the conidia are pale brown, transparent, erect, simple, and thick-walled, connected at the bottom to the foot cells. At the apex they are spherical vesicles bearing conical heads called phialides, which bear brown or black spores at their apex. The outer frame of the colony is white and regular in shape, figure (2).

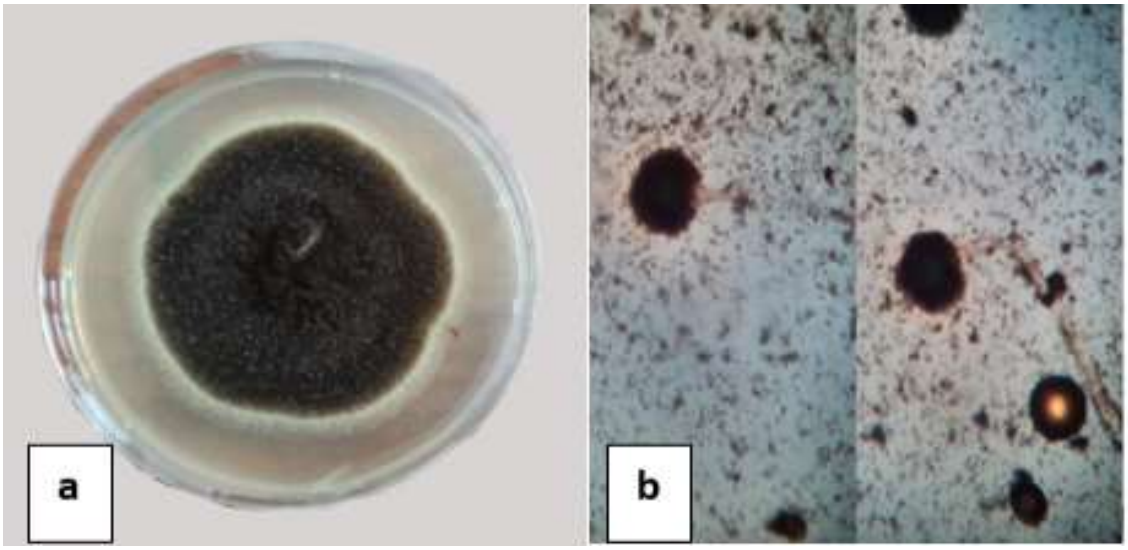

Figure 2: *Aspergillus niger* a- conidia under 40X direct microscopic examination, b- on PDA agar after incubation at 25C for 3-5 days

The concentrations of TPHs in water samples treated with *A. niger* in figure (3) ranged from 1.850 to 1.023 µg/l, in water before treatment and after treatment respectively. The removal percentages achieved by the addition of *A. niger* to contaminated water samples was 44.7%, where the concentrations of TPHs in water samples treated with *A. fumigatus* ranged from 1.059 to 0.476 µg/l, in water before treatment and after treatment respectively. The removal percentages achieved by the addition of *A. fumigatus* to contaminated water samples was 55.05%.

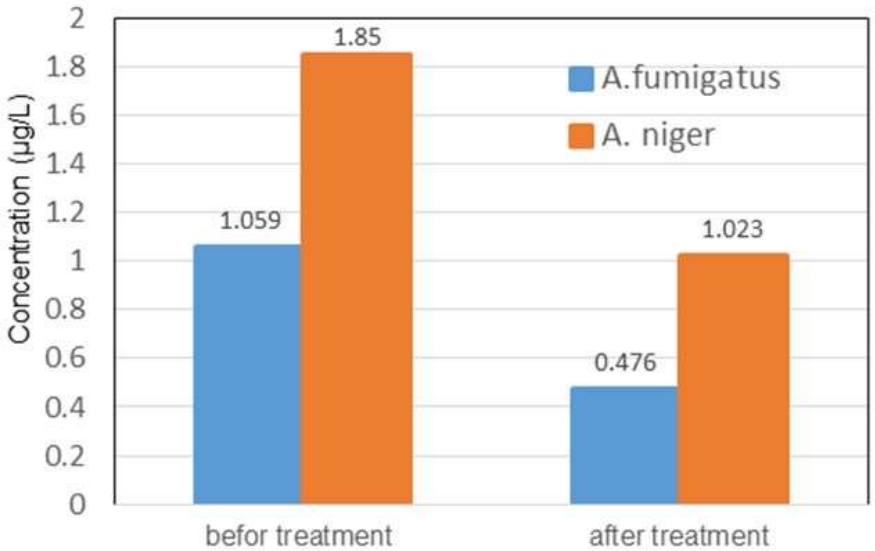

Figure 3: concentrations of TPHs (µg /l) in water samples before and after treatment with *Aspergillus* spp.

After adding *Aspergillus* sp., polycyclic aromatic hydrocarbons (PAHs) in water samples contaminated with various crude oil concentrations decreased significantly throughout all phases.

3.2. PAHs in water

The remaining PAHs were identified using GC-MS. The 10 unmodified PAHs (EPA 10 PAHs) on the priority list of pollutants are the most commonly studied PAHs, their concentrations are typically expressed in oil. Among the 10 PAHs, acenaphthene, naphthalene, acenaphthylene, fluorene, phenanthrene, pyrene, anthracene, fluoranthene, and dibenzo[a,h]anthracene were identified in the raw oils in this study. According to Table (1), *Aspergillus fumigatus* has a higher degree of degradation of PAHs than *Aspergillus niger.*

Table 1: Determination of the percentage of degradation of individual PAH components in crude oil by two Aspergillus isolates by GC-MS analysis

Name of compound	Treatment with A.*niger*	Treatment with *A.fumigatus*
Naphthalene	80%	84%
Acenaphthylene	100%	100%
Acenaphthlene	70%	73.3%
Fluorene	79%	82%
phenanthrene	85%	88%
Anthracene	ND	ND
fluoranthene	ND	ND
pyrene	60%	63%
Benzo[k]fluoranthene	74%	77%
Dibenzo[a,h]anthracene	96%	99%

ND: Not detected

Table (2) showed the ratios of individuals PAHs which were decreased after treatment with *Aspergillus* spp. These results treatments unit with *Aspergillus fumigatus* fungus has been shown in table (3), it showed that it was effective in removing heavy metals and improving most physical and chemical properties and nutrients in industrial waste waters analyzed.

Table 2. Ratios of individuals PAHs in water samples before and after bioremediation.

The equation	Before treatment	Treatment with *A.niger*	Treatment with *A.fumigatus*
∑Low PAHs	6070.21	440.12	350.22
∑high PAHs	11227.9	749.1	578.3
LPAHS/HPAHs	0.56788	0.0031	0.0050

Table 3. Water treatment by *Aspergillus fumigatus*

Physico-Chemical Parameter	Before treatment	After treatment
Conductivity (µS/cm)	800	712
pH	5.8	6.8
salinity	0.8	0.4
Temperature (˚C)	30	25.6
Alkalinity (mg/L)	25	10
Hydrocarbonates	67	nil
Calcium hardness	400	150
Magnesium hardness	280	75
Phenol	0.03	0.005
Total PAH	0.09	0.0001

Nil: none or zero.

The results treatment unit with *Aspergillus niger* fungus has been shown in Table (4), suggested that it was effective in removing heavy metals and improving most physical and chemical properties and nutrients in industrial waste waters analyzed.

Table 4. Water treatment by *Aspergillus niger*

Physico-Chemical Parameter	Before treatment	After treatment
Conductivity (µS/cm)	800	610
pH	5.8	7
Salinity	0.8	0.38
Temperature (˚C)	30	24
Alkalinity (mg/L)	25	8.8
Hydrocarbonates	67	2
Calcium hardness	400	132
Magnesium hardness	280	66
Phenol	0.03	0.008
Total PAH	0.09	0.0003

3.3. Discussion

This investigation identified two prevalent *Aspergillus* species isolated from water contaminated with crude oil: *A. niger* and *A. fumigatus*. The result concurred with Al-Dhabaan *et al.,* [28]. A total of 22 fungal isolates were assessed for their ability to biodegrade crude oil. Only three isolates demonstrated hydrocarbon oxidation capabilities and exhibited a promising biodegradation pattern, indicated by the alteration of Czapek's broth color from blue colorless. These isolates were *A. fumigatus and A. niger. A. niger* exhibits the greatest degrading potential among fungal isolates. In the research conducted by Ahmed *et al.,* [29] fungal variety indicated that *Aspergillus* constituted the highest prevalence at 60% of contaminated samples. This genus encompasses a varied array of species capable of adapting to numerous conservational conditions and severe to extreme habitats, including deserts and soils polluted with dangerous substances such as oil. *Aspergillus* generates numerous asexual conidia, capable of withstanding stress, and produces sexual ascospores that facilitate its extensive dissemination [30,31]. The variations in the prevalence of fungal genera may be attributed to their resilience and adaptability to harsh conditions, tolerance to diverse temperature ranges, capacity to secrete enzymes that facilitate the decomposition of various substrates for energy and growth, and their prolific production of reproductive units that promote environmental dissemination [32]. Furthermore the findings of the present study align with those of Minati and Mohammed-Ameen [33], wherein *Aspergillus* exhibited the highest prevalence among the isolated taxa in their investigation. Subsequent studies have shown that several fungi are capable of degrading a variety of PAHs such as naphthalene, phenanthrene, fluoranthene, pyrene, pyrene, and benzo[a]pyrene [34, 35]. The research conducted by Barnes *et al.* [27], shows that the fungal strains obtained had a higher propensity to consume oil and its associated PAHs as a source of carbon and energy. Additionally, the fungi employed in this research are marine-based, which means they have a greater chance of survival and adaptation in the presence of polluted environmental conditions, as a result, their tolerance to high salinity, wide pH values, and temperature fluctuations is significantly considerable. All results of this study demonstrated that elevated concentrations of crude oil in water samples correspond with a reduction in the percentage of polycyclic aromatic hydrocarbons (PAHs) removed by *Aspergillus* sp., as hydrocarbon pollutants inhibit the enzymatic activity of microorganisms with increasing pollution levels [36].

These findings are consistent with the findings of Hadibarata and Tachibana [37], revealed that increased concentrations of crude oil had a negative impact on the rates of decomposition. This is largely due to the capacity of fungi to degrade hydrocarbons decreases as the concentration of crude oil increases. Following the introduction of fungal genera (*Penicillium* sp. and *Aspergillus* sp.), the decrease in polycyclic aromatic hydrocarbons (PAHs) in soil samples that were contaminated with crude oil can be attributed to the fact that these fungi use hydrocarbons as their sole source of carbon and energy for cell wall synthesis. This results in increased biomass as well as the production of

carbon dioxide and water as byproducts. Fungal genera have a remarkable capacity to break down hydrocarbon molecules into smaller components through the use of their extracellular enzymatic systems, which produce specific enzymes for the purpose of performing this activity [38]. Sari *et al.* [39] conducted an experiment to evaluate the effectiveness of fungal species (*Penicillium* sp. and *Aspergillus* sp.) in the degradation of polycyclic aromatic hydrocarbons (PAHs) in crude oil samples obtained from Siak Petroleum Company in Riau, Indonesia. *Aspergillus* species have been shown to be more effective than *Penicillium* species in the process of breaking down polycyclic aromatic hydrocarbons (PAHs), according to several pieces of research, there are several factors that influence the biodegradation of crude oil, including the specific species of fungi involved and the concentration of crude oil. Another study conducted by Vanishree and colleagues [40] investigated the ways in which the increase in the global population of humans has led to an increase in the demand for petroleum products and a variety of industrial chemicals, because of this, a sizable amount of potentially hazardous compounds have been released into the atmosphere, water, and soil, which has the potential to represent a threat to the ecosystem. Relatively, the processing and distribution of petroleum hydrocarbons, in conjunction with the usage of petroleum products, have led to the contamination of the environment and have contributed to the emission of polycyclic aromatic hydrocarbons (PAHs) into the environment in a localized manner[41].

Previous reports have shown that Aspergillus spp. are more efficient at degrading low molecular weight PAHs [42]. Low molecular weight PAHs are more volatile and soluble in water and are therefore more susceptible to degradation. On the other hand, high molecular weight PAHs are often more difficult to remove because of their low bio-availability and affinity to soil organic matter, studies have employed low molecular weight PAHs as the sole source of carbon, but this is uncommon for high molecular weight PAHs [43] . Other researchers demonstrated that the degradation of high molecular weight PAHs is facilitated by the presence of additional carbon sources, such as glucose, that are available in abundance [27]. Most importantly this investigation examined the fate of PAHs as a mixture of different hydrocarbons, with oil as the sole source of carbon. The capacity of fungi to take in PAH as their sole source of carbon and energy has previously been documented. [44]. However, there are also reports that degradation of PAHs by fungi occurs through cometabolism with another carbon source [45].

4. Conclusions

Arguably, fungal species, notably *Aspergillus*, can thrive in crude oil-polluted water because their extracellular enzyme systems breakdown hydrocarbon molecules, their primary source of carbon and energy for cell wall formation. Fungi can bioremediate crude oil-polluted water.

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