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RESEARCH ARTICLE

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Factors affecting polycyclic aromatic hydrocarbon biodegradation by *Aspergillus flavus*

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Abstract

This study investigated the ability of fungi isolated from highly contaminated soil to biodegrade polycyclic aromatic hydrocarbon (PAH) compounds, as well as the effect of several parameters on the biodegradation ability of these fungi. The isolated fungi were identified using ITS rDNA sequencing and tested using 2,6-dichlorophinolendophenol to determine their preliminary ability to degrade crude oil. The top-performing fungi, *Aspergillus flavus* and *Aspergillusfumigatus*, were selected to test their ability to biodegrade PAH compounds as single isolates. After 15 days of incubation, *A. flavus* degraded 82.7% of the total PAH compounds, with the complete degradation of six compounds, whereas *A. fumigatus* degraded 68.9% of the total PAHs, with four aromatic compounds completely degraded. We also tested whether different temperatures, pH, and nitrogen sources influenced the growth of *A. flavus* and the degradation rate. The degradation process was optimal at a temperature of 30°C, pH of 5.5, and with nitrogen in the form of yeast extract. Finally, the ability of the fungal candidate, *A. flavus*, to degrade PAH compounds under these optimum conditions was studied. The results showed that 95.87 of the total PAHs, including 11 aromatic compounds, were completely degraded after 15 days of incubation. This suggests that *A. flavus* is a potential microorganism for the degradation of PAH compounds in aqueous cultures.

1 | INTRODUCTION

The transportation, refining, and consumption of petroleum hydrocarbons result in their widespread environmental contamination, the previous factors have a significant effect on living organisms (Mrchand, St-Arnaud, Hogland, Bell, & Hijri, 2017). Crude oil is a combination of diverse compounds including polycyclic aromatic hydrocarbons (PAHs), which are considered one of the main harmful materials in the environment, with long half-lives (Williams, Mahler, & Van Metre, 2013). The molecular structure of PAHs generally includes two to seven aromatic rings. They can potentially accumulate in the environment to substantial levels. Furthermore, owing to their nature, PAHs may negatively affect the physicochemical properties of soil, including texture and water holding capacity. They can also migrate into the groundwater and enter the food chain, with severe consequences to living organisms, especially owing to the mutagenic or carcinogenic properties of some PAHs (Clemente, Anazawa, & Durrant, 2001; Govarthanan, Fuzisawa, Hosogai, & Chang, 2017). Different methods have been applied for the remediation of PAH-contaminated sites, including physicochemical and biological approaches (Gan, Lau, & Ng, 2009; Rayu, Karpouzas, & Singh, 2012). Physicochemical methods are highly efficient in the removal of PAH compounds, but they have a negative effect on the environment as they alter soil texture and reduce soil biota, thereby reducing essential nutrients necessary for plant life (Gan et al., 2009; Khan, Kuek, Chaudhry, Khoo, & Hayes, 2000). Previous research have focused on natural processes such as biodegradation, which uses a microbial consortium, to degrade PAHs in the environment (Hassanshahian, Emtiazi, & Cappello, 2012). With respect to biodegradation, fungi possess certain advantages over bacteria, owing to the nature of their growth patterns and their resistance to PAHs, as demonstrated by several studies (Al-Hawash, Zhang, & Ma, 2018; Li et al., 2008; Mauti, Onguso, Kowanga, & Mbak, 2016; Mrchand et al., 2017). Filamentous fungi have been readily isolated from oil-contaminated sites and have demonstrated the ability to degrade PAHs. *Aspergillus* spp., one of the most commonly isolated fungi,

has recently received more attention as potential tools for degradation of different PAH compounds (Ali, Khalil, & Abdelghany, 2012; Mauti, Onguso, Kowanga, & Mbaka, 2016; Ye et al., 2011; Zhang, Xue, Gao, Ma, & Wang, 2016). Fungi should grow optimally and produce sufficient biomass to efficiently degrade and mineralize PAHs. Fungal growth is affected by several factors, including species type and concentration, as well as temperature, pH, and nutrient availability (Govarthanan et al., 2017; Hamzah, Zarin, Hamid, & Omar, 2012).

The goals of this study were, first, to evaluate the ability of fungi isolated from oil-contaminated soil to biodegrade PAHs, and second, to study the effects of varying temperature, pH, and nutrients on the degradation process.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Eight soil samples contaminated with oil sludge were obtained from the surface layer (0–15 cm in depth) in the Al-Zubair oil fields, South–West Basra Province, Iraq (30°N [21′27"], 47°E [37′59"]). More than 50 years of oil production operations resulted in contamination of these fields by different oil products. The soil texture is sandy. The PAH concentrations in the soils were determined following the method described by Goutx and Saliot (1980). The PAH data are summarized in Table 1. Soil samples were collected and stored in sterile plastic bags at 4°C until further use.

2.2 | Chemicals

Regular Basra crude oil was purchased from a southern oil company (Basra, Iraq). All additional chemicals were purchased from Hi-Media (Mumbai, India) and Bioneer (Daejeon, South Korea).

2.3 | Isolation of hydrocarbon-degrading fungi

Hydrocarbon-degrading fungi in the soil samples were isolated according to the procedure described by Wicklow and Wittingham

TABLE 1 Polycyclic aromatic hydrocarbon (PAH) concentrations (ng g^{-1}) in soil samples

	Location							
PAHs compounds	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Naphthalene	20.583	ND	ND	ND	4.361	3.1480	5.748	ND
Acenaphthylene	19.540	ND	ND	ND	4.648	4.843	ND	ND
Acenaphthene	ND	ND	ND	12.074	9.534	13.170	ND	ND
Fluorene	ND	13.933	ND	7.311	23.258	6.811	ND	3.743
Phenanthrene	ND	2.224	4.829	2.625	7.532	2.683	ND	ND
Anthracene	6.755	3.880	2.950	14.634	4.581	4.364	ND	14.244
Fluoranthene	13.442	33.031	7.361	6.028	4.973	2.316	ND	ND
Pyrene	ND	3.726	3.024	11.489	8.220	13.930	ND	17.982
Benzo[a] anthracene	ND	5.261	9.854	5.991	3.527	9.488	ND	ND
Chrysene	ND	6.445	6.102	3.859	14.483	4.605	ND	ND
Benzo[b] fluoranthene	ND	8.404	8.099	14.033	10.382	17.051	ND	3.436
Benzo[k] fluoranthene	ND	20.95	11.662	6.619	16.423	5.637	ND	10.086
Benzo[a]pyrene	18.848	25.196	6.306	8.725	23.281	7.190	ND	17.395
Dibenzo[a,h] anthracene	ND	14.335	19.409	6.591	41.184	7.736	35.365	7.969
Benzo[g,h,i] perylene	6.695	11.017	16.494	36.148	4.006	30.232	24.746	11.573
Indeno[1,2,3-c,d] pyrene	ND	4.0659	12.805	11.436	2.566	9.218	6.807	ND
16 PAHs (ng g^{-1})	82.863	152.472	96.0912	147.599	182.959	142.422	72.666	86.428

Abbreviations: 16 PAHs (ng g^{-1}), the sum of the results of each compound listed above; BLAST, basic local alignment search tool; HPLC, high-performance liquid chromatography; ND, non detect; PCR, polymerase chain reaction.

(1974), using the dilution plating method with mineral salts agar (MSA) medium supplemented with 0.1% sterile Regular Basra crude oil. The medium composition was as follows (in g L^{-1}): NaCl, 10.0; $MgSO_4 \cdot 7H_2O$, 0.42; KCl, 0.12; KH_2PO_4, 0.83; $NaNO_3$, 0.42; Na_2HPO_4, 1.25; and agar, 20.0, supplemented with streptomycin (30 μg ml^{-1}) to inihibit bacterial growth. Each soil sample (10 g) was suspended in 100 ml of sterile physiological saline solution, shaken well for 10 min, and diluted up to $\times 10^4$. Approximately 1 ml from each dilution was transferred to a sterile Petri dish, to which 15 ml of sterile MSA medium was added. The components of the Petri dish were mixed well before solidification, and the Petri dish was incubated at 25°C for 2 weeks. Pure cultures were produced from all visible fungal colonies and preserved on potato dextrose agar at 4°C for molecular identification.

2.4 | Molecular identification of isolated fungi

Pure cultures from the isolated fungal colonies were subcultured on Czapek Dox agar medium and allowed to grow for 7 days. DNA extraction and PCR amplification were carried out according to the method described by Mirhendi, Makiumura, Khoramizadeh, and Yamagushi (2006). The isolates were identified by amplifying the internal transcribed spacer (ITS) regions of the ribosomal DNA with a forward primer, ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′), and a reverse primer, ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (19 and 20 base pairs [bp] long, respectively). The initial amplification stage involved denaturation at 95°C for 1 min; followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 45 s, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. The PCR yielded distinctive products of approximately 400–850 bp in length. The purification of the PCR products for the recovery of isolates was carried out at Macrogen (Seoul, South Korea). The resulting products were aligned and identified using the basic local alignment search tool (BLAST) program.

2.5 | Preliminary screening for fungal isolates

To evaluate the potential of fungal isolates to degrade hydrocarbons, redox indicator 2,6-dichlorophenol-indophenol (2,6-DCPIP) technique was used, where the degree of color change of the indicator from deep blue to colorless indicated the degree of degradation. (Al-Nasrawi, 2012). This experiment was carried out in 100-mL conical flasks containing 50 ml of Bushnell–Haas broth medium supplemented with 0.008 mg 2,6-DCPIP, 0.1% (v/v) Tween-80, and 0.1% (v/v) crude oil as the carbon source. The medium composition was as follows (in g L^{-1}): $MgSO_4$, 0.2; $CaCl_2$, 0.02; KH_2PO_4, 1.0; K_2HPO_4, 1.0; NH_4NO_3, 1.0; and $FeCl_2$, 0.05. Two plugs were collected from the edge of 7-day-old fungal colonies using a 5-mm cork borer and added to each flask. Negative controls were set up using the medium and crude oil with 2, 6-DCPIP only without any fungal inoculum. The flasks were incubated in a combined rotary shaker incubator maintained at 115 rpm and 25°C for 7 days. Changes in the medium color were observed and recorded.

2.6 | Screening of crude oil degradation by single fungal isolates

The fungal isolates that presented the best results in the previous experiment were further tested to assay their ability to degrade crude oil. These isolates were subcultured on Czapek Dox agar medium for 7 d, the medium composition was as follows (in g L^{-1}): $NaNO_3$, 2.0; $MgSO_4$, 0.5; KCl, 0.5; K_2HPO_4, 1.0; $FeSO_4$, 0.01; and agar, 20.0.

Three plugs were collected using a 5-mm cork borer from the periphery of each selected fungal colony and added to 250-mL conical flasks, each containing 100 ml of mineral salts broth (MSB) medium (pH 4.5). Crude oil (1 ml) was added to each flask as the sole carbon source. The culture flasks were incubated at 25°C in a combined rotary shaker incubator maintained at 115 rpm for 15 days. Uninoculated medium containing only crude oil served as a control. All experiments were carried out in triplicate (Okerentugba & Ezeronye, 2003).

2.7 | Effects of physicochemical parameters on the biodegradation process

The effects of different parameters on fungal biodegradation ability were evaluated, according to the procedure described by Hamzah et al. (2012), using the fungal species which showed the highest degradation rate in the previous experiment described in Section 2.6.

2.7.1 | Incubation temperature

Two sets of flasks, containing the fungal isolate, the same (MSB) medium as previously described, and crude oil (pH 4.5), were prepared. One set was incubated at 30°C and the other at 35°C, in a combined rotary shaker incubator maintained at 115 rpm for 15 days. Uninoculated medium containing only crude oil served as a control. All the experiments were carried out in triplicate.

2.7.2 | PH

The effects of pH on the biodegradation process were evaluated at two different pH values. The pH of the growth medium in one set of flasks was adjusted to 5.5 and the other set to 6.5. Uninoculated medium containing only crude oil served as a control. The flasks were incubated, in triplicate, at 25°C in a combined rotary shaker incubator maintained at 115 rpm for 15 days.

2.7.3 | Nitrogen source

The nitrogen source in the MSB medium was $NaNO_3$ and was replaced in one set of experiments by an inorganic nitrogen source, that is, $(NH_4)_2SO_4$, and in a second set by an organic source, that is, yeast extract. Control flasks containing uninoculated medium with only crude oil were used. The flasks were all incubated at 25°C, in triplicate, in a combined rotary shaker incubator maintained at 115 rpm for 15 days.

2.8 | Effect of biostimulation on PAH biodegradation

To evaluate the outcome of biostimulation on the PAH degradation process by single fungal isolates, 250-ml conical flasks, each containing 100 ml of MSB medium, were maintained under the optimal temperature, pH, and nutritive conditions determined from the previous experiments. Each flask was inoculated with a single isolate of the best fungal candidate, as previously determined. Control flasks containing uninoculated medium with only crude oil were used. The flasks were set up, in triplicate, for two incubation periods, 7 and 15 days, in a combined rotary shaker incubator maintained at 115 rpm and 25°C.

2.9 | Extraction and qualitative analysis of PAHs

After the completion of each experiment, the contents in each flask were extracted with 60 ml of dichloromethane in a separating funnel, with continuous shaking. This process was repeated three times. The liquid fraction was left for a period of time until it settled, yielding two layers: an aqueous layer and an organic dichloromethane layer containing residual crude oil. The organic layer was transferred to a clean glass container and air dried (Parsons, Matia, & Malli, 1985).

The aromatic fraction was separated by passing the extracted crude oil through a silica gel column. The air-dried samples were redissolved in 5 ml of n-hexane and the soluble samples were passed through a silica column. The PAH fraction was separated with 30 ml of benzene. To identify aromatic compounds, the samples were evaporated to dryness using a rotary evaporator and redissolved in 5 ml of the solvent, then injected into the HPLC system (LC type; Shimadzu) under the following settings: C18 column (250 mm, 25 cm, 4.6 mm); portable phase: acetonitrile/water (90:10 v/v); flow rate: 0.5 ml min^{-1}; injection volume: 20 µl, and UV-visible wavelength: 254 nm.

The PAH degradation percentage was determined according to Li et al. (2008), as follows: $D\% = 100 \times (MI - MF) \times MI^{-1}$, where MF was the final concentration of PAHs at the end of each incubation period, and MI was the initial concentration in each treatment.

2.10 | Statistical analysis

Minitab version 16 software was used to analyze the results using one-way analysis of variance. Relative least significant difference values were calculated to determine significant differences between the fungal processes. A completely randomized design was used.

3 | RESULTS AND DISCUSSION

3.1 | Fungal identification and colorimetric screening

PCR-amplified genomic DNA was analyzed using the BLAST program to identify related species based on similarities in the DNA nucleotide sequences. Six fungal species, belonging to the genera *Alternaria*, *Aspergillus*, and *Penicillium*, were identified. Four of these species belonged to the genus *Aspergillus* (Figure 1). The results further showed that all the isolated fungi in this study were related to the dematiaceous anamorphic fungi. This group of fungi is common and widespread in the environment, and most of them produce reproductive units in large numbers and possess resistant reproductive structures such as chlamydospores and sclerotia. These features enable them to resist unfavorable environmental conditions such as low humidity, high temperatures, and high crude oil concentrations in the soil, providing them a competitive advantage for survival in such environments. Their high enzymatic activity also enables them to degrade a wide range of organic sources (Schwarz et al., 2018; Serna-Chavez, Fierer, & Van Bodegom, 2013; Wu et al., 2016). Indigenous microorganisms isolated from polluted areas were more effective in the biodegradation of PAHs than those isolated from uncontaminated soils. Here, this was apparent in the 2,6-DCPIP test, in which most of the isolated fungi were able to tolerate and biodegrade crude oil. All the fungal species, except *Asprgillus niger*, presented a positive reaction with the redox indicator 2,6-DCPIP. *Aspergillus flavus* and *A. fumigatus* showed relatively more intensive reactions and completely changed the medium color from blue to colorless (Table 2).

This test elucidated the potential of fungi to utilize crude oil as their only source of carbon and energy. The change in 2,6-DCPIP color from deep blue (oxidized) to colorless (reduced) indicated that crude oil was utilized (Obi, Atagana, & Adeleke, 1946). This method has been employed in previous studies to identify microorganisms with a high potential for biodegradation (Al-Nasrawi, 2012; Moustafa, 2016).

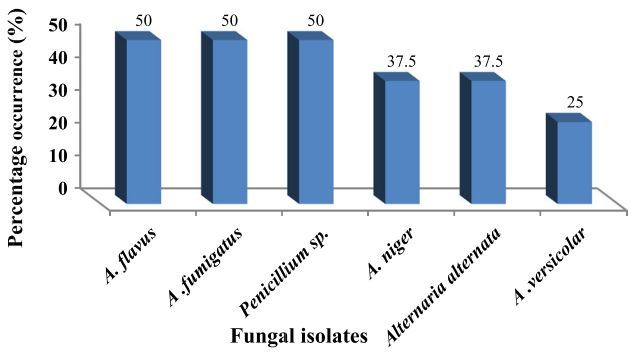

FIGURE 1 Occurrence percentage of the fungi isolated in this study [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Preliminary screening for fungal biodegradation ability using 2,6-DCPIP

No.	Fungi species	Degradation degree
1	*Aspergillus flavus*	++
2	*Aspergillus fumigatus*	++
3	*Aspergillus versicolor*	+
4	*Alternaria alternata*	+
5	*Penicillium* sp.	+
6	*Aspergillus niger*	–

Abbreviations: –, no color change; +, mild color change; ++, high color change.

3.2 | PAH biodegradation using single isolates

The two species with the highest potential among the fungal isolates, *A. flavus* and *A. fumigatus*, were selected to test their ability to degrade PAHs as single isolates. These two fungi belong to the genus *Aspergillus*; many species of this genus have been previously isolated and have demonstrated their ability as oil degraders (Al-Hawash et al., 2018; Al-Nasrawi, 2012; Mittal & Singh, 2008; Ponnapalli, Sura, Sudhakar, Govindarajalu, & Sijwali, 2018). In the present study, a significant amount of total PAHs was degraded after 15 days of incubation compared with the control treatment. There were significant differences ($p < .01$) in the degradation percentage between the treatments and the experimental control, as well as between treatments with the two species of fungi. The HPLC results showed that *A. flavus* exhibited a relatively higher degradation ability than *A. fumigatus* after 15 days of incubation. *A. flavus* was able to completely degrade six aromatic compounds (naphthalene, acenaphthylene, fluoranthene, pyrene, chrysene, and benzo[b]fluoranthene), whereas *A. fumigatus* was able to completely degrade four compounds (naphthalene, phenanthrene, fluoranthene, and benzo[b]fluoranthene). The total concentration of the 16 PAH compounds decreased considerably, to 946.147 and 1701.046 μg L^{-1} after incubation with *A. flavus* and *A. fumigatus*, respectively, compared with the control (5470.906 μg L^{-1}). *A. flavus* exhibited 82.7% PAH biodegradation compared with 68.9% by *A. fumigatus* (Figure 2). The high surface area of the fungal mycelia can maximize both enzymatic

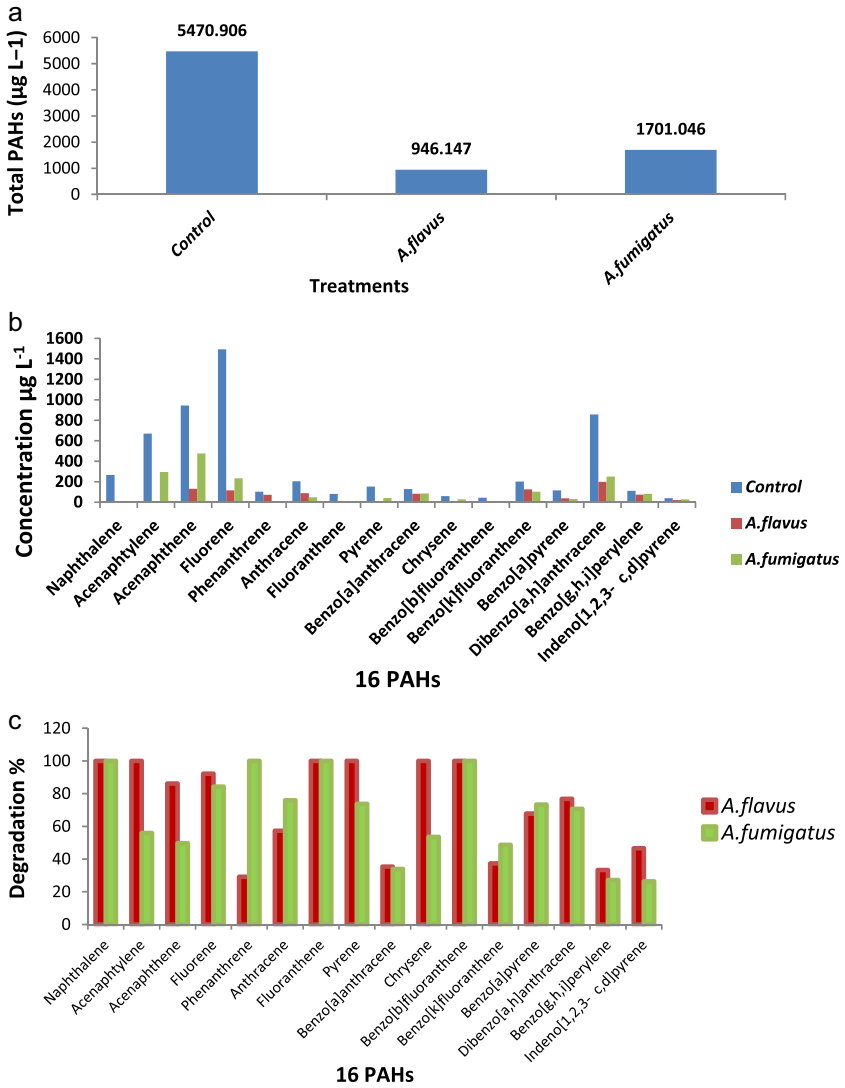

FIGURE 2 Degradation of polycyclic aromatic hydrocarbon (PAH) compounds by fungi after 15 days. (a) Total concentration of PAH compounds, (b) concentration of different PAH compounds, and (c) PAH degradation percentage [Color figure can be viewed at wileyonlinelibrary.com]

and mechanical contact with PAH compounds, and this may explain the high degradation rate exhibited by these fungi (Mohsenzadeh, Rad, & Akbari, 2012; Potin, Veignie, & Rafin, 2004). In addition, fungi have a higher tolerance to the toxicity of hydrocarbons owing to their physiology, allowing them to exhibit an effective ability for degradation (Barnes, Khodse, Lotlikar, Meena, & Damare, 2018). *A. flavus* exhibited the highest degradation rate at 82.7% and was able to completely degrade six aromatic compounds. The ability of this species to produce more than one type of degradative enzyme in high quantities may increase its potential to degrade PAH compounds (Barnes et al., 2018; Mohsenzadeh et al., 2012). These results are consistent with those of other studies in which fungi especially the genus *Aspergillus* has a great degradation ability for hydrocarbons (Al-Hawash et al., 2018; Ponnapalli et al., 2018; Ye et al., 2011).

3.3 | Optimization of PAH degradation using *A. flavus*

The fungus *A. flavus* was chosen to investigate the effects of temperature, pH, and nitrogen source on the biodegradation process of PAHs, for a period of 15 days. One of the most important parameters that can affect fungal growth is temperature (Delille, Coulon, & Pelletier, 2004). In the present study, *A. flavus* grew over a range of temperatures, 25–35°C, with the optimal temperature for growth and degradation being 30°C. At 30°C, the total concentration of PAH compounds decreased considerably from an initial concentration of 5470.906 μg L^{-1} for the control to 414.8584 μg L^{-1} for the treatment at 30°C, the total concentration of PAH compounds decreased considerably from an initial concentration of 5470.906 μg L^{-1} for the control to 414.8584 μg L^{-1} for the treatment at 30°C, which represents 92.41% degradation (Figure 3). corresponding to 92.41% degradation (Figure 3). This finding is consistent with that of several studies (Al-Hawash et al., 2018; Govarthanan et al., 2017; Hamzah et al., 2012), which reported that the optimum temperature for fungal growth during the oil degradation processes was 30°C. This may be because of an increase in fungal growth, which potentially improved energy efficiency in the system by facilitating contact between the cell surfaces and the energy source. However, at higher temperatures, this process may slow down, perhaps owing to the deformation of cell surfaces available as sites for substrate attachment (Al-Asheh & Duvnjak, 2018).

Fungi favor acidic conditions for growth(Rousk, Brookes, & Bååth, 2009). In this experiment the optimum pH was 5.5, with the fungus degrading 91.81% of the total PAHs, and considerably decreasing the total PAH concentration to 448.025 μg L^{-1} at this pH (Figure 3). A pH of 6.5 negatively affected the degradation process, probably because an increase in hydroxyl ions in the culture media inhibited the adsorption of PAHs to cation-binding sites (Jalali, Ghafourian, Asef, Davarpanah, & Sepehr, 2002).

The nitrogen source is considered one of the main factors that affect the growth and metabolism of fungi, and it is, therefore, important to accomplish a C:N balance, to ensure their effective growth and enhance their biodegradation ability by selecting the optimum nitrogen source to accelerate these processes (Jin & Fallgren, 2007). The results showed that the organic nitrogen source, yeast extract, accelerated the degradation process to a higher extent than the inorganic source, $(NH_4)_2SO_4$. The highest degradation rate was recorded in the medium containing yeast extract as the nitrogen source, with 93.13% degradation; the total PAH concentration decreased to 375.829 μg L^{-1}. Whereas $(NH_4)_2SO_4$ negatively affected the degradation process (Figure 3). This may be because of the high nitrogen content in the yeast extract, which was readily available to the fungi for growth. In contrast, the inorganic source $(NH_4)_2SO_4$ exerted a harmful effect on the growth of fungal mycelia by possibly decreasing the pH of the medium, thereby slowing the degradation process (Ruiz-Aguilar, Fernández-Sánchez, Rodríguez-Vázquez, & Poggi-Varaldo, 2002). Similar results were reported by Hamzah et al. (2012), who illustrated that culture conditions directly affected microorganism growth and the degradation process. There were significant differences ($p < .01$) in the PAH concentrations recorded under conditions of varying temperatures, pH, and nitrogen sources.

3.4 | Biostimulation of PAH degradation

The biostimulation process involved the adjustment of environmental conditions to stimulate the growth and enzymatic activity of the existing microorganisms. The optimum conditions, that is, 30°C, pH 5.5, and yeast

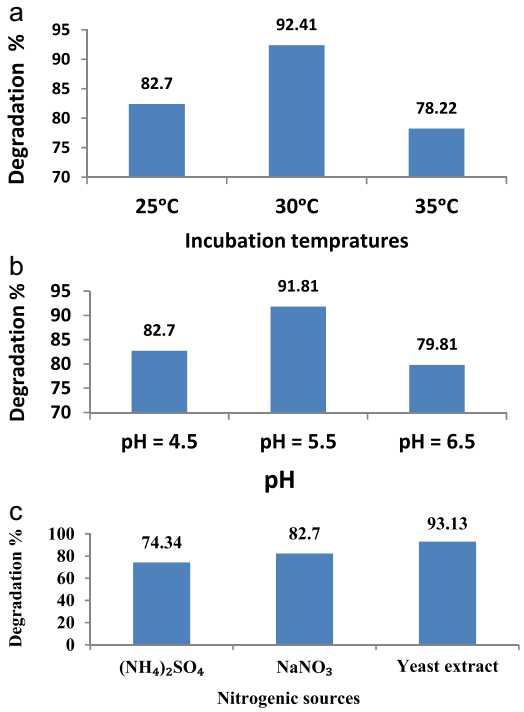

FIGURE 3 Effect of different parameters on polycyclic aromatic hydrocarbon degradation. (a) Effect of different temperatures, (b) effect of different pH, and (c) effect of different nitrogenic sources [Color figure can be viewed at wileyonlinelibrary.com]

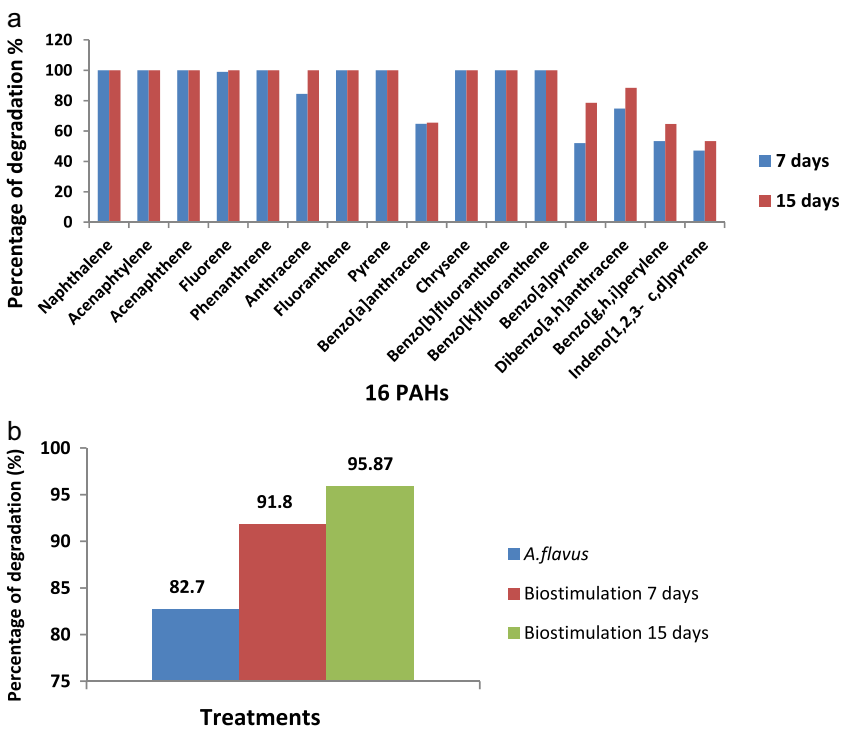

FIGURE 4 Effect of biostimulation on the polycyclic aromatic hydrocarbon (PAH) degradation process. (a) PAH degradation percentage after 7 and 15 days, and (b) comparison of total degradation percentage of PAHs before and after biostimulation [Color figure can be viewed at wileyonlinelibrary.com]

extract as a nitrogen source, were used together to study their effect on PAH degradation by *A. flavus*. The results showed that the total PAH concentrations decreased to 588.796 μg L^{-1} after 7 days, and the total degradation percentage increased to 91.80%, (Figure 4). A high biodegradation efficiency was achieved with biostimulation, where approximately 91.8% of PAH compounds were degraded in just 7 days; this was higher than the degradation percentage exhibited by the same fungus in the experiment without biostimulation. This suggests that subjecting the fungi to the optimum temperature, pH, and nutrient type resulted in higher enzymatic activity and growth, with a higher number of fungal mycelia coming in contact with the crude oil, thereby enhancing the degradation process more readily (Couto, Monteiro, & Vasconcelos, 2010; Garapati & Mishra, 2012). The results of the current study were consistent with those of several earlier studies (Garapati & Mishra, 2012; Kuappi, Sinkkonen, & Romantschuk, 2011; Wu et al., 2016).

A higher degradation efficiency was observed after 15 days of incubation, with the PAH concentrations decreasing to 225.443 μg L^{-1}, a degradation percentage of 95.87%. With biostimulation, *A. flavus* was able to completely degrade 11 PAHs after 15 days of incubation (Figure 4). There were significant differences ($p < .01$) in the degradation percentage between the fungus in this experiment and that in the previous experiment without biostimulation. It appeared that the longer incubation time presented more efficient results; that is, a higher fungal biomass in contact with the PAH compounds leads to higher levels of PAH degradation (Hamzah, Manikan, & Abd Aziz, 2017; Satti, Shah, Marsh, & Auras, 2018). Several reports state that the degradation process was maximized once optimal conditions for growth were achieved (Ali et al., 2012; Govarthanan et al., 2017; Hamzah et al., 2012; Wu et al., 2016).

4 | CONCLUSIONS

The fungus *A. flavus* exhibited a high degradation potential under laboratory conditions, with the ability to degrade 82.7% of total PAHs after 15 days of incubation. Biostimulation under the optimum temperature, pH, and nitrogen source conditions stimulated the degradation process to a higher degree. In addition, the incubation period also affected the biodegradation process, with approximately 95.87% of PAHs being degraded after 15 days of incubation. Our study indicated that *A. flavus* is a potential biodegrading agent that can be utilized in the bioremediation of PAHs in the environment.

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