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Hydrocarbon-Degrading Fungi Isolated from Oil-Contaminated Sites in Northern Peninsular Malaysia

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ABSTRACT

Improper waste management from automobile workshops has contributed markedly to environmental contamination. Areas within the vicinity of these workshops are exposed to high amounts of waste engine oils and other hydrocarbon wastes. Bioremediation may provide a practical solution due to better cost-effectiveness and high probability of total mineralisation without causing secondary pollution. Thus, this study aims to isolate, characterise and identify fungi that can utilize and degrade hydrocarbon. The research was conducted by collecting soil and water samples from the oil-contaminated sites including workshops, households and a sewage treatment plant in the Northern region of Peninsular Malaysia. Hydrocarbon-degrading ability was screened by growing fungi on selective agar containing waste engine oil (hydrocarbon) as the sole carbon source. The fungal colonies that grow on the selective agar were streaked and subcultured onto potato dextrose agar until pure isolates were obtained. Further screening by 2,6-dichlorophenol indophenol (DCPIP) assay was carried out to confirm the ability of all fungal isolates to utilise hydrocarbon. The isolated fungi were identified based on morphological characterisation and microscopic observation. Four fungal isolates from an oil-polluted environment were identified as *Aspergillus sydowii* USM-FH1, *Aspergillus westerdijkiae* USM-FH3, *Curvularia lunata* USM-FH6 and *Chaetomium globusum* USM-FH8. These fungal isolates showed good potential to be applied in the bioremediation of hydrocarbon-contaminated sites.

Keywords: hydrocarbon-degrading fungi, biodegradation, bioremediation, isolation, oil-contaminated environment, waste engine oil

INTRODUCTION

Since more than 8 billion tonnes of oil are used for industrial production each year via refining, exploration, and transportation, petroleum hydrocarbon pollution has become a global environmental issue (García-Olivares et al., 2017; Yuan et al., 2018; Liu et al., 2023). This large volume of oil field wastewater is becoming one of the major environmental concerns as it gives rise to potential hazards to human health due to the presence of various toxic compounds such as alkanes, BTEX (benzene, toluene, ethylbenzene, and xylene), heavy metals, and polycyclic aromatic hydrocarbons (PAHs) (Zhou et al., 2023). Among the recalcitrant compounds, polycyclic aromatic hydrocarbons (PAHs) are among the most persistent environmental pollutants, with a wide range of toxicity and highly bioaccumulated in the environment. Unlike many other pollutants, PAHs are emitted not only by anthropogenic activities but also by natural processes (Akash et al., 2022).

Incomplete combustion, either naturally or anthropogenically derived, has been identified as the single largest contributor of PAHs to the environment (Xenia & Refugio, 2016). The fused ring structure of PAHs confers their toxicity, mutagenic, and carcinogenic effects on humans and other living creatures (Cerniglia & Sutherland, 2010). The toxicity of these compounds remains in the environment for a long time, contaminates air, soil and groundwater and finally enters the food chain (Bansal & Kim, 2015). Consequently, the disposal of these contaminants contributes to the heavy discharge of hydrocarbon into the environment, thus threatening nature, the ecosystem, and public health.

Environmental degradation is a serious negative outcome of increasing urbanization that significantly impairs sustainable development and ecological balance. To date, many technologies are being used to remediate petroleum hydrocarbon-contaminated soils, including electrokinetic oxidation, thermal treatment, chemical oxidation, and bioremediation (Sajid et al., 2023). The most used technology is through chemical and mechanical methods where chemical and mechanical means of hydrocarbon removal from contaminated sites have limited effectiveness and are often non-cost effective (Bajagain & Jeong, 2021).

The major concern about these treatments is they can lead to secondary pollution such as the greenhouse effect (Sajid et al., 2023). Moreover, these methods are not suitable for treating contaminated soil as they will cause more damage to the soil via the creation of hydrocarbon residues in the subsoil, which eventually mix with groundwater. Generally, improperly disposed waste engine oils from automobile workshops caused oil contamination in water streams and absorbed oil contaminants in soil close to the area. Consequently, this may lead to soil infertility, rendering the soil unsuitable for plant growth. Additionally, oil contaminants also flow into water bodies such as lakes, streams, ponds and oceans which will eventually be dispersed in the area, leading to terrestrial and aquatic pollution.

Microbial remediation technology has been widely used to subside toxic hydrocarbons in constructed wetlands, seawater, soils, and intertidal zones (Meng et al., 2019; Dai et al., 2020; Behera et al., 2022). Bacteria are the most common organisms used in biological wastewater treatment systems to abate organic contaminants. Fungi, on the other hand, have received far less attention than bacteria for bioremediation treatment (Negi et al., 2020). However, at present, the concept of using filamentous fungi as bioremediation agents for waste oils has received a lot of attention (Jasme et al., 2022). Fungi make a good candidate for bioremediation as they have a higher potential to survive harsh environments, such as extreme pH, temperature and moisture, compared to other microorganisms (Kiama 2015). Fungi are also remarkably aerobic, despite their ability to grow in environmentally stressful conditions such as low pH and poor nutrient status. Furthermore, it can provide a robust approach as fungi can thrive in a wide range of environments (Jasme et al., 2022).

Fungal hyphae can penetrate contaminated soil, subsequently facilitating the biodegradation process. Moreover, fungi can travel through the air as spores (Barnes et al., 2018)such as mangrove sediments, Arabian Sea sediments, and tarballs. Out of the ten isolates, six belonged to Aspergillus, two to Fusarium and one each to Penicillium and Acremonium as identified using ITS rDNA sequencing. The selected ten fungal isolates were found to degrade the long-chain n-alkanes as opposed to short-chain n-alkanes from the crude oil. Mangrove fungus #NIOSN-M126 (Penicillium citrinum. For instance, white rot fungi as documented by Aust et al. (2004) can survive the toxic level of organ pollutants and can secrete extracellular lignin modifying enzymes; lignin peroxidase, Mn-dependent peroxidase, and laccase. These enzymes, which are rarely produced by bacteria, have low substrate specificity and are stimulated by nutrient limitation. They can degrade a wide range of recalcitrant compounds that are similar to lignin (Pointing, 2001; Cajthaml et al., 2002; Mansur et al., 2003; Hamman, 2004)pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, bleach plant effluent, synthetic dyes, synthetic polymers, and wood preservatives. Factors relating to the feasibility of using white-rot fungi in bioremediation treatments for organopollutants are discussed.","author":[{"dropping-particle":"","family":"Pointing","given":"S. B.","non-dropping-particle":"","parse-names":false,"suffix":""}],"container-title":"Applied Microbiology and Biotechnology","id":"ITEM-1","issue":"1-2","issued":{"date-parts":[["2001"]]},"page":"20-33","publisher":"Springer","title":"Feasibility of bioremediation by white-rot fungi","type":"article","volume":"57"},"uris":["http://www.mendeley.com/documents/?uuid=7ae0e00f-fb5c-328d-97ff-877b038a9239"]},{"id":"ITEM-2","itemData":{"DOI":"10.1016/S0021-9673(02. In addition, Singh (2006) also has found several groups of fungi that have the potential to degrade crude oil hydrocarbons including *Penicillium*, *Aspergillus* and *Cephalosporium*. Moreover, Chaillan et al., (2004) have reported that fungal genera, namely, *Neosartorya*, *Amorphoteca* and *Talaromyces* that were isolated from petroleum-contaminated soil were found to be potential degraders of hydrocarbon.

Hence, this study aims to identify and evaluate the hydrocarbon-degrading ability of the isolated fungi. Fungi that can utilize and break down hydrocarbon contaminants are good candidates for bioremediation, without negatively affecting the physical and chemical properties of the contaminated sites. Accelerated hydrocarbon biodegradation may be carried out by autochthonous microorganisms or bioaugmentation, with the appropriate nutritional and environmental conditions (Xu & Obbard, 2004; Xenia & Refugio, 2016)low bioavailability of the contaminants, or scarcity of PAH-biodegrading microorganisms. This study focused on addressing the limitation of nutrient availability for PAH biodegradation in oil-contaminated beach sediments. In our previous study, three nutrient sources including inorganic soluble nutrients, the slow-release fertilizer Osmocote (Os; Scotts, Marysville, OH.

MATERIALS AND METHOD

Sample Collection

Water samples were collected from three oil-contaminated sites into sterile Schott Duran bottles, namely drainage near an automotive workshop (6.4542° N, 100.1572° E) and a household area (6.4417° N, 100.1560° E) in Kangar, Perlis and the Batu Ferringhi Sewage Treatment Plant (STP), Penang, Malaysia. Soil samples were collected from oil-contaminated soil near the automotive workshop area in Kangar, Perlis, Malaysia (6.4542° N, 100.1572 °E). The soil samples were collected 5 cm below the surface using a sterile spoon into a sterilized container. All samples were stored at 4°C before use.

Isolation of Hydrocarbon-Degrading Fungi

All collected samples were labelled and subjected to serial dilution from 10^{-1} to 10^{-7}. One mL of every water sample and 1.0 g of soil sample were each transferred into 9.0 mL of distilled water, respectively. Selective mineral salt agar, Bushnell-Haas (BH) agar, containing hydrocarbon as the sole carbon source was used for screening and isolation of hydrocarbon-degrading fungi. BH agar was prepared following the composition as follows (per litre): 1.0 g KH_2PO_4, 1.0 g K_2HPO_4, 0.2 g $MgSO_4$, 0.02 $CaCl_2$, 1.0 g NH_4NO_3, and 0.05 g $FeCl_3$. The selective agar medium was supplemented with 3% (v/v) waste engine oil obtained from an automotive workshop and 25 mg mL^{-1} chloramphenicol. A volume of 0.1 mL suspension from each dilution tube was spread on the selective BH agar using a sterile L-shaped glass spreader in triplicates incubated at room temperature (25 – 28 °C) for 15 days (Khan et al., 2017; Jasme et al., 2022). Colonies obtained were sub-cultured on potato dextrose agar (PDA) and incubated for 7 days. Pure cultures of each fungal isolate were maintained on PDA by sub-culturing every four weeks.

Morphological Characterisation of Hydrocarbon-Degrading Fungi

Pure cultures obtained from the isolation and screening exercise were observed macroscopically for the colour, shape, size, texture, colony appearance, pigmentation and exudate formation after 7 days of incubation. Subsequently, the microscopic characteristics of the isolates were observed using a compound light microscope (Olympus CX21, Japan). The mycelial structure and spore arrangement were observed under 40X and 100X magnifications (oil immersion) using lactophenol cotton blue wet mount. This observation enables characterisation of the spore type, mycelia and the presence of other fruiting bodies.

Glass Slide Culture Technique

The glass slide culture technique was used to further determine the characteristics of fungal growth without disturbing the spore of the fungi. A small amount of sterile PDA was cut and put onto a sterile glass slide. Then, a needle was used to place a small portion of fungal growth into the edge of the agar block. The cover slide was put on top. The plates were incubated at room temperature (25 – 28°C) for 7 days, after which it was subjected to microscopic characterisation under a light microscope (Olympus CX21, Japan) and a scanning electron microscope (SEM).

Microscopic Observation Under Scanning Electron Microscope (SEM)

The morphology of each fungal isolate was examined under SEM to observe the spore shape, spore surface and spore chain morphology. To produce the samples for microscopic observation, each fungal isolate was grown on PDA for 7 days at room temperature (25 – 28°C). A piece of dried agar (5 mm x 5 mm) with mycelial growth and spore mass was cut using a Topaz micronized blade. The sample was subjected to vapour fixation by exposing it to 2% osmium tetroxide (OsO_4) vapour for 1 – 2 h in a fume hood. Then, the sample was plunged into slushy nitrogen (–210°C) and freeze-dried for 10 h. After the freeze-drying process, the sample was kept in a desiccator to dry. After drying, the sample was mounted onto the SEM specimen stub with a double-sided sticky tape, coated with 5 – 10 nm gold and viewed under SEM.

2,6-Dichlorophenol Indophenol (DCPIP) Assay

Ten per cent (v/v) of inoculum was inoculated into 50 mL BH medium supplemented with 3% (v/v) waste engine oil as the sole carbon source. The inoculated medium was incubated in an orbital shaker agitated at 150 rpm for 48 h at ambient room temperature (25 – 28°C). Then, 1% (v/v) redox indicator (DCPIP) was added to the culture. A colour change from dark blue to colourless indicates the ability of the culture to utilise hydrocarbon as the carbon source.

RESULTS AND DISCUSSION

Isolation of Hydrocarbon-Degrading Fungi

Fungi can degrade hydrocarbon pollutants and are better degraders than bacteria, offering high tolerance to a wide range of pH and temperature (Kiama, 2015). Bushnell-Haas (BH) agar was used as a selective media for the screening of possible fungi, as it provided all the necessary nutrients except the carbon source. The supply of waste engine oil to the fungi will be utilised by fungi as an energy and carbon source for growth (Hock et al., 2018). Therefore, fungi that were able to grow in BH agar supplemented with waste engine oil are considered potential fungi due to the capability of utilising waste engine oil as a carbon source. The effectiveness of the fungi in degrading waste engines in a laboratory environment over 15 days was observed.

After 15 days of incubation, seven pure isolates of fungi with different morphological characteristics have been successfully isolated from the four different types of samples; Batu Ferringhi Sewage Treatment Plant, drain near automotive workshop, drain near household and soil near automotive workshop. All these fungal isolates were able to grow on the Bushnell Haas (BH) agar containing 3% (v/v) of waste engine oil, indicating their ability to utilize hydrocarbon as their sole carbon source (El Hanafy et al., 2015)two fungal isolates were selected amongst 15 oil degrading strains. Analysis of ITS-1, ITS-2 and amplicon pyrosequencing studies of fungal diversity revealed that these strains belong to Penicillium and Aspergillus species. Two strains that proved to be the most efficient in degrading crude oil was Aspergillus niger (54%. All of the pure fungal isolates were sequentially subcultured onto MEA or PDA and subjected to morphological characterisation. Based on the macroscopic and microscopic morphological characters, among the seven fungal isolates two were identified as *Aspergillus sydowii*, three were *Chaetomium globusum* and the rest were *Aspergillus westerdijkiae* and *Curvularia lunata*, respectively. Consequently, four different species of fungi have successfully been isolated. All isolates were coded as FH, namely, *Aspergillus sydowii* USM-FH1, *Aspergillus westerdijkiae* USM-FH3, *Curvularia lunata* USM-FH6 and *Chaetomium globusum* USM-FH8 as summarised by Table 1.

Table 1 Four fungal isolates obtained from water and soil samples

Isolates / Sites	*Aspergillus sydowii* USM-FH1	*Aspergillus westerdijkiae* USM-FH3	*Curvularia lunata* USM-FH6	*Chaetomium globusum* USM-FH8
W		√		
X	√			√
Y				√
Z	√		√	√

* W (Batu Ferringhi Sewage Treatment Plant), X (drain near workshop), Y drain near household) and Z (soil near workshop)

Most of the fungi were isolated from the soil sample. Although fungi can be terrestrial and aquatic, fungi dominate 75% of soil microbial biomass (Ritz & Young, 2004). In addition, soil microorganisms have a different range of optimal pH that they can tolerate to efficiently remove PAHs (Brito et al., 2015). These locally isolated fungi could potentially be grown in mass quantities to treat hydrocarbon contamination at small and large contaminated sites (Hock et al., 2018).

Each isolate showed different pigmentation and colony appearance on BH agar, malt extract agar (MEA) or potato dextrose agar (PDA), as the medium composition affects pigmentation (da Costa Souza et al., 2016)in cosmetics and textiles, and because of the important biological activities of these compounds. In this context, the objectives of this study were to select pigment-producing fungi, identify these fungi based on internal transcribed spacer sequences, evaluate the growth and pigment production of the selected strains on four different media, and characterize the major coloured metabolites in their extracts. Of the selected fungal strains, eight were identified as Aspergillus sydowii (CML2967. The source of vitamins and coenzymes in MEA can promote the development and pigmentation, especially for *Aspergillus* sp. (Pradeep & Pradeep, 2013), subsequently, enhancing more sporulation to occur compared to growing on PDA. Hence, this property is important for identification purposes.

Generally, growth was slower when grown on BH agar supplemented with waste engine oil. Colony diameter was smaller on BH agar compared to those on PDA or MEA, which are complex and nutrient-rich media. The degradation of hydrocarbon substrate can lead to several challenges related to the nature of the substrate. Hence, a longer time is required for the fungi to break down and utilize the substrate, which influences their growth and survival. This can be seen when the fungal colonies started to grow after 15 days of incubation on BH agar. In contrast, the growth and sporulation of PDA and MEA were observed after 5 – 7 days of incubation.

Identification of Isolated Hydrocarbon-Degrading Fungi

The isolated fungi were identified based on their morphological characterisation when grown on microbiological media, such as size, colony formation and spore mass colour. The spore chain morphology and orientation were also observed by microscopic observation. The morphological analysis and identification of fungi is useful for the identification of isolates at family or genus level (Wang et al., 2016)145 isolates belonging to Chaetomiaceae were cultured from air, swab and dust samples from 19 countries. Based on the phylogenetic analyses of DNA-directed RNA polymerase II second largest subunit (rpb2. Table 2 exhibits the macroscopic and microscopic observations of four isolated hydrocarbon-utilising fungi under a light microscope and scanning electron microscope (SEM).

Table 2 Characteristics of four isolated hydrocarbons utilizing fungi under light microscope and scanning electron microscope observation

Isolate	Identified fungal isolates	PDA or MEA			Conidia	
		*Diameter (cm)	Upper surface	Lower surface	Length (µm)	Shape
FH 1	*Aspergillus sydowii*	2.6	Blue-green powdery	Creamy yellow	3-4	Globose to sub globose
FH 3	*Aspergillus westerdijkiae*	3.7	Yellow conidia surrounded by whitish mycelia	Creamy brown	2.5-3	Globose to sub globose
FH 6	*Curvularia lunata*	8.2	Woolly brown	Black	17	Ovoid, obclavate
FH 8	*Chaetomium globusum*	7.9-8.1	Olive-greyish	Creamy yellow, blackish at centre	5	Lemon-shaped

*After 7 days of incubation

Aspergillus spp. are among the most frequently reported filamentous fungi that can grow on hydrocarbons (Husaini et al., 2008). Figure 1a displays the morphology of *Aspergillus sydowii* USM-FH1 when grown on MEA. The colony's upper surface was blue-green and powdery with a creamy yellow bottom surface. The conidiophores and conidia of *A. sydowii* USM-FH1 were observed under SEM as shown in Figure 1b. The phialides were cylindrical with short necks, whereas the conidia were rough and circular. Under macroscopic observation, *Aspergillus* sp. and *Penicillium* sp. look similar as their colonies are powdery and blue-green. Microscopic characterisation is essential to differentiate them. The conidiophores of *Aspergillus* sp. had a swollen apex (vesicle) and phialides borne directly on the vesicle. In contrast, the conidiophores of *Penicillium* sp. developed as an erect branch from the vegetative mycelium and its sterigmata (flask-shaped phialid) borne directly on the metulae, forming a paintbrush-like structure. *A. sydowii* is a common fungus, halo-tolerant and can tolerate low pH (Butinar et al., 2011). Mt al.ancera-Lopez et al. (2007) reported that *A. sydowii* has been isolated in silty loam soil contaminated with a complex solid mixture of hydrocarbon.

Aspergillus westerdijkiae USM-FH3 was isolated from a domestic sewage treatment plant in Batu Ferringhi, Penang, Malaysia. Generally, sewage is the world's largest contributor to organic pollution of water resources and surrounding environments. As sewage sludge is a highly heterogeneous substrate, for microbes that can metabolise a wide range of organic substances and can also grow by consuming their nutrients indicating that these microbes can adapt and tolerate harsh environments. Fakhrul-Razi et al. (2002) reported that the second most isolates that were found in Indah Water Konsortium (IWK) in Malaysia that were involved in the biological treatment of domestic waste sludge was *Aspergillus* species. This fungus is also halo-tolerant and is commonly found in warmer climates (Butinar et al., 2011). As illustrated in Figure 2a, the yellowish colonies of *A. westerdijkiae* USM-FH3 are surrounded by white mycelial mats. The upper surface looks powdery while the bottom is creamy brown when grown on MEA. Figure 2b shows a micrograph of *A. westerdijkiae* USM-FH3 under SEM with a biseriate conidial head and cylindrical phialides tapering to a distinct neck, while the conidia are globose and smooth-walled.

The colonies of *Curvularia lunata* USM-FH6 had woolly brown upper surface and black lower surface when grown on PDA as shown in Figure 3a. Figure 3b shows a micrograph of the conidiophore attached to five conidia. The conidia were ellipsoidal with 4-distoseptate, ovoid and obclavate. The third cells are often curved or larger than the rest with dark brown septate hyphae. Mohsenzadeh et al. (2010) reported the isolation of *C. lunata* from the soil in petroleum-polluted areas. About 80 species of *Curvularia* can be found in soil and as a plant pathogen.

In addition, another isolate was identified as *Chaetomium globusum* having hairy fruiting bodies, ascospores and lemon-shaped spores. This fungus is ubiquitous, occurring on a wide variety of substances, in the air and marine environments (Wang et al. 2016)145 isolates belonging to Chaetomiaceae were cultured from air, swab and dust samples from 19 countries. Based on the phylogenetic analyses of DNA-directed RNA polymerase II second largest subunit (rpb2. The conidia of *C. globusum* USM-FH8 appeared as small green-black dots without any mycelial form. When fungi are stressed, they can simplify their asexual life cycle by only germinating conidia without having the mycelial form (Jung et al. 2014). This fungus belongs to *Ascomycota*. The fruiting bodies (ascomata) formed after 1 – 2 weeks, giving the colonies an olive-greyish appearance as shown in Figure 4a. The perithecia are dark brown, globose to ovoid with perithecial hairs, numerous, flexuous or even coiled as illustrated by Figure 4b. In addition, the ascospores are brown and lemon-shaped (Figure 4c). This is the first report on *C. globusum* as the potential hydrocarbon degraders.

Hydrocarbon Utilization by Fungal Isolates

All isolates obtained were subjected to 2,6-dichlorophenol indophenol (DCPIP) assay. DCPIP is an enzyme-catalysed oxidation electron acceptor that is blue in oxidised form and colourless when in reduced form (Bidoia et al., 2010). During microbial oxidation of the carbon source, electrons were transferred to the electron acceptor. The presence of DCPIP in the culture medium allows the ability of the microorganism to utilize the carbon substrate to be observed by the colour change of DCPIP from blue (oxidised) to colourless (reduced).

Table 3 Time taken for the fungal isolates to utilise waste engine oil in BH medium indicated by the reduction of DCPIP

Fungal isolates	Time taken for DCPIP to turn colourless (hours)
Aspergillus sydowii USM-FH1	48
Aspergillus westerdijkiae USM-FH3	48
Curvularia lunata USM-FH6	72
Chaetomium globusum USM-FH8	48

Table 3 shows the time taken for each isolate to utilise the waste engine oil in the BH medium. The control flask containing BH medium without culture which represented negative control, remained blue until the end of the experiment. Out of four species, three species, namely *A. sydowii* USM-FH1, *A. westerdijkiae* USM-FH3, and *C. globusum* USM-FH8 can utilise the waste engine oil within 48 hours. Other fungi have also been reported to degrade hydrocarbon substrates but not as effectively as these newly locally isolated strains. For instance, a study by Tiwari and Saraf (2017) reported that *Aspergillus nidulans, Aspergillus flavus* and *Aspergillus fumigatus* were capable to utilise crude oil represented by the change in colour of DCPIP to colourless after 7 days of fermentation. This result showed that different species have different rates of utilisation towards hydrocarbon.

Fungal growth morphology varies among different species when grown in BH broth. *Aspergillus sydowii* USM-FH1, *A. westerdijkiae* USM-FH3 and *C. lunata* USM-FH6 grew in clumps and no discernible free droplet of waste engine oil was observed as these fungi adhered to the oil, as shown in Figure 5a and 5b. In contrast, *C. globusum* USM-FH8 grew in pellets with obvious free microdroplets of oil in the culture broth as shown in Figure 5c.

Presumably, different isolates have different mechanisms of oil uptake; either by modifying their cell surface hydrophobicity, producing exopolysaccharide (EPS) to form biofilm, or producing biosurfactants or enzymes. Initially, the cells would attach to the surface of the carrier via hydrophobic forces in the surroundings. Then, the production of EPS may aid the microbial cells to attach to the hydrophobic surface, which is responsible for the cohesion of cells and particulate nutrients in their surroundings. Increased cell growth often ensues due to improved nutrient diffusion and gaseous exchange, which subsequently, increases the degradation activities (Hazaimeh et al. 2014).

Based on this study, it can be summarized that the indicators of hydrocarbon utilisation and biodegradation by the isolated fungi were shown by the change of colour culture broth from blue to colourless during DCPIP assay and the fungal biomass growth throughout fermentation which correlated to the utilisation of waste oil as the sole carbon source in the liquid (Nasrawi, 2012).

However, it is important to note that one of the drawbacks of this study is the identification of fungal species was made only based on morphology and microscopic characterization. Further identification of the isolates shall be carried out through a molecular approach in subsequent work.

CONCLUSION

Four fungal species with a high potential for degrading waste engine oil have been isolated from the local hydrocarbon-contaminated sites. These isolates were identified as *A. sydowii* USM-FH1, *A. westerdijkiae* USM-FH3, *C. globusum* USM-FH6 and *C. lunata* USM-FH8. These isolates demonstrated the ability to utilise and degrade hydrocarbon within 48 to 72 h in BH medium. Hence, they showed a good potential to be applied for bioremediation of hydrocarbon-contaminated environments.

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CONFLICT OF INTEREST

The authors declared that this study was performed in the absence of any conflict of interest.

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