

Original Research Article

The Use of Fungi and Biotechnology in Treating Environmental Pollution

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Abstract: Environmental pollution from crude oil and its derivatives poses a significant environmental challenge due to the presence of toxic hydrocarbons such as benzene, phenols, and polycyclic aromatic hydrocarbons (PAHs). Bioremediation has emerged as an environmentally friendly alternative to conventional chemical and physical methods. In this study, the filamentous fungus *Aspergillus niger* was used to synthesize silver nanoparticles (AgNPs) using cell filtrate as a green, low-cost, and sustainable approach. The resulting nanoparticles were characterized via visual observation, ultraviolet-visible (UV-Vis), and FTIR spectroscopy, confirming their formation and stability. Subsequently, the efficacy of *A. niger* alone and in combination with AgNPs in treating crude oil-contaminated water samples was evaluated. The results demonstrated a significant reduction in total petroleum hydrocarbon (TPH) concentrations and a significant degradation of PAHs. Treatment with the *A. niger*-AgNP mixture demonstrated superior performance compared to using fungi alone, particularly in degrading complex and stubborn hydrocarbon compounds. These results confirm the potential of nano-assisted fungal biotechnology as a promising option for sustainable environmental remediation.

Keywords: Bioremediation, TPH, *Aspergillus Niger*, Hydrocarbon Compounds.

INTRODUCTION

Since the discovery and use of crude oil, problems with environmental contamination have emerged and are now posing a threat to human existence. Environmental contamination issues, including the potential for oil spills and other oil wastes to move to water surfaces, began to materialise as oil production and consumption rose. Because of the large amounts of its derivatives and toxic compounds that have extremely negative effects on both humans and animals, one of the worst types of pollutants is oil [1]. Oil is harmful because it contains a lot of hydrocarbons, including the carcinogenic chemical benzene. Furthermore, ether and gasoline, which are hazardous substances in large amounts, are found in oil. Furthermore, the oil's phenolic chemicals are one of the things that cause allergy when inhaled. When combined, these effects have the potential to kill aquatic life. Hydrogen sulphide and other harmful gases are also present in oil, particularly crude oil, and are released when hydrocarbon particles disintegrate or evaporate [2]. One of the most significant and secure methods for removing oil contaminants from water is biodegradation [3]. Microorganisms, such as bacteria, fungus, and algae, accomplish this process by consuming the hydrocarbons found in the oil. The type of microbe determines the extent of intake [4]. Biological treatment is conducted through the breakdown of oil hydrocarbon compounds by microorganisms and conversion into simple and environmentally friendly chemicals such as carbon, carbon dioxide, nitrogen, phosphorous and others [5]. The use of fungi has recently been witnessed in the field of biological treatment and disposal of the environmental pollutants that are caused by oil waste, such as *Aspergillus*. It was discovered that these animals frequently eat the hydrocarbons found in petroleum products [6]. The process of nanotechnology works with particles that are between 1 and 100 nm [7]. Physically, chemically, and structurally, nanomaterials are different from their larger counterparts. Their nanoscale shape and size are responsible for their remarkable magnetic, electrical, and optical properties as well as their surface activity [8]. Nanotechnology, which enhances the qualities of fungi at a low cost, is one of the new and creative techniques that have been presented recently to regulate and minimise environmental pollution [9]. The goal of this work is to create and analyse silver nanoparticles using an extract of the fungus *Aspergillus niger*. These NPs' efficacy in cleaning up oil contaminants in water were assessed.

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MATERIALS AND METHODS

Maintenance of Fungal Strains

The *A. niger* strain of fungus was obtained and its acquisition was through a soil sample, which was identified by PCR method. The fungus was subcultured on Potato Dextrose Agar (PDA) (Oxoid, India) at 28 °C for 96 hours and then preserved at 4 °C until used in the biosynthesis of AgNPs.

Production of Fungal Biomass

A. niger was grown aerobically in potato dextrose broth in order to preset the fungal biomass to give nanoparticles. As an antibacterial agent, chloramphenicol (50 µg/mL) was added to the soup. The flasks of the said media were incubated at 28 °C, in a 7 days shaking incubator (Lab Tech, India) and stirred at 100 rpm. Isolation of fungal mycelia Fungal mycelia were then isolated on broth through filtration with sterile Whatmann filter paper No. 1 and the isolated mycelia rinsed (3 times) with sterile distilled water to remove any medium constituents that may still interact with metal ions. A fungal mass of 20 grammes was put in 200 ml deionized water and allowed to stand at 72 hours and agitated in the same manner. The cell filtrate was then obtained through filtration after the incubation. The filtrate was then used to obtain nanoparticles [10].

Preparation of Nanoparticles by Biomimic

In order to prepare silver nanoparticles (AgNPs) 50 ml of cell-free filtrate was added to 50 ml of 1 mM AgNO₃ in a 250 ml Erlenmeyer flask and left to incubate in a shaking incubator at 150 rpm and 28°C. At the same time, a positive control of cell filtrate without metal salts and the negative control of only metal salt solutions were also performed in parallel with the experimental flasks [11]. The mixtures of all reactions were kept in the dark in order to avoid any photochemical reaction throughout the experiment.

Characterization of Biosynthesized Silver Nanoparticles

UV-Vis Spectroscopic Study

AgNPs were identified mainly by the colour change of fungus filtrate after the treatment with silver nitrate that was performed by the visual examination. The appearance of a dark brown color in the fungal cell filtrate is an indication of the production of silver nanoparticles as a result of reduction of pure silver ions. To confirm the production of AgNPs, a dual beam UV-Visible spectrophotometer (SPEKOL1300, Germany) was used to sample 1 cm³ of reaction solution at different time intervals and scan the absorbance spectra at wavelengths 300-700 nm at a wavelength resolution of 1 nm.

Ultraviolet Spectroscopy (UV Spectroscopy)

Using an FTIR spectrophotometer (Bruker Tensor 27, Germany) in the 500–4000 cm⁻¹ range, the interaction between the biosynthesized AgNPs and the biomolecules responsible for their reduction, capping, and stabilisation in colloidal solution was examined.

Improving the Production of AgNPs

The most efficient and effective production of AgNPs was achieved by standardising a number of variables, including growth medium, temperature, pH, silver nitrate concentration, and duration. For the sake of repeatability, the experiments were carried out three times. The solutions with different reaction conditions were monitored using a UV Visible spectrophotometer.

Water Collection

In september 2024, water samples were taken from two different locations. Dark glass vials were used to collect water samples, which were subsequently cooled and transported to the laboratory for analysis.

Biological Treatment of Industrial Wastewater Using *Aspergillus Fungi* and *A. Nigar*-AgNP

Aspergillus niger were selected as follows:

- 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. Then a sample of the growing fungi in the above paragraph (T) was taken and cultivated in 500 ml transparent glass bottles, then 250 ml of industrial wastewater was added to it, and 10 ml of PDB medium was added and incubated for five days at a temperature of 28-25 degrees Celsius, then it was filtered with 0.45 micrometer filter paper and physical, chemical and heavy elements tests were conducted on it. The same procedure was done to the *A.niagr* only and *A.nigar*-AgNP which was used in the experiment in

two replicas. The nanoparticle was added to the mixture twice. Moreover, the negative control (water with *A. niger* but without nanoparticles) and positive control (water with nanoparticles) were used.

Extraction of Hydrocarbon Compounds from Samples

Hydrocarbon chemicals were extracted from water samples using the UNEP [12], technique.

PAHs Sources

The following ratios were used to assess the sources of PAHs:

- The ratio of molecular weights, measured in ratio of low to high (LPAHs/HPAHs). Crude oil and its byproducts are considered petrogenic sources when the value is greater than one, and pyrogenic sources when the value is less than one [13].
- The ratio of phenanthrene to anthracene Ratio values below one indicate pyrogenic hydrocarbon origins, while ratio values over one indicate petrogenic origins [14].
- The fluoranthene to pyrene ratio. The sources that are considered petrogenic are those with a ratio below one, whereas those that are considered pyrogenic are those with a ratio greater than one [15].

Analysis of Samples

The spectrophotometric method was used to determine the quantities of TPHs in the samples, while the shimadzo method was used to determine the concentrations of PAHs Following the method outlined by Barnes *et al.*, [16] chromatography is used to test pollutants, and the fungus's purifying effectiveness is determined by comparing the data.

Estimating the Efficiency of the Treatment Unit

The percentage of removal was calculated according to the following equation

$$\text{Percentage of removal} = \frac{\text{Concentration of the pollutant before treatment} - \text{Concentration of the pollutant after treatment} \times 100}{\text{Concentration of the pollutant before treatment}}$$

RESULTS

The goal of this work was to optimise SNPs through biosynthesis, characterization, and optimisation. The cell-free filtrate of *A. niger* was used to reduce aqueous silver ions (Ag^+), allowing for the biological synthesis of silver nanoparticles (SNPs). Assessment of silver nanoparticles (SNPs) produced through biological means: After the *A. niger* growth phase was over, the cell-free filtrate was used to make silver nanoparticles (SNPs). The filtrate had a little yellowish hue at first. After the filtrate was mixed with AgNO_3 , the mixture turned a deeper yellowish-brown colour. After the reaction with Ag^+ ions was complete, it turned a dark brown. The presence of SNPs in the reaction mixture was confirmed by the colour shift, which was exclusively seen in the test flask. No colour change was observed in the two control flasks that were incubated under the same conditions: the one with the aqueous AgNO_3 solution and the one with the fungal filtrate that did not include AgNO_3 (Figure 1).

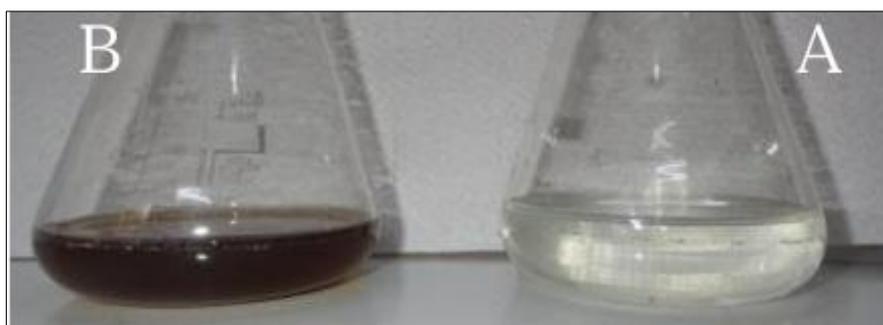


Figure 1: Culture flasks containing (A) 1mM of AgNO_3 solution, (B) Mixture of fungal cell-free filtrate with 1mM AgNO_3 .

Characterization of Ag Nanoparticles Using UV-Visible Analysis

As shown in Figure 2, the UV-Vis spectra of the silver nanoparticles synthesized by *A. niger* extract shows a distinct peak at 436 nm, which is a result of the surface plasmon resonance (SPR) of such nanoparticles. This indicates fairly homogeneous distribution of particle sizes and a range of 20-50 nm size of nanoparticles. In addition, there is a minor peak at approximately 290 nm. Possible chemical constituents in the *A. niger* extract that absorb UV light in this region include phenolics and aromatic compounds, which could explain this peak. It could also be the result of artefacts in the instrument or ambient noise.

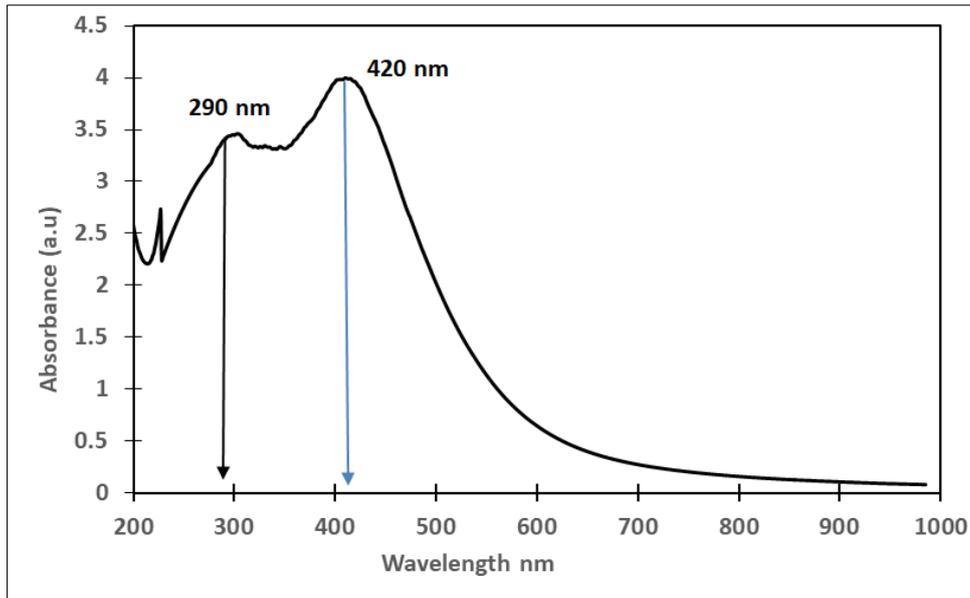


Fig. 2: The characteristic surface plasmon resonance (SPR) peak at about 410 nm is visible in the ultraviolet-visible spectra of silver nanoparticles produced with *A. nigar* extract.

Characterization of Ag Nanoparticles Using FTIR

Figure 3 shows the FTIR spectrum of silver nanoparticles synthesised by *A.nigar* extract. IR spectroscopy of the *A.nigar* extract indicated the existence of functional group alcohols, phenols, amides and probably amines at 3500 cm^{-1} (O-H stretching), 2100 cm^{-1} (C-H stretching), 1637 cm^{-1} (C=O stretching) and 1541 cm^{-1} (N-H bending). The small signal at 420 cm^{-1} is possible M-O bending vibrations which can be used to suggest the interaction of these biomolecules with the surface of the silver nanoparticles.

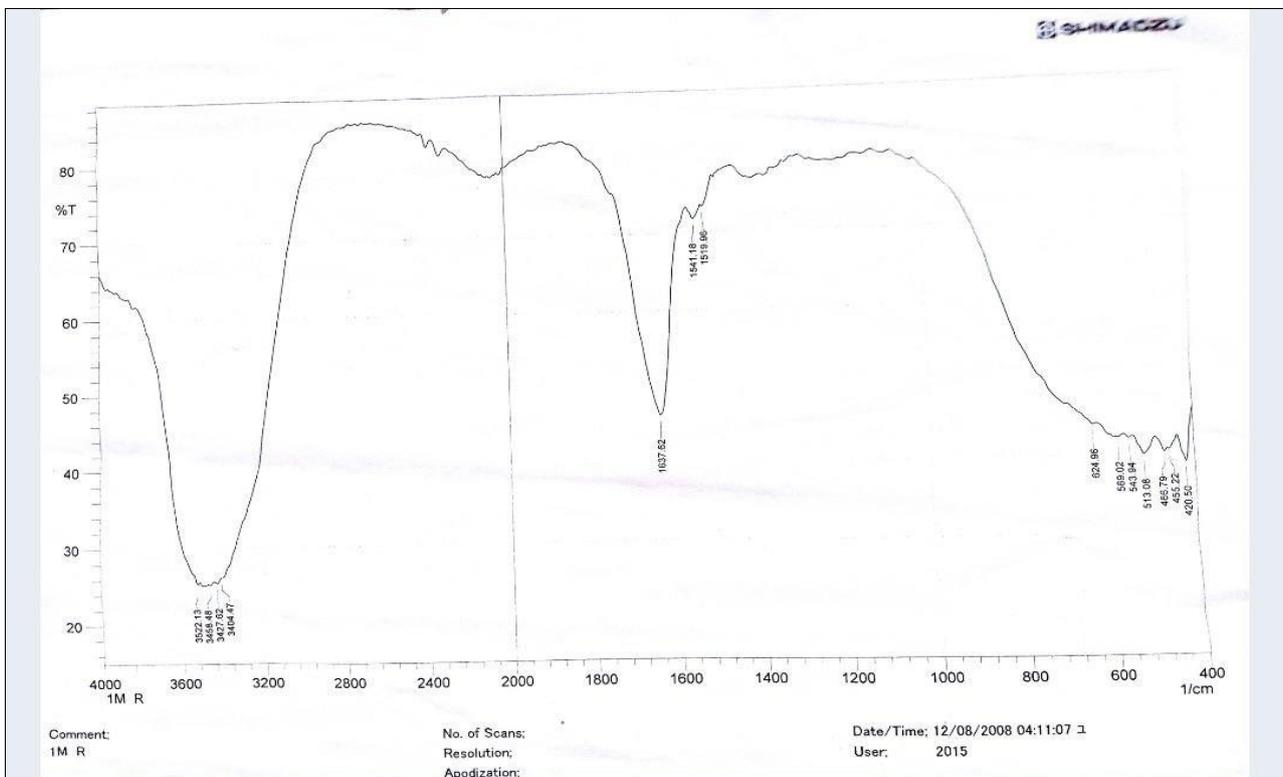


Fig. 3: The FTIR spectrum of silver nanoparticles synthesized using *A. nigar* extract

Before and after treatment with *A. nigar* and AgNP, the amounts of TPHs in the water samples shown in figure (4) varied between 2.6 and 1.21 $\mu\text{g/l}$, respectively.

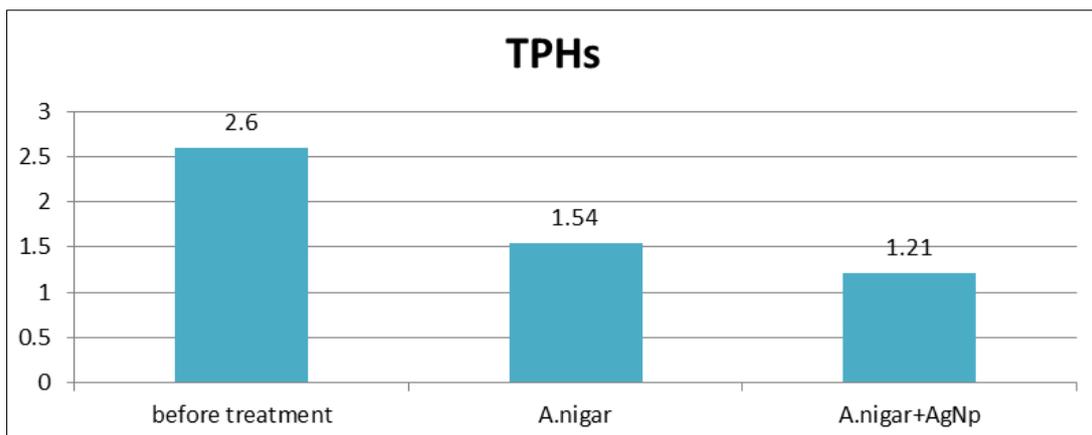


Figure 4: concentrations of TPHs (µg /l) in water samples before and after treatment with *A. nigar*. and *A.nigar-AgNP*

PAHs in Water

The GC-MS was used to examine the remaining PAHs. The amounts of the ten unsubstituted PAHs (EPA 10 PAHs) that are on a priority pollution list vary from one oil to another, and they are the most often tested PAHs. The crude oil utilized in this investigation contained ten of these PAHs: acenaphthene, naphthalene, acenaphthylene, fluorene, phenanthrene, pyrene, Benzo[k]fluoranthene, Dibenzo[a,h]anthracene were.

Table(1) shows that when comparing *A. nigar*, and *A.nigar-AgNP* the former had a higher percentage of PAH decomposition.

Table 1: The percentage of breakdown for each component of the PAH fraction of crude oil as determined by gas chromatographic mass spectrometry

Name of compound	Treatment with <i>A. nigar</i>	Treatment with nanoparticles
Naphthalene	70%	74%
Acenaphthylene	95%	94%
Acenaphthlene	77%	75%
Fluorene	80%	78%
phenanthrene	82%	83%
pyrene	65%	67%
Benzo[k]fluoranthene	77%	74%
Dibenzo[a,h]anthracene	100%	97%

Table (2) showed the ratios of individuals PAHs which were decreased after treatment with *Aspergillus nigar* and nanoparticles.

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

The equation	Before treatment	Treatment with <i>B. nigar</i>	Treatment with <i>A.nigar-AgNP</i>
ΣLow PAHs	6000.01	450.12	370.22
Σhigh PAHs	9237.3	766.1	555.3
LPAHS/HPAHs	0.6788	0.0061	0.0040
Phe/Ant	0	0	0
Flu/Pyr	0	0	0

DISCUSSION

This study found *Aspergillus nigar* and silver nanoparticles effective in treating crude oil-contaminated water. The outcome matched Al-Dhabaan *et al.*, [17]. Biodegradability of crude oil was tested on 22 fungus isolates. Only three isolates showed hydrocarbon oxidation and potential biodegradation, as Czapek's broth from blue to colourless. The isolates were *A. fumigatus* and *A. niger*. *A. niger* degrades the most among fungal isolates. *Aspergillus* was the most common fungus species at 60% in Ahmed *et al.*, [18]. This genus contains a wide range of plants that can survive in deserts and oil-polluted soils. Asexual conidia can resist stress, and sexual ascospores help *Aspergillus* spread [19]. Fungal genera'

resilience and adaptability to harsh conditions, tolerance to diverse temperature ranges, ability to secrete enzymes that decompose substrates for energy and growth, and prolific production of reproductive units that promote environmental dissemination may explain their prevalence [20]. Similar to Minati and Mohammed-Ameen [21], *Aspergillus* was the most common isolated species in their study. Numerous fungal species decompose PAHs like naphthalene, phenanthrene, fluoranthene, chrysene, pyrene, and benzo[a]pyrene [22]. According to Barnes *et al.*, [16], fungal isolates exposed to crude oil and related PAHs grew and used PAHs better, making them a better carbon and energy source. The marine-derived fungi used in this study had an advantage in adapting to and living in the contaminated environment due to their exceptional tolerance for high salinity, broad pH, and temperature and change. All results showed that *Aspergillus* sp. removed fewer polycyclic aromatic hydrocarbons (PAHs) from water samples as crude oil concentrations increased, as hydrocarbon pollutants inhibit microorganism enzymatic activity [23].

These results are consistent with Hadibarata and Tachibana [24], who concluded that crude oil concentration reduced the breakdown rates. The higher the concentration of crude oil the lower the hydrocarbon breaking ability of fungi. Because *Penicillium* sp. and *Aspergillus* sp. rely on hydrocarbons as the only source of carbon and energy to synthesize their cell walls, the amount of polycyclic aromatic hydrocarbons (PAHs) in soil samples contaminated with crude oil went down after the introduction. Increasing biomass produces carbon dioxide and water as byproducts. Special enzymes produced by fungal extracellular enzymatic systems break down hydrocarbon molecules into smaller components [25]. Sari *et al.*, [26], tested *Penicillium* and *Aspergillus* sp. to degrade polycyclic aromatic hydrocarbons (PAHs) in crude oil samples from Siak Petroleum Company in Riau, Indonesia. The study appeared in Environmental Research Letters. Several studies have indicated that *Aspergillus* species break down polycyclic aromatic hydrocarbons (PAHs) better than *Penicillium* species. Fungi species and crude oil concentration affect crude oil biodegradation. Previous research suggests *Aspergillus* sp. can break down LMW PAHs better [27]. LMW PAHs breakdown faster due to their increased volatility and water solubility. However, heavy metal ion PAHs have low bioavailability and high organic matter sorption rates, making them difficult to remove from soil [28]. Using crude oil as the only carbon source, this study studied PAHs' complex hydrocarbon fate. Several fungi may use PAHs entirely for carbon and energy synthesis [29]. Previous studies have shown that PAH is degraded by fungi with another carbon source [30].

Many biological materials have been used in nanoparticle biosynthesis investigations. Shah *et al.*, synthesised AgNPs from *Silybum marianum* plant extract [31], and Abd-Elhady *et al.*, biologically synthesised extracellular AgNPs from *Streptomyces aizuneusis* [32]. Fungi-mediated nanoparticle (NP) synthesis has many advantages over previous approaches. Adjusting reaction settings to optimise NP characteristics is a major benefit. This method could be revolutionary because to the high surface-to-volume ratio of the NPs and their dual role as antibacterial and bioactive fungal capping agents. During AgNPs biosynthesis reaction parameter optimisation, a brown colour indicated colloidal silver particle production in the medium. The reduction of silver ions in the aqueous solution may increase the number of synthesised nanoparticles, increasing colour intensity [33].

CONCLUSION

This study demonstrated the successful synthesis of silver nanoparticles using the fungus *Aspergillus niger* and their effective application in the treatment of crude oil-contaminated water. The combination of fungi and nanoparticles enhanced the removal efficiency of both total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAHs) compared to using fungi alone. This nano-fungal remediation approach offers multiple advantages, including lower cost, environmental friendliness, and the ability to target complex hydrocarbon contaminants. Therefore, the integration of fungal biotechnology with nanotechnology represents a promising direction for developing sustainable and widely applicable solutions to reduce environmental pollution caused by crude oil and its derivatives.

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