

1 **RESEARCH ARTICLE**

2 **Bioprospecting of endophytic fungi *Colletotrichum acutatum* and**
3 ***Aspergillus oryzae* for their diverse role in crude oil biodegradation**

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Abstract

The purpose of the current study was to assess the degree to which endophytic fungi can degrade crude oil. The growth capability of many fungal isolates was investigated using *Colletotrichum acutatum* and *Aspergillus oryzae* on a 1% crude oil in a low-salinity medium as the carbon source. As far as we are aware, the isolated species has never been found in contaminated soil samples in Saudi Arabia's eastern area. *A. oryzae* and *C. acutatum* have demonstrated their ability to digest crude oil by eliminating 78.1 and 59.7 %, respectively. It is noteworthy that they were able to lower the surface tension to 50.3 and 40.8 mN/m, respectively. In addition, the hydrophobicity and emulsification activity were found to be 46.7, 54.1, and 41.2, respectively, and 62.4. solvent extraction, acid precipitation zinc sulfate, ammonium sulfate were among the recovery assays. These methods all shown a correlation between the amount of biosurfactants and the hydrocarbons under examination. Finally, our research sheds new information on the fungal resources present in terrestrial ecosystems that are continuously contaminated. The safe removal of petroleum-oil contamination, bioremediation, and other industrial applications will benefit from this understanding. In addition, this study is the first to demonstrate the crude oil biodegradation potential of endophytic *C. acutatum* and *A. oryzae* fungi in Saudi Arabia, revealing their significant biosurfactant production and applicability in petroleum bioremediation.

Keywords crude oil, DCIP, endophytic fungi, biosurfactant, biodegradation

1. Introduction

Contamination of the environment is a significant issue caused by chemicals, solvents, hydrocarbons and heavy metals. In addition to being extremely detrimental to all living things, including humans, they also indirectly result in financial losses for developing countries [1]. Petroleum oil and its products' extraction, processing, and transportation provide particularly significant environmental issues on a global scale. Despite precautions, a significant amount of the more than billion tons of petroleum oil transported annually seeps into the environment due to accidents or operational issues [2]. Recovering from the ecological problems will take years brought on by a spill, and crude oil is a diverse range of organic molecules, including simple and complex hydrocarbons, and hydrocarbon deposits found in nature, all of which have varying potentials for toxicity, danger, and cancer [3]. Remediation of crude oil-contaminated soils has drawn a lot of interest in recent decades. In the past, crude oil was mineralized using costly and dangerous chemicals such as potassium permanganate/hydrogen peroxide as chemical oxidizing agents, putting the lives of many species in impacted areas at risk [4].

For in situ applications, renewable sources have emerged, especially those based on the biological remediation capacity of plants and microorganisms, to address the problem of rising prices and the limited efficacy of traditional procedures [5, 6]. The term bioremediation refers to the use of microorganisms to break down or purify organically contaminated areas. The field of environmental microbiology has experienced rapid expansion due to safety, low cost, and increasing popularity [7]. Regions contaminated by crude oil can be difficult to bioremediate due to there are insufficient local microorganisms with suitable substrates and/or poor biodiversity of native microflora needed to break down the different hydrocarbons found in polluted areas [4].

Fungi and bacteria both contributed to the biodegradation of oil products. These microorganisms possess the metabolic machinery required to consume crude oil carbon to create cells, unlike any other living species [8]. In comparison to bacteria, fungi are better degraders of certain environmental pollutants, such as halogenated phenolic compounds, phenols, petroleum hydrocarbons, polychlorinated biphenyls, polyethylene, polycyclic aromatic compounds, and [9, 10]. Their sensitivity to changes in toxicity of hydrocarbon in the environment and their physiology give them a greater tolerance [7]. Pawłowska et al. [11] claim that fungus release extracellular enzymes that facilitate their quick ramification, absorption of complex polysaccharides, and subsequent substrate digestion.

Furthermore, their mass manufacturing is possible under severe environmental circumstances, such as stress [12]. Furthermore, separation through filtration is made simple by the filamentous structure of fungi [11]. While cell walls

have significant metal-binding capacity, fungi also display a number of tools or traits that influence the toxicity and metals mobility, including the capacity to create organic and inorganic precipitation, metal-binding proteins, intracellular compartmentalization, and active transport [13].

With the growth of the shipping and oil industries, there is a significant risk to the environment from oil spills and pollution from seaward oil stages, such as offshore oil platforms and large oil ships. Saudi Arabia is a major producer of petroleum, hence many studies have been conducted there to look at the presence of different oil biodegraders. Three large oil-biodegraders—*Aspergillus spelaeus*, *A. niger*, and *A. polyporicola*—were found in soil samples close to Saudi Arabian crude oil reserves [14].

In order to demonstrate the bioremediation properties of crude oil, the study's primary goals were to isolate, identify, and characterize fungal communities from contaminated locations and look into the possibility that indigenous fungi could use it as a special carbon source. Here, the goal was to get more knowledge regarding the evaluation of fungal bioremediation as a viable, efficient, and environmentally responsible alternative to physical-chemical methods for cleaning up hydrocarbon-contaminated areas.

2. Materials and methods

2.1. Samples collection and fungal isolates

Four distinct crude oil reservoirs in the eastern region of the Kingdom of Saudi Arabia, Ras Tanura (26°38'38 N and 50°9'33 E), provided soil samples for the current study. At a depth of about 10 cm, 500 g of soil samples were collected and stored at 4°C until needed in sterile wide-mouth short-profile transparent glass jars (Thermo Fisher Scientific Inc., Waltham, MS, USA). 2.5 mm pore-size sieves were used for screening the collected samples for any soil debris. Two endophytic fungal isolates, *Aspergillus oryzae* and *Colletotrichum acutatum*. The prior internal transcribed spacer (ITS) region approach was used to identify these isolates.

2.2. Preparation of fungal inoculum

The investigated fungal isolates were cultivated separately in 250 ml conical flasks using potato dextrose broth (PDB, Merck, India) and Tween-80 (0.1 ml). After that, the flasks were shaking for seven days at 25°C at 100 rpm/min. Using a hemocytometer, the concentration of spore inoculum was set to 1×10^6 spores/ml to create the fungal inoculum. For experiments on the biodegradation of crude oil, the fungal suspension functioned as a biodegradable agent [15].

2.3. Biodegradation crude oil assay

Utilizing a adjusted the redox indicator DCPIP (2,6-dichlorophenol indophenol) test, two fungal isolates (*C. acutatum*, *A. oryzae*) from the prior experiment were chosen and evaluated for their ability to decompose crude oil [16, 17]. For the detection of hydrocarbon primary oxidation, this method has a good sensitivity. DCPIP is a frequently used colorimetric technique. When oxidized, it is blue, but when reduced, it is colorless. The molecular structure changes, which results in the color shift. Concisely, the autoclaved MSM was mixed with 0.6 mg/ml of the DCPIP, 0.1% (v/v) Tween 80, and 1% (V/V) crude oil. A 3 to 5 ml of different fungal inoculum growing in liquid medium are then added to the previously created crude oil degradation media, and the mixture is cultivated for 6 days at 25°C in a shaking incubator. To mitigate the impacts of oil weathering, the flasks were covered and shielded from light, temperature changes, and aeration. By boosting metabolism, the surfactant Tween 80 was utilized to bio-stimulate and speed up the creation of biosurfactants [18]. The colorimetric analysis for the change in DCPIP color was then measured at 420 nm using a spectrophotometer Plus (Japan). The color shift is directly correlated with crude oil deterioration. To determine whether growth and decolorization were occurring simultaneously, the fungal isolates' growth was also measured in terms of dry mass. The degradation % was calculated in accordance with Subathra et al. [19], and each experiment was carried out in triplicate.

2.4. Extraction and analysis of crude oil

By using degradation media and an equivalent volume of chloroform, the remaining crude oil was gradually removed from each sample. We employed anhydrous sodium sulfate to extract the crude oil's moisture content. The chloroform was evaporated in a water bath set at 55°C with a rotating evaporator. The solvent was finally evaporated using nitrogen gas. To analyze the extracted/treated crude oil, gas chromatography was used.

2.5. Gas chromatography

Additionally, the GC investigation was implemented using a MIDI-Sherlock GC (Agilent, USA) equipped with a flame ionization detector (FID) to verify that each isolate, *C. acutatum*, *A. oryzae*, was capable of biodegrading the crude oil. Crude oil was separated on the DB-1 capillary column (30 m × 0.25 mm × 0.25 μm) under the following circumstances. 50°C/min was the program for the inlet temperature. The oven was maintained at 35°C for the initial temperature and 28°C for the end temperature, for five min. The oven's final hold time was 15 min, and its temperature program was 10°C/min. Nitrogen was employed as a carrier gas. The operating temperature of the detector was 280°C. Air and hydrogen gas flowed at 40 and 400 ml/min, respectively. With a total run time of 58 minutes, 1 μl of sample was injected at a split ratio of 1:50.

2.6. Cell-free supernatant preparation

Isolates were cultivated in MSM broth medium containing 1% crude oil at 25±2°C for 10 days in a shaking incubator to produce CFS. Following incubation, the cells were centrifuged for 30 min at 4°C at 10,000 rpm in order to extract them. The supernatant (CFS) was filtered and sterilized using a sterile membrane with pore sizes of 0.45 µm. Investigations were conducted into CFS's capacity to generate different types of biosurfactants. Every experiment was carried out three times.

2.7. Emulsification activity

To find out if the fungal isolates might use crude oil as a hydrophobic substrate to emulsify, the emulsification activity test was conducted [20]. The cell-free culture broth and crude oil were mixed in equal parts in a test tube, vortexed for 2 min, and then allowed to sit at room temperature for 24 h. Another tube was filled with distilled water to act as a negative control. Divide the emulsified layer's height (mm) by the solution column's overall height (mm), then multiply the result by 100 to determine the percentage emulsification activity.

2.8. Fungal adherence to hydrocarbons (FATH)

A 3.2 ml of Sigma benzene and 6.8 ml of CFs were mixed together in test tubes that had been acid-washed. The mixture was vortexed for one minute in compliance with the procedures of a previous investigation by Pruthi and Cameotra [21]. Hydrophobicity was calculated using the formula below:

$$\text{FATH} = \frac{\text{initial absorbance} - \text{absorbance after phase separation}}{\text{initial absorbance}} \times 100$$

2.9. Surface tension

To measure the surface tension, a Kruss K7 tensiometer (Germany) was used. A temperature of 25°C was established for the sample. Each sample was put into a 20 ml volume in the sample container of the tensiometer. subsequently in triplicate, the surface tension was determined in three distinct biological runs and expressed in mN/m units. An uninoculated medium and deionized distilled water were used as controls [22].

2.10. Biosurfactants Recovery

To detect and quantify the amount of biosurfactants produced by fungal isolates, various techniques were used. The acid precipitation approach [23] involved adding 6N HCl, a powerful acid, to 3 ml of each fungal solution to bring the pH down to 2. A 24-hour incubation period at 4°C was then conducted. After receiving a solution of chloroform and methanol (2:1), each tube (v/v) was re-incubated for a further 24 hours at room temperature. The precipitate was light brown and collected after 30 minutes of spinning down at 10,000 rpm and 4°C.

After drying, the precipitate was weighed. A chloroform, acetone, methanol combination in equal volume (1:1:1) was applied to the fungal growth as part of the solvent extraction process [24]. For 5 h, the mixture was homogenized at 200 rpm and 30°C. A white precipitate develops at the bottom of the tube. They gathered, dried, and documented this precipitate. Furthermore, two precipitation procedures were used: zinc sulfate and ammonium sulfate [25]. In short, fungal suspensions were treated with 40% (w/v) zinc sulfate or ammonium sulfate overnight at 4°C. After that, the mixture was centrifuged for 30 min at 10,000 rpm at 4°C. A light-brown precipitate was formed, separated, dried, and weighed in the zinc sulfate mixture. The precipitate was collected using acetone and dried in a fume hood while the ammonium sulfates combination was being prepared. This resulted in a white cream-colored powder that was noted. Every experiment used distilled water as a negative control, swapping out the fungus samples.

2.11. Statistical analysis

The trials were conducted in triplicate, and the mean±standard deviation of the three trials (n = 3) was used to display the data. The analysis of variance method was used to examine the data using SPSS v21.0, a statistical program (SPSS, Inc., Chicago, IL).

3. Results and discussion

Millions of liters of natural and synthetic oil enter the environment annually [26]. Although a variety of physical and chemical remediation methods are available for oil spill cleanup, they are often economically unfeasible. This highlights the critical need to identify and isolate indigenous microbial species capable of tolerating extreme environmental conditions while efficiently degrading crude oil. While bacterial biodegradation of crude oil has been extensively studied [27–30], the mechanisms by which fungi native to contaminated environments metabolize hydrocarbons remain poorly understood. Microorganisms capable of degrading crude oil are typically isolated from oil-polluted habitats [31]. Our findings establish a new benchmark for crude oil degradation using endophytic fungi isolated from Saudi Arabia, specifically *Colletotrichum acutatum* and *Aspergillus oryzae*.

3.1. Crude oil degradation

The ability of *C. acutatum* and *A. oryzae* to break down crude oil was then evaluated by observing how the color of culture media enriched with 0.1% DCPIP changed. Since DCPIP decolorization, a drop in crude oil content, and a mass of fungal growth are the three main indicators that influence the biodegradation phase of fungal isolates. As part of their method, fungi biodegrade crude oil using an electron acceptor like DCPIP [32, 33]. When DCPIP is added to our media, the reagent's color changes from blue (oxidized) to colorless (reduced), which indicates that a

degradation process has started [34]. The two fungal isolates used for crude oil breakdown in MSM were shown to be potential degraders, as illustrated in Figs. 1A and B. *C. acutatum* and *A. oryzae* degraded crude oil by 79.45% and 79.11%, respectively, during the course of a 2-day incubation. Additionally, the degradation level somewhat increased during the course of the 6-day incubation period.

Crude oil was significantly degraded by *C. acutatum* and *A. oryzae* following this incubation period, with percentages of 80.4% and 94.8%, respectively. For the components of crude oil to be analyzed, a carbon source must be oxidized. DCPIP decolorization, a reduction in crude oil production, and fungal growth all supported a similar study conducted in the Gulf of Mexico that found some fungus might degrade crude oil [35]. Another study from Pernambuco, Brazil, discovered that the biodegradation of diesel oil, which led to the discoloration of the DCPIP, was induced by *Rhodotorula aurantiaca* and *Candida ernobii* isolated from soil samples polluted with petroleum [36]. In addition to *C. acutatum* and *A. oryzae*'s ability to breakdown crude oil, all of these studies provided evidence that the isolated fungal isolates could oxidize the crude oil, which resulted in its electronic transfer to DCPIP and subsequently decolorization [14].

3.2. GC evaluation of the biodegradation of crude oil

The GC chromatograms of the crude oil treated by *C. acutatum* and *A. oryzae* are shown in Fig. 2 in contrast to the control (Fig. 2a), which shows the residual hydrocarbon in the crude oil with hydrocarbon compound peaks in the C₁₂–C₂₄ range. In contrast to the untreated control, the fungal isolates effectively used the alkane, as seen in Figs. 2b. Nevertheless, compared to *A. oryzae*, we discovered that *C. acutatum* marginally outperformed it in lowering the aliphatic component peaks in the crude oil. Similarly, these fungal isolates most likely produced the graph's receding peaks of C_n alkanes and other low-mass peaks by Crude oil biodegrading produces a variety of residues, which the fungi may have used to thrive.

A. oryzae may use C₁₂ to C₂₄ as the only carbon source and energy source, while *C. acutatum* can break down all n-alkanes from C₁₆ to C₂₀, according to study. Because of their effective degradative enzyme systems, fungal isolates were able to attack both short and long chain alkanes with remarkable vulnerability. It was shown that the selected fungal isolates were efficient degraders of crude oil. While new peaks indicated the breakdown of products or presumed metabolites, the treated crude oil substrate showed a dip in the vicinity of some peaks, indicating its degradation. This finding aligns with previous research demonstrating that microorganisms that break down hydrocarbons preferentially break down alkanes [37].

Furthermore, the *Aspergillus oryzae* KR029081 isolate was identified by Mallikarjuna et al. [38] as being especially unique and breaking down with a hydrocarbon from C₁₄ to C₂₇. GC is without a doubt a very helpful method for tracking and monitoring hydrocarbons and chemical contaminants in the environment. Hydrocarbon by-products, such as C₆₀, can be detected using it [39].

3.3. Emulsification activity, surface tension and Cell surface hydrophobicity

Surface tension was measured after 14 days of incubation at 30°C, and FATH, emulsification activity tests were performed for *C. acutatum* and *A. oryzae* (Table 1). *A. oryzae* and *C. acutatum* both reported high FATH levels of 40.8% and 41.2%, which were nearly identical. Crude oil degradation may be significantly influenced by this characteristic [40]. *A. oryzae* and *C. acutatum* significantly decreased surface tension by 50.3 and 40.8 mN/m, respectively, demonstrating a clear association between surface tension reduction and fungal isolates' biodegradation of crude oil.

This finding suggested that surface tension was decreased as a result of the biosurfactants produced by these isolates. Ghosal et al. [41] isolated *Aspergillus* sp. RFC-1 crude oil-degrading fungal strains from Rumaila field oil in Basra, Iraq, and our results were consistent with their findings. They discovered that the strength of the fungi's crude oil degradation ability was inversely correlated with a reduction in surface tension. Additionally, by studying eight hydrocarbonoclastic oil field bacteria from Tunisia, Mnif et al. [42] found that the surface tension decreased to 35.1 mN/m, indicating the production of biosurfactant.

Furthermore, Varadavenkatesan and Murty [43] found that the use of MSM in the crude oil resulted in a biosurfactant output over 96 hours, reducing surface tension to 36.1 mN/m. Table 1 shows that *A. oryzae* (62.4%) had marginally more emulsion activity than *C. acutatum* (54.1%). Accordingly, we may infer from the data that there is a relationship between fungal biodegradation and crude oil that depends on increasing the rate of fungal growth on crude oil, lowering surface tension, and increasing emulsification activity. Furthermore, fungi have a significant impact on how hydrophobic cell surfaces develop.

As emulsifying agents, *C. acutatum* and *A. oryzae* can improve crude oil biodegradation in this scenario by reducing surface tension in two distinct ways. Mycelial pellet structures are formed by emulsifying hydrophobic substances, which is encouraged by the first pathway. By increasing the hydrophobicity of the cell surface, the second mechanism enhances the direct physical interaction between cells and substrates that are weakly soluble in water [44,45].

3.4. Assessment of the weight of biosurfactants extracted from fungal isolates

The present investigation focused on the amount of biosurfactants generated by *C. acutatum* and *A. oryzae* in the presence of crude oil in order to confirm the obtained fungal isolates' ability to displace oil. Four different recovery methods were applied. The dry weight of biosurfactants in the presence of *C. acutatum* was 4.5 g, followed by *A. oryzae* (4.2 g), according to the results of the acid precipitation experiment, which are shown in Fig. 3. Similar to this, the solvent extraction test was used to separate the soluble and insoluble components that came from the reaction of fungal isolates with crude oil; interactions between *C. acutatum* (4.1 g) and *A. oryzae* (5.3 g) with crude oil were responsible for a significant portion of the dry weight precipitate (Fig. 3).

However, when the crude oil was treated with *C. acutatum* (1.1 g) and *A. oryzae* (1.4 g), it produced ammonium sulfate precipitation, as shown in Fig. 3, two additional techniques of zinc and ammonium sulfate precipitation. The zinc sulfate precipitation method's dry weight of the precipitate showed that crude oil's reaction with *C. acutatum* (2.02 g) and *A. oryzae* (3.01 g) was what produced the biosurfactant. Remarkably, the most important characteristic of a suspected hydrocarbon degrader is its ability to produce microbial biosurfactants, which are made up of a mixture of organic (proteins, fatty acids, and exopolysaccharides) components [46].

Biosurfactants increase the solubility of greasy contaminants, making them more accessible to microorganisms as carbon sources and hastening their breakdown. Furthermore, by emulsifying the hydrocarbon by reducing surface tension, these components can withstand oil bioremediation and oil recovery [47]. The emulsification of the crude oil used in this investigation was enhanced by *C. acutatum* and *A. oryzae*. Crude oils produced a significant amount of biosurfactants, according to the amount produced by *C. acutatum* and *A. oryzae*.

According to our research, fungal biosurfactants have a strong capacity to biodegrade oil, that might be explained by the production of enzymes that could destroy chemical contaminants. Numerous studies have shown that certain fungal species are capable of producing degrading enzymes, including as laccases, peroxidases, and catalases, which speed up the breakdown and immobilization of pollutants [48, 49].

Furthermore, although the effects of microbial biosurfactants on the bioremediation of different hydrocarbons from other countries have drawn a lot of attention, little study has been done in Saudi Arabia on *C. acutatum* and *A. oryzae*. Using crude oil, *Pseudomonas aeruginosa* generated biosurfactant that, after 35 days, broke down 82% of the petroleum hydrocarbons, according an Indian study [50]. A follow-up study conducted in India discovered that 97% of crude oil samples could be broken down by *Bacillus subtilis* biosurfactants (4.85 g/l). This capability was ascribed to the development of two biodegradative enzymes, alkane hydroxylase and alcohol dehydrogenase [51].

Following 30 days, the biosurfactants produced by *Trichoderma atroviride*, *A. sydowii*, and *A. nidulans* that were extracted several samples of polluted soil shown effective crude oil breakdown (66.9–83.5%) [52]. 73.6% of crude oil was successfully biodegraded in the Saudi Arabia study using a combination of biosurfactants produced by *T. harzianum*, *Alternaria alternative*, *A. terreus*, *A. flavus* [53]. Our results and all of these studies suggest that fungal biosurfactants have strong oil-biodegradative capabilities, which could be explained by the emergence of degradative enzymes that lessen pollution of hydrocarbon in contaminated areas.

According to our findings, only trace amounts of biosurfactants were recovered when using zinc sulfate and ammonium sulfate precipitation methods. This limited recovery may be attributed to the influence of pH and the presence of divalent cations, such as magnesium ions, which are known to affect microbial emulsification activity and biosurfactant stability [54]. Notably, *Pseudomonas aeruginosa* was reported to produce higher concentrations of rhamnolipid biosurfactants through organic solvent extraction (7.37 ± 0.81 g/L) compared to zinc sulfate precipitation (5.83 ± 0.02 g/L), a trend that aligns with our observations [55]. The variation in biosurfactant yields among fungal isolates can likely be explained by physicochemical properties of the biosurfactants themselves—specifically, their ionic charge and solubility characteristics in different solvents—which have been identified in prior studies as key factors influencing recovery efficiency [58].

The biodegradation of petroleum hydrocarbons by fungi and other microorganisms is essential for mitigating environmental contamination caused by oil spills, particularly in oil-rich regions such as Saudi Arabia. Indigenous fungi and yeasts isolated from local ecosystems exhibit promising potential for use in bioremediation strategies, as they are well-adapted to the unique climatic and ecological conditions of the region [59].

Several studies have demonstrated the capacity of fungal strains to degrade complex hydrocarbons effectively. For example, fungal isolates obtained from mangrove sediments along the Red Sea coast—including *Alternaria alternata*, *Aspergillus terreus*, and *Cladosporium sphaerospermum*—showed significant diesel oil degradation. These fungi produced elevated CO₂ levels during incubation, indicating active metabolic breakdown of hydrocarbons [40]. These findings corroborate the role of mangrove-associated fungi in hydrocarbon degradation, as previously reported in similar coastal ecosystems worldwide [43].

In contaminated soils from Dhahran, *Aspergillus niger* exhibited the highest degradation efficiency among isolated strains, reducing hydrocarbon content by approximately 58% and generating substantial CO₂ emissions [60]. The robust enzymatic machinery of *A. niger*—including laccases and peroxidases—likely underpins its effectiveness in

breaking down petroleum hydrocarbons, consistent with its documented biodegradation abilities in other geographical contexts. Similarly, fungal isolates from the Red Sea region such as *Penicillium commune* and *Aspergillus niger* demonstrated effective degradation of a wide spectrum of hydrocarbons, confirmed through spectrophotometric and GC-MS analyses [61]. This suggests that these fungi possess versatile metabolic pathways suitable for the bioremediation of complex oil pollutants prevalent in Saudi Arabian environments. Moreover, yeast isolates from the Khafji oil field, including *Candida tropicalis*, have been identified as efficient crude oil degraders, with degradation rates exceeding 50%. The capacity of yeasts to metabolize hydrocarbons complements fungal and bacterial activities, particularly under extreme environmental conditions such as high salinity and temperature fluctuations common in the region [62].

4. Conclusions

Polluting the environment from oil spills is one of the main problems of the twenty-first century, and it can be addressed with all the resources at hand. This study presents the potential of the recently identified Saudi Arabian endophytic fungi *C. acutatum* and *A. oryzae* in the biodegradation of crude oil. The finding is both practically and academically relevant, particularly in Saudi Arabia, a major oil-producing nation. Future research should explore the metabolic mechanisms behind the fungal degradation process, particularly the role of degrading enzymes and genomic methods used to further reveal the genes involved in the degradation process. Further research into the applications of biosurfactants in real-world environments would be beneficial, especially regarding their potential industrial value. Consider researching the co-degradation of other types of pollution (such as heavy metal contamination) to explore the fungi's potential in degrading complex pollutants.

Conflict of Interest

Authors don't have any conflict of interest

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Table 1 Surface tension, cell surface hydrophobicity and emulsification activity of fungal isolates after 14 days of incubation (Mean $\pm$ SD, n = 3)

Fungal isolates	Cell hydrophobicity (%)	Emulsification activity (%)	Surface tension (mN/m)
<i>Colletotrichum acutatum</i>	44.9 $\pm$ 1.98	36.7 $\pm$ 2.51	41.2 $\pm$ 0.72
<i>Aspergillus oryzae</i>	53.4 $\pm$ 1.26	35.9 $\pm$ 3.26	35.9 $\pm$ 2.64
Control (distilled water)	0	-	66 $\pm$ 0.62
Control (un-inoculated media)	0	15.3 $\pm$ 2.1	59 $\pm$ 0.69

In all of these assays, un-inoculated medium and distilled water served as the negative controls.

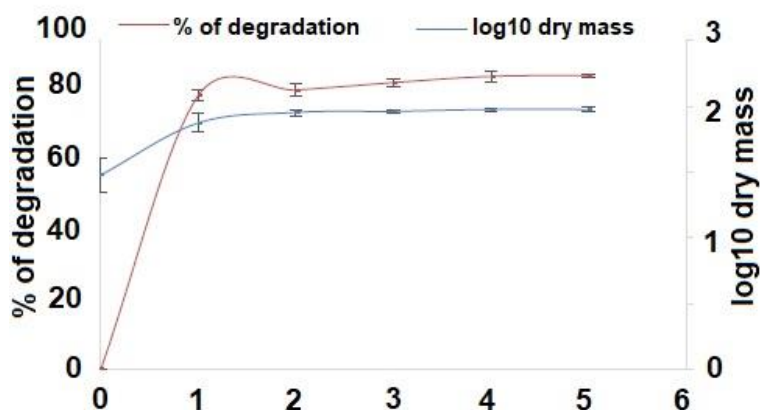
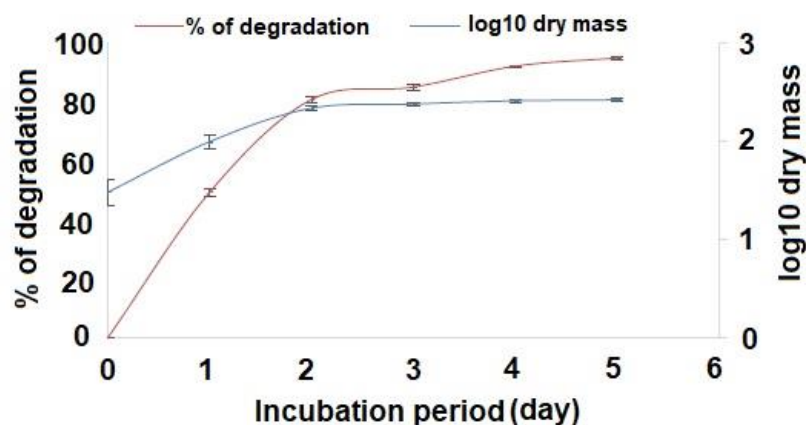


Fig. 1 (A) The oil biodegrading capacity of *C. acutatum* and (B) *A. oryzae* on crude oil. The colorimetric changes in DCPIP were measured at 420 nm during a 6-day incubation period at 25°C with the fungal isolates grown in MSM supplemented with 0.1% Tween 80 and 0.6 mg/ml DCPIP. The mean $\pm$ SD of three separate experiments determines the way the results are displayed.

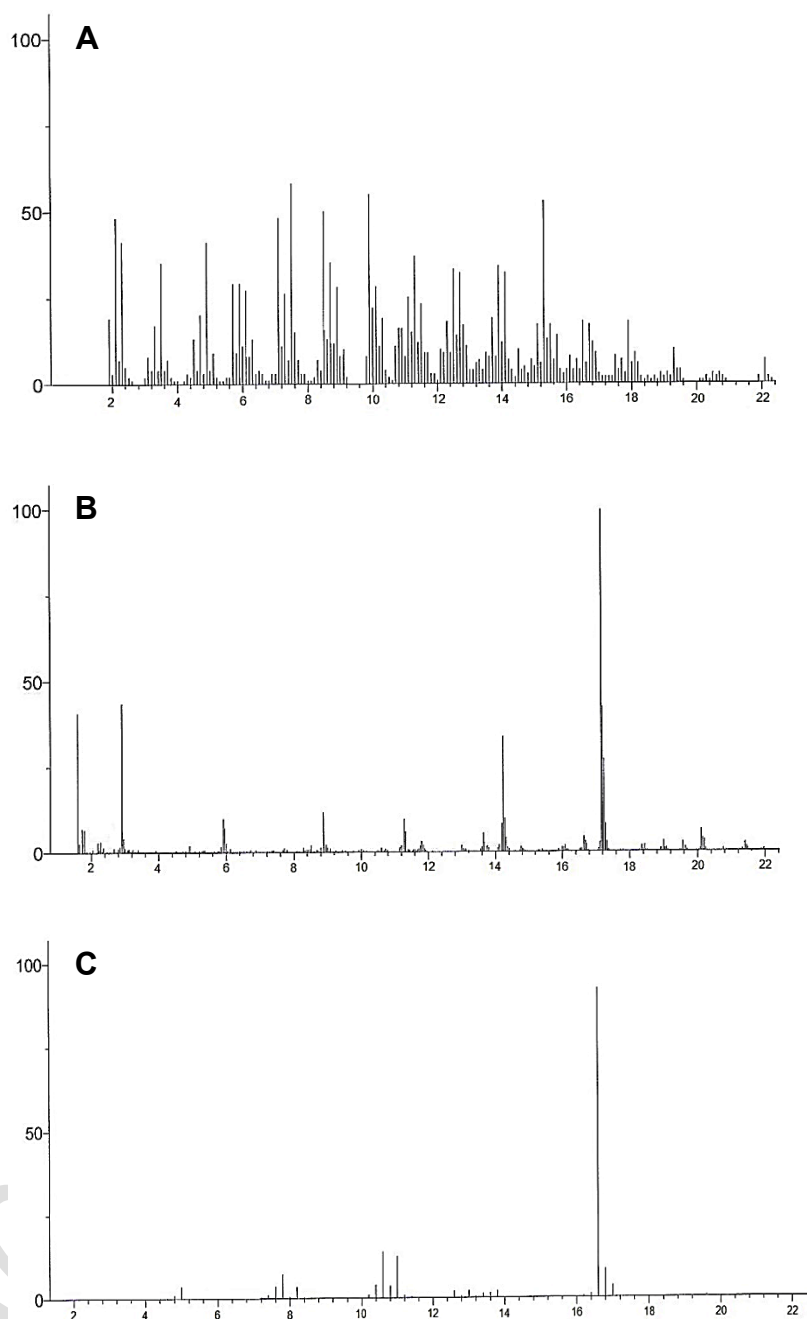


Fig. 2 Gas chromatography analysis of crude oil degradation from the untreated control (A), and treated with *C. acutatum* (B), *A. oryzae* (C) after 10 days of incubation.

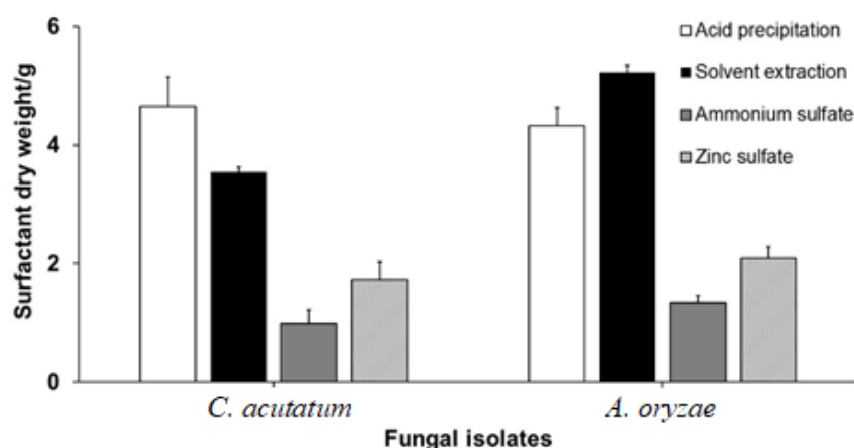


Fig. 3 Solvent extraction, ammonium sulfate, Acid precipitation, and zinc sulfate precipitation assays are used to recover biosurfactants of *C. acutatum* and *A. oryzae*. The mean $\pm$ SD of three separate experiments is how the results are displayed.