International Journal of Plant & Soil Science

Volume 36, Issue 5, Page 450-462, 2024; Article no.IJPSS.115286
ISSN: 2320-7035

Crude Oil Biodegradation Performance in Natural Seawater of a *Trichoderma* species from Decaying Mangrove Wood in East Kalimantan

Sitompul Afrida [a,b*], Prapti Ira Kumalasari [b], Jan Friadi Sinaga [b], Amiruddin [b], Dede Heri Yuli Yanto [c], Sakae Horisawa [a] and Yutaka Tamai [d]

[a] School of Engineering Science, Kochi University of Technology, Kami City, Kochi, 782-8502, Japan.
[b] STT Migas, Jalan Soekarno Hatta Km. 8, Balikpapan, East Kalimantan, 76125, Indonesia.
[c] Research Center for Applied Microbiology, National Research and Innovation Agency (BRIN), Soekarno Science and Technology Park, Cibinong Science Center, Jalan Raya Bogor Km. 46, Cibinong, Bogor, 16911, Indonesia.
[d] Department of Forest Science, Hokkaido University, N9W9, Kita-ku, Sapporo, 060-8589, Japan.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2024/v36i54542

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/115286

Original Research Article

Received: 20/01/2024
Accepted: 27/03/2024
Published: 29/03/2024

ABSTRACT

Large-scale petroleum production poses environmental and human health risks, particularly due to petroleum contamination in marine environments. The aim of this study was to isolate and screen fungi inhabiting decaying mangrove wood in East Kalimantan with their ability to degrade crude oil in seawater. In the initial step, fungi were isolated using standard methods and screened for crude oil degradation and biomass production. Selected fungi were based on their significant biomass

**Corresponding author: E-mail: afrida.sitompul0214@gmail.com, afrida@kochi-tech.ac.jp;*

production. Subsequently, the selected fungus was cultured in three types of media based on natural seawater and crude oil at initial concentrations ranging from 1% to 5% (v/v). The biodegradation of crude oil was assessed spectrophotometrically at 420 nm. Out of the 17 decaying wood fungi, MG-07 exhibited significantly higher biomass production compared to the control medium (without crude oil). Gas chromatography-mass spectrometry analysis indicated that MG-07 degraded 20%–80% of the normal aliphatic compounds in the tested crude oils within 2 weeks. Furthermore, the growth and degradation efficiency of MG-07 was significantly enhanced when cultured in a natural seawater medium containing crude oil, supplemented with glucose (20 g/L) and Tween 80 (1% v/v). The highest observed biomass gains were 867%, with a biodegradation efficiency of 31.6% at a crude oil concentration of 1%. MG-07 was identified as *Trichoderma* sp. based on the internal transcribed spacer region. The results obtained from this study demonstrate that MG-07 has significant potential for degrading crude oil in saline environments. These findings provide valuable insights for the application of fungi in marine oil spill remediation.

Keywords: Marine environment; bioremediation; fungi; biodegradation; petroleum; health risks; coastal area; mangrove forests.

1. INTRODUCTION

Economic and social activities (e.g., commercial activities, electric generation, industrial activities, residential activities, and transportation) depend on petroleum hydrocarbon products (crude oil, diesel, and motor oil) [1,2]. Large-scale petroleum production can pose environmental and human health risks because petroleum is carcinogenic, mutagenic, and toxic and can accumulate through the food chain [3]. One of the most important issues is the occurrence of petroleum pollution in the marine environment [4]. Most events in which the marine environment becomes contaminated with petroleum occur during petroleum use, offshore drilling, production, refining in coastal areas, shipping, and unintentional spills [1–5]. Oil spills at sea often extend into coastal areas, reaching and spreading within salt marshes and mangrove forests [6,7]. Petroleum hydrocarbons have limited solubility in water and are readily adsorbed onto particles that can enter and persist for long periods in marine sediment, harming benthic aquatic communities [8].

Bioremediation employs microorganisms to break down and neutralize harmful environmental pollutants, converting them into carbon dioxide and water, biomass, and by-products with lower toxicity than the original compounds [8,9]. Bioremediation of petroleum in marine environments using microorganisms is significantly influenced by various environmental factors, including bioavailability, nutrient availability, salinity, pH, and concentration of hydrocarbon [2]. Microorganisms require essential nutrients such as carbon, nitrogen, phosphorous, sulfur, oxygen, and hydrogen for their growth and energy. Among these, carbon plays a crucial role in bioremediation, enhancing the metabolic activity of microorganisms and, consequently, accelerating pollutant degradation [10]. Petroleum hydrocarbons are hydrophobic pollutant compounds characterized by high-carbon components such as saturates, aromatics, asphaltenes, and resins, which microorganisms can utilize as a carbon and energy source [11]. However, these components resist dissolving in water, making it challenging for microorganisms to degrade and mineralize them [9,11]. To facilitate microbial adaptation to environments with limited carbon bioavailability, the addition of a simple carbon source, such as glucose, as a co-metabolic substrate can be effective [12]. In addition to glucose, microorganisms can accelerate the degradation and mineralization of petroleum pollutants by utilizing surfactants like Tween 80 [13]. A field study demonstrated that the addition of surfactants to support *Pseudomonas* sp. in the restoration of petroleum-contaminated soil can both decrease the levels of petroleum hydrocarbons and stimulate the growth of other soil microbes [14].

Numerous fungi from saline environments have been isolated and characterized for crude oil degradation [15]. Recent studies have increasingly focused on exploring the capability and effectiveness of halophilic fungi in the degradation of crude oil and its derivatives, such as genera belonging to *Penicillium, Aspergillus, Fusarium, Alternaria,* and *Trichoderma* [1,2,16]. These fungi can adapt through diverse mechanisms, such as ion homeostasis, accumulation of compatible solutes, maintaining plasma membrane fluidity, and utilizing the high

osmolarity glycerol (HOG) signaling pathway [17].

On 31 March 2018, Balikpapan Bay in East Kalimantan experienced an accidental crude oil spill from a cracked underwater pipeline operated by the Indonesian national oil and gas company [6]. The spill, estimated at 40,000 barrels, affected 120 km^2 of Balikpapan Bay, including mangrove areas. Balikpapan City hosts a crude oil refinery that supplies 26% of the fuel in Indonesia, making Balikpapan Bay one of the busiest ports in the Kalimantan ports for cargo, passenger, and tanker traffic. These activities pose significant risks of crude oil contamination to both land and marine environments. Conversely, East Kalimantan is a tropical region rich in natural resources, including fish, forests, microorganisms, oil, and gas. Despite this richness, little attention has been given to the biota of East Kalimantan, particularly microorganisms [18]. Therefore, the aim of this study was to isolate and screen fungi from East Kalimantan with the ability to degrade crude oil. Additionally, the influence of glucose and Tween 80 on the ability of the selected fungus to degrade crude oil degradation in natural seawater was investigated.

2. MATERIALS AND METHODS

2.1 Chemical

Three types of crude oil were obtained from Pertamina Refinery Industry Unit V, Balikpapan, Indonesia: crude oil A (COA, from Bekapai oil field, East Kalimantan), crude oil B (COB, from Handil oil field, East Kalimantan), and crude oil C (COC, from Banyu Urip oil field, Central Java). Data on the crude oils as provided by the company is presented in Table 1. Toluene (purity 99.9%) was purchased from Merck (Darmstadt, Germany). All other chemicals were from HiMedia Laboratories (Mumbai, India) or were purchased from SIP Chemicals Industries (Mumbai, India).

2.2 Sources and Isolation of Fungi

Sample of crude oil contaminated decaying mangrove wood in Balikpapan (East Kalimantan, Indonesia; 1°13'34"S 116°49'24"E) was collected and cut into small pieces. Fraction of the pieces were inoculated directly onto mineral salt potato dextrose agar (MSPDA) medium [19]. Inoculated medium were incubated at 28 ± 3 °C for 4–7 d. The composition of the MSPDA medium is presented in Table 2. The medium was sterilized by autoclaving at 121 °C for 15 min. A pure culture was obtained by subculturing an inoculum two or three times in a sterile medium. Stock cultures of the isolates were maintained on PDA slants at 28 ± 3°C.

2.3 Screening for Potential Crude-oil-Degrading Fungi

The abilities of isolated fungi to degrade crude oil were assessed by inoculating the fungi into mineral salt medium containing 20 g/L glucose, 1 % (v/v) Tween 80, and 1 % (v/v) of COA, COB, or COC. The total volume in each flask was 20 mL. The control was a mixture containing no crude oil. Each flask was plugged with air-permeable silicone sponge T-24 (Nichiden-Rika Glass Co., Japan) and autoclaved, then the medium was inoculated with a 1 cm × 1 cm piece of fungal isolate that had previously been sub-cultured on PDA at 28 ± 3 °C for 7 d. The flasks were incubated at 28 ± 3 °C for 2 weeks in a dark room. The contents of each flask were then collected, and each suspension was passed through a Whatman No. 1 filter (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA) to collect the mycelia. The fungal biomass was determined from the mass of mycelia collected, and the liquid was analyzed by gas chromatography-mass spectrometry to assess the degree of biodegradation of normal aliphatic hydrocarbons in crude oil [20].

2.4 Determination of the Fungal Biomass

The mycelia collected from a test suspension were dried at 105 °C for at least 8 h until a constant mass was reached. The fungal biomass (in grams per liter) was determined from the dry mass of the mycelia and the volume of medium the mycelia were collected from. The biomass gain was defined as the difference between the fungal biomasses in the treatment and control samples.

2.5 Identification of Crude-oil-degrading Fungi

The selected isolate was identified from the nucleotide sequences for parts of the genes for small and large subunits of RNA and the complete sequence for the internal transcribed spacer region [21,22]. The genomic DNA for the isolate was extracted using a Plant DNA Isolation Reagent (Takara Bio, Shiga, Japan). The DNA was amplified using primers NS7 (5'

GAGGCAATAACAGGTCGTGATGC 3') and NL4 (5' GGTCCGTGTTTCAAGACGG 3'). The PCR products were purified using NucleoSpin Gel and PCR Clean-up (Macherey-Nagel, Duren, Germany) and sequenced using the Sanger method. The DNA sequences were then compared with the International Nucleotide Sequence Database at the BLAST program at the US National Center for Biotechnology Information. Alignments and the phylogenetic tree were determined using MEGA version 11 software [23].

2.6 Determining the Effect of Media Type and Crude Oil Concentration on Hydrocarbon Degradation by the Fungus

The abilities of the selected fungus to degrade crude oil at concentrations of 1%, 2%, 3%, 4%, and 5% in three different media were assessed. The basal medium was natural fresh seawater (3.5% w/v salinity, pH 8.1), sourced from the coastal area in Balikpapan (1°16'42"S 116°50'00"E) and supplemented with crude oil (COC). This basal medium was referred to as Seawater Oil (SO) medium and used as a control. The second medium was SO with 20 g/L glucose added (SOG). The third medium was SO with 20 g/L glucose and 1% Tween 80 added (SOGT). Each test was performed in a 100 mL Erlenmeyer flask at 28 ± 3 °C and incubated for 2 weeks. Each flask contained 20 mL of medium and the inoculum, which was taken from a culture grown on PDA for 7 d. At the end of the incubation period, 20 mL of toluene was added to each flask and then the mixture was passed through a Whatman No. 1 filter. The mycelium was dried and the remaining crude oil concentration in the liquid was determined by spectrophotometry using a wavelength of 420 nm [24]. The difference between the initial and final total hydrocarbon concentrations was defined as the Extent of hydrocarbon degraded (EHD) and biodegradation efficiency of the fungus. EHD and efficiency were calculated using the equations shown below [25].

EHD (%) = [(Initial concentration – Final concentration) / Initial concentration] × 100

Biodegradation efficiency (%) = EHD of the treatment (SOG or SOGT) – EHD of the control (SO)

2.7 Determining the Effect of Incubation Time, Glucose Concentration, Tween 80 Concentration, and Salinity on Hydrocarbon Degradation by the Fungus

A series of tests were performed each using a 100 mL Erlenmeyer flask containing 20 mL of basal medium (natural seawater containing 1% crude oil (COC), 20 g/L glucose, and 1% Tween 80). The effects of incubation times of 1–8 weeks, glucose concentrations of 1, 5, 10, 15, and 20 g/L, Tween 80 concentrations of 0.5%, 1%, 2%, 3%, 4%, and 5% (v/v), and salinities of 1%, 2%, 3%, 4%, and 5% (w/v) were determined. The effects of salinity were investigated by performing tests using artificial seawater (HiMedia). The amount of artificial seawater powder required to give the desired salinity was dissolved in distilled water. The control was a mixture of COC, glucose, and Tween 80 in distilled water. Control tests for the effects of different glucose and Tween 80 concentrations were performed with no glucose or Tween 80 added. The flasks were incubated at 28 ± 3 °C for 2 weeks in a dark room. At the end of the incubation period, each flask was treated as described in the section above (Effects of three types of media and crude oil concentration).

Table 1. Physicochemical characteristics of the crude oils

Parameter	Method	Unit	COA	COB	COC
API gravity at 15.56°C	ASTM D1298		38.4	36.3	32.1
Asphaltenes (Heptane insoluble)	IP 143	mass %	0.14	0.69	0.20
Viscosity Kinematic at 37.8°C	ASTM D445	mm^2/s	2.55	3.70	10.5
Metal content: Ni	IP 470 / UOP 391 / ASTM D5708	mass ppm	1 / - / -	-/ - / 1.93	- / - / 0
V	IP 470 / UOP 391 / ASTM D5708	mass ppm	1 / - / -	-/ - / 0.68	- / - / 0
Fe	IP 470 / UOP 391 / ASTM D5708	mass ppm	3 / - / -	-/ - / 5.47	- / - / 0
Na	IP 470 / UOP 391	mass ppm	17 / -	- / 0.40	- / 3

Table 2. Composition of the mineral salts PDA medium

Components	Concentration (mg/L)
$CaCl_2.2H_2O$	1.47
$CuSO_4.5H_2O$	0.025
$FeSO_4.7H_2O$	5.56
KCl	7.46
KH_2PO_4	136
$MgSO_4$	246.5
$MnSO_4.H_2O$	0.34
NaCl	5.35
NH_4Cl	5.35
$ZnSO_4.7H_2O$	0.29
Potato dextrose agar (PDA)	39,000

The type and concentration of the mineral salts were adapted from Ameen et al [15] with slight modifications

2.8 Statistical Analysis

Each test was performed in triplicate. Each result is presented as the mean ± standard deviation of triplicate tests or controls. Differences between the results for different experimental groups were identified by performing one-way analysis of variance using SPSS version 25 software. Differences between the results for different treatments were identified by performing post hoc Bonferroni tests using the significance level $p< 0.05$.

3. RESULTS

3.1 Increase in Fungal Biomass

The increases in biomass after a 2-week incubation period with different crude oil samples are presented in Table 3. Six strains (MG-02, MG-04, MG-06, MG-07, MG-12B, and MG-13A) exhibited a significant increase in biomass compared to other strains. The biomasses of these six strains increased by more than 100% in the presence of crude oil compared to the control (absence of crude oil). The biomass of MG-07 increased more than that of the other five strains in the presence of all three crude oil samples, particularly in the case of COC, where the biomass increased by 633% compared to the control. Meanwhile, MG-07 exhibited biomass gains of 300% and 367% for COA and COB, respectively. Statistically, the growth of MG-07 in the three crude oil samples was not significantly different ($p> 0.05$).

3.2 Crude Oil Degrading Ability of the Fungus (MG-07) having the Highest Increase in Biomass

The ability of MG-07 to degrade COA, COB, and COC is presented in the chromatograms in Fig. 1. Comparisons of test and control chromatograms show that there was degradation of some hydrocarbon components. The intensities of crude oil peaks varied among COA, COB, and COC controls, indicating different hydrocarbon concentrations. Treatment with MG-07 led to decreased areas of hydrocarbon peaks, signifying degradation. MG-07 exhibited lower degradation efficiency (~20%) for COA aliphatic hydrocarbons than COB and COC. Comparing Fig. 1 with fungal biomass growth in Table 3 revealed that MG-07 obtained less biomass in the presence of COA. MG-07 effectively degraded C_{16}–C_{22} aliphatic hydrocarbons in COB, completely degrading C_{16} and ~80% of C_{17}–C_{22}. More than 50% of C_{23}–C_{30} aliphatic hydrocarbons in COB were degraded. Remarkably, MG-07 extensively degraded COC, reducing major and minor peak areas by ~80%. Short-chain hydrocarbons were fully degraded, while long-chain hydrocarbons were partially degraded, leading to a decrease in chain length range.

3.3 Identity of Fungal Isolate MG-07

The relatedness of MG-07 to other fungal genotypes is illustrated in Fig. 2. Branching in the phylogenetic tree indicated a high similarity between MG-07 and *Trichoderma harzianum* KC330218 (96 % similarity). The DNA sequences have been deposited in the DNA Data Bank of Japan (DDBJ), and the assigned accession number is LC797959.

3.4 Effects of Media Types and Crude Oil Concentration on the Ability of MG-07 to Degrade Crude Oil

In the medium containing natural seawater and crude oil (SO), MG-07 exhibited slow growth with no observed crude oil degradation (Tables 4 and 5). However, in the presence of glucose (SOG), MG-07 demonstrated an increased biodegradation efficiency ranging from 7% to 14%. When both glucose and Tween 80 (SOGT) were present, MG-07 exhibited a biodegradation efficiency approximately two times higher than that in the SOG medium at an initial crude oil concentration of 1% to 5%. More fungal growth was also produced when both glucose and

Tween 80 were present than when neither was present or when only glucose was present. The results show that the biodegradation efficiency of MG-07 was facilitated by its growth in the culture medium. The highest observed biomass gains were 867%, with a biodegradation efficiency of 31.6% at a crude oil concentration of 1%. Thus, the order of increasing biomass gains and biodegradation efficiency of MG-07 on the three types of media is as follows: SOGT > SOG > SO.

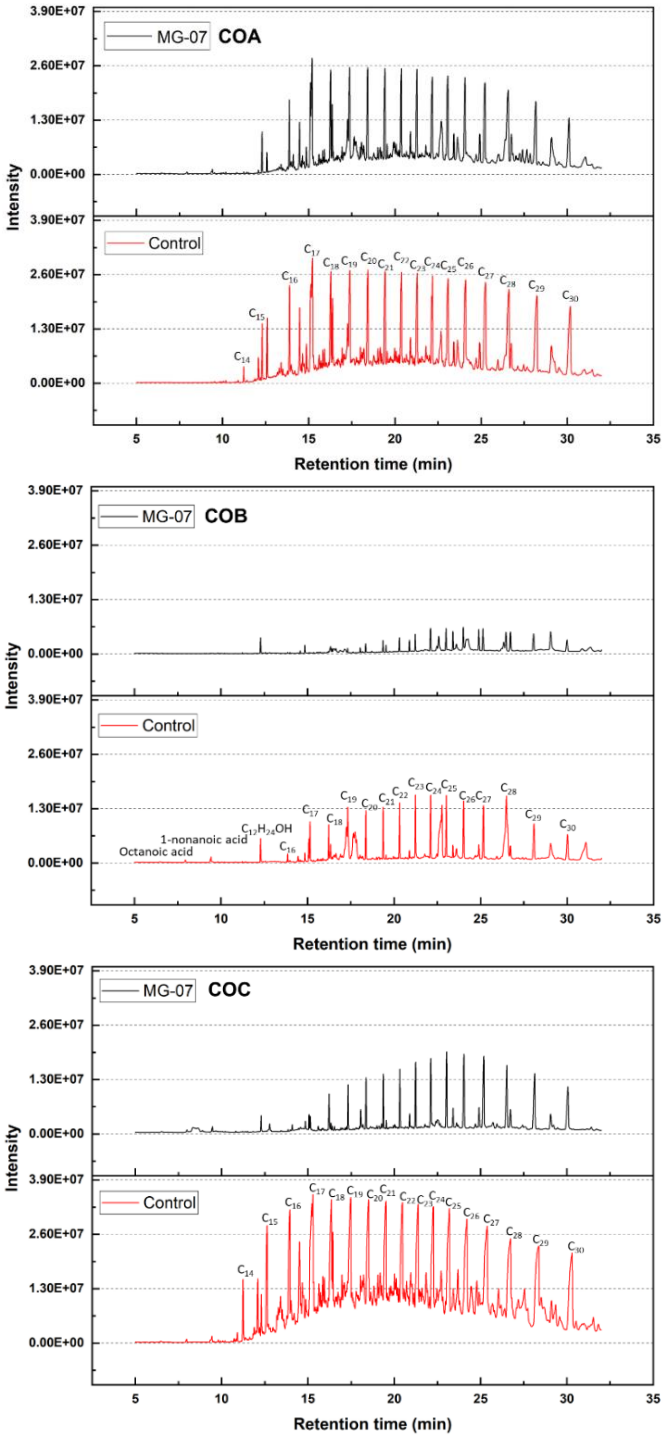

Fig. 1. Gas chromatography-mass spectrometry chromatograms indicating degradation of crude oil samples COA, COB, and COC

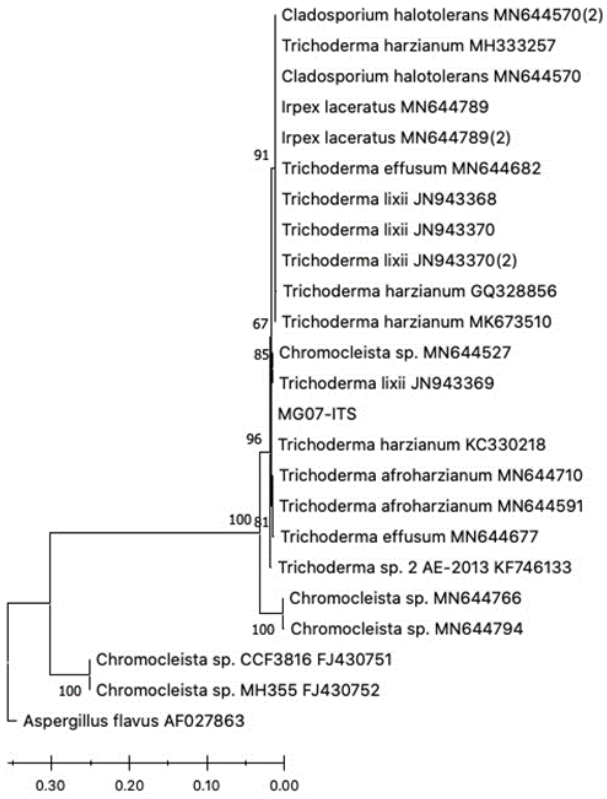

Fig. 2. Phylogenetic of fungal isolate MG-07 and closely related fungi

Table 3. Biomass gains of the fungal isolates

No	Isolate	Biomass gain (%)		
		COA	COB	COC
1	MG-02	250 ± 50.0a*; a**	183 ± 76.4a; ab	217 ± 28.9a; b
2	MG-04	167 ± 115.5a; ab	250 ± 50.0a; a	300 ± 86.6a; b
3	MG-05	17.0 ± 14.4b; c	117 ± 28.9a; b	125 ± 50.0a; bc
4	MG-06	189 ± 50.9ab; ab	244 ± 50.9a; a	100 ± 57.7b; bc
5	MG-07	300 ± 200a; a	367 ± 152.8a; a	633 ± 115.5a; a
6	MG-07A	3.00 ± 2.50a; c	3.00 ± 3.60a; d	8.00 ± 4.20a; c
7	MG-07B	117 ± 28.9b; b	33.0 ± 38.2c; c	167 ± 38.2a; bc
8	MG-08DW.1	13.0 ± 30.6b; c	80.0 ± 20.0a; bc	93.0 ± 30.6 a; bc
9	MG-08DW.2	21.0 ± 14.4a; c	0.00 ± 0.00b; d	33.0 ± 19.1a; c
10	MG-08FB	57.0 ± 14.3a; bc	57.0 ± 42.9a; bc	76.0 ± 29.7a; bc
11	MG-10	144 ± 50.9a; ab	78.0 ± 117.1a; bc	156 ± 19.2a; bc
12	MG-11A	28.0 ± 9.60b; c	61.0 ± 9.6a; bc	83.0 ± 33.3a; bc
13	MG-11B	83.0 ± 76.4b; bc	0.00 ± 0.00c; d	283 ± 160.7a; b
14	MG-12A	17.0 ± 16.7b; c	67.0 ±16.7 a; bc	56.0 ± 34.7a; bc
15	MG-12B	267 ± 28.9a; a	217 ± 76.4a; a	317 ± 152.8a; b
16	MG-13A	158 ± 14.4a; ab	167 ± 14.4a; ab	242 ± 57.7a; b
17	MG-13B	50.0 ± 25.0b; bc	0.00 ± 0.00c; d	125 ± 66.1a; bc

** The first letter after a result (a, b, or c) indicates statistically significant differences between the results for the same isolate in the presence of the different types of crude oil. The same letter indicates that the results were not significantly different.*

***The second letter or pair of letters (a, b, c, and/or d) indicates statistically significant differences between the isolates in the presence of the same type of crude oil.*

Table 4. Extent of hydrocarbon degradation (EHD) and biodegradation efficiencies of fungus MG-07 (*Trichoderma* sp.)

Initial oil concentration	Final oil concentration (%)			Extent of hydrocarbon degradation (%)			Biodegradation efficiency (%)
(%)	SO	SOG	SOGT	SO	SOG	SOGT	
1	1.00 ± 0.12	0.84 ± 0.12	0.68 ± 0.10	0.00	14.1	31.6	14.1
2	2.00 ± 0.17	1.78 ± 0.13	1.50 ± 0.34	0.00	11.1	25.2	11.1
3	3.00 ± 0.06	2.73 ± 0.31	2.33 ± 0.06	0.00	9.12	22.2	9.12
4	4.00 ± 0.01	3.70 ± 0.08	3.38 ± 0.22	0.00	7.59	15.4	7.59
5	5.00 ± 0.01	4.64 ± 0.43	4.42 ± 0.06	0.00	7.18	11.7	7.18

Table 5. Biomass gain by MG-07 in the different media

Initial oil concentration	Mycelia dry weight (g/L)			Biomass gain (%)	
(%)	SO	SOG	SOGT	SOG	SOGT
1	0.50 ± 0.50	3.50 ± 0.87	4.83 ± 0.29	600	867
2	1.33 ± 0.29	3.00 ± 0.50	4.17 ± 0.76	125	213
3	2.00 ± 0.29	2.33 ± 0.29	3.50 ± 0.00	17.0	75.0
4	1.83 ± 0.29	2.00 ± 0.50	3.00 ± 0.50	9.00	64.0
5	2.50 ± 0.76	2.67 ± 1.15	3.67 ± 0.58	7.00	47.0

3.5 Effects of the Incubation Time, Glucose Concentration, Tween 80 Concentration, and Salinity on Crude Oil Degradation

The results from the incubation time effect indicate a noticeable increase in the proportion of crude oil degraded by MG-07 as the incubation time extends (Fig. 3A). However, there was a decrease in degradation efficiency in week 6, followed by an increase again from week 7 until week 8. The highest level of biodegradation occurred at 8 weeks, resulting in a 22.2% decrease in crude oil concentration in the medium. Nevertheless, there were no significant differences ($p > 0.05$) observed in the MG-07 biomasses after incubation for 1–8 weeks (Fig. 3B), with the average growth of MG-07 ranging from 2.00 g/L to 3.33 g/L.

The addition of glucose increased both the biodegradation ability and biomass of MG-07 at 2 weeks of incubation (Fig. 3C and 3D). MG-07 exhibited growth in the medium containing 1 g/L of glucose, but no crude oil degradation was detected. When glucose was added at concentrations of 5 or 10 g/L, MG-07 reduced the crude oil concentration by 10.0%–13.4%. Furthermore, adding glucose at concentrations of 15 or 20 g/L enabled MG-07 to decrease the crude oil concentration by 33.1%. There was no significant difference in crude oil concentration reduction when glucose was added at concentrations of 15 or 20 g/L ($p > 0.05$). However, at glucose concentrations of 15 or 20 g/L, there was a significant decrease in crude oil concentration compared to a glucose concentration of 1–10 g/L ($p < 0.05$). As shown in Fig. 3D, the biomass in the medium increased with the rising glucose concentration, reaching its peak at 4.00 g/L when the glucose concentration was 20 g/L.

The addition of Tween 80 markedly increased ability of MG-07 to degrade crude oil, particularly at concentrations of 4% or 5%, where 87.0%–97.3% of the crude oil was removed (Fig. 3E). The addition of Tween 80 had no negative effect on MG-07 biomass; in fact, an increase in Tween 80 concentration corresponded to a marked increase in MG-07 biomass (Fig. 3F). Macroscopic observations revealed that, at high Tween 80 concentrations (4%–5%), MG-07 exhibited more extensive growth and a thicker appearance on the surface of the liquid medium compared to lower Tween 80 concentrations (1%–3%). MG-07 had limited growth in the medium without Tween 80 (control) or at a concentration of 0.5%. The biomass of MG-07 reached 6.67 g/L and 7.67 g/L at Tween 80 concentrations of 4% and 5%, respectively, while the MG-07 biomass in the control (with no Tween 80 present) was 1.67 g/L.

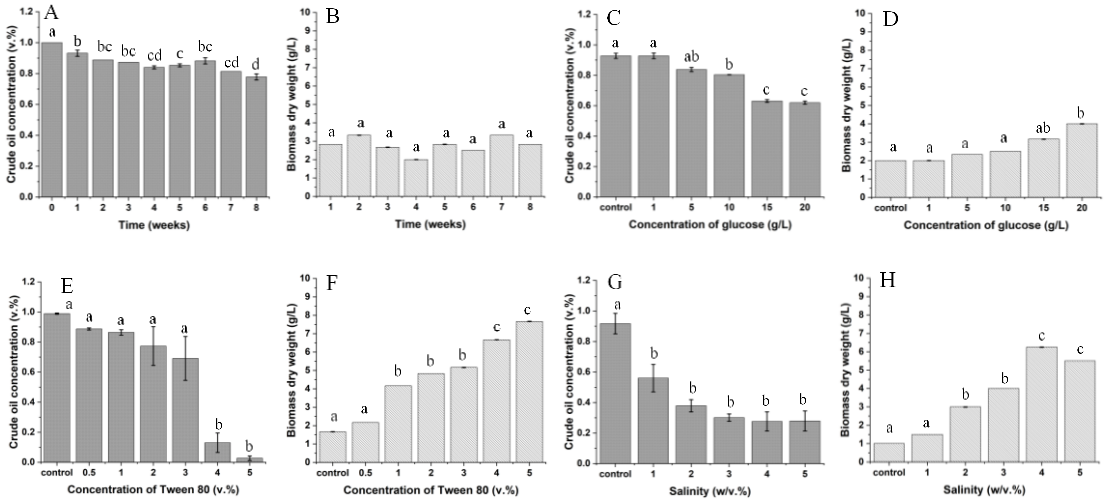

Fig. 3. Effects of (A and B) incubation time, (C and D) glucose concentration, (E and F) Tween 80 concentration, and (G and H) salinity on crude oil degradation by MG-07 and MG-07 biomass. Different lowercase letters above the bars indicate statistically different results

The results of crude oil degradation at various salinities are presented in Fig. 3G. The biodegradation efficiency remained consistent across salinities from 1% to 5%. No significant differences in biodegradation efficiencies were observed at various salinities ($p > 0.05$), with values ranging from 39.0% to 69.6%. This suggests that salinity did not significantly impact the biodegradation ability of MG-07. However, the biomass of MG-07 was influenced by salinity, as illustrated in Fig. 3H. MG-07 exhibited slow growth at a salinity of 1% (1.50 g/L) compared to salinities of 2%–5% (3.00–6.25 g/L) ($p < 0.05$). Mycelial growth of MG-07 increased with higher salinity levels. The mycelial mass was slightly lower at a salinity of 5% than at a salinity of 4%, although this difference was not statistically significant ($p > 0.05$).

4. DISCUSSION

Biodegradation by fungi is strongly affected by environmental conditions (e.g., the types and bioavailability of the pollutants present, salinity, nutrient concentrations, and competition with autochthonous microbes). Isolating and selecting potent indigenous fungal strains and re-introducing the strains into the area in which they originated can improve the adaptation and proliferation of useful strains [26]. This method offers more promise for degrading pollutants than by introducing externally obtained strains to a contaminated area [11]. MG-07 was isolated from decaying mangrove wood in East Kalimantan that had been contaminated in the past. This enables MG-07 to survive and adapt easily when applied to similar areas.

The isolation and screening of petroleum-degrading fungi are commonly carried out in a mineral salt medium. A study by Ameen et al. [15] reported that fungi isolated from marine environments, when incubated with diesel hydrocarbons for 4 weeks in a mineral salt medium, exhibited biomass accumulations of 43.4% for *Eupenicillium hirayamae* and 40% for *Cladosporium sphaerospermum*. MG-07 exhibited remarkable performance, producing a biomass increase of 633% after a 2-week incubation period. The growth rate of microorganisms during incubation with pollutants is an important indicator of the success of bioremediation. This signifies that these fungi can endure stressful environmental conditions and are capable of accumulating pollutants as a source of carbon and energy [1,27]. Previous studies have also revealed that fungi and bacteria capable of generating substantial biomass in a medium containing hydrocarbon pollutants exhibit a robust capacity to degrade crude oil and its derivatives.

MG-07 exhibited lower growth and degradation efficiency in COA compared to COB and COC, possibly attributable to the high metal compound content in COA (Table 1). Among the three oil samples, COC had the least metal content, and MG-07 exhibited good growth in this medium. Metals play a crucial role in supporting the metabolism of microorganisms; however, at high

concentrations, they can inhibit cellular functions [10]. Furthermore, the presence and speciation of metals influence the biodegradation of organic matter by microorganisms through both direct and indirect mechanisms.

The chromatogram showed that the degradation of normal aliphatic hydrocarbons (C_{14} to C_{30}) in COA, COB, and COC by MG-07 exhibits a preference for short chains over long chains. These results align with previous studies, confirming that some fungi can preferentially degrade shorter-chain aliphatic hydrocarbons, and the degradation proportion decreases with increasing carbon chain length [13,20]. As suggested by Pandolfo et al. [28], microorganisms prefer short chains over long chains, possibly because short chains are more soluble and bioavailable. Moreover, toxicity typically increases with the molecular weight of the hydrocarbon. Another contributing factor may be related to the presence of specific enzymes in petroleum-degrading microorganisms [4].

MG-07 was identified as *Trichoderma* sp., a genus of filamentous fungi within the *Ascomycota* division. *Trichoderma* is known to adapt to a wide range of environmental conditions in both marine and terrestrial environments [29]. An increasing number of studies on the bioremediation of toxic materials by *Trichoderma* have been conducted, given its resistance to persistent pollutants such as heavy metals, polycyclic aromatic hydrocarbons (PAHs), and pesticides [29,30]. However, the effectiveness of *Trichoderma* species in bioremediating crude oil in aquatic environments is limitedly studied [31], as these species are typically employed for bioremediation in soil [13,30].

Natural seawater was utilized to gain a better understanding of the ability of MG-07 to degrade crude oil, providing more relevant insights for its field application. MG-07 exhibited limited growth in the SO medium, probably due to its inability to utilize crude oil as a carbon and energy source because of low bioavailability. Consequently, metabolism is disrupted, and the secretion of secondary metabolites is hindered. As mentioned by Essabri et al. [9], one of the conditions that must be met for efficient pollutant biodegradation is the presence of sufficient nutrients to support the growth and enzyme production of fungi. Numerous studies have shown that *Trichoderma* sp. and other fungi rely on enzyme and secondary metabolite secretion for pollutant degradation [5,13,31].

The presence of glucose (SOG) eases the adaptation of MG-07 by utilizing glucose as a co-metabolic substrate for carbon and energy, supporting its growth [12]. Nazifa et al. [31] observed that adding glucose as a carbon source resulted in higher biodegradation efficiency in *Trichoderma reesei* compared to galactose and fructose. In the ocean, glucose availability, typically derived from phytoplankton, is a crucial factor for fungal growth, serving as the primary source of energy for eukaryotic microorganisms to carry out metabolic processes [32]. Hence, the findings of this study can serve as a basis for determining the nutrient concentrations that MG-07 can convert into glucose, thereby functioning as a potential energy source when applied in the field. This is because adequate nutrients play a crucial role in helping microorganisms adapt to petroleum-contaminated environments [2,10]. MG-07 requires at least 15 g/L to achieve maximum biodegradation efficiency in a natural seawater medium.

The presence of glucose and Tween 80 in SO (SOGT) increased the degradation performance of MG-07 by two times compared to SOG. This enhancement may be attributed to the presence of a simple carbon source (glucose) that facilitates co-metabolism, and simultaneously, an increase in carbon bioavailability due to the solubility of crude oil by Tween 80. Tween 80 is a nonionic synthetic compound, that enhances the mobility and bioavailability of crude oil by reducing surface and interfacial tension, significantly affecting the biodegradation performance of some fungi [16]. The increased solubilization of oil contaminants leads to greater availability and accessibility of hydrocarbons, consequently enhancing biodegradation activities through the affinity between the cellular membrane of fungi and hydrocarbon compounds [3].

Variations in Tween 80 concentration (0.5%–5%) revealed that higher concentrations led to enhanced crude oil degradation by MG-07. The findings of this study indicate that MG-07 requires a high surfactant concentration to achieve optimal performance in a natural seawater environment, but applying Tween 80 at concentrations of 4%-5% may be considered too high for field use. To make the bioremediation carried out by MG-07 more environmentally friendly in the future, the application of co-culture between fungi and biosurfactant-producing bacteria appears very promising [33].

The growth and biodegradation efficiency of MG-07 continuously decreased as the crude oil concentration increased from 1% to 5%, consistent with previous studies demonstrating that higher crude oil concentrations reduce fungal degradation ability [1,16]. High concentrations of hydrocarbons lead to limited microbial growth over time, primarily due to increasing levels of toxic compounds, such as naphthalene [2]. These elevated hydrocarbon levels also result in decreased microbial biodegradation activity, attributed to restricted nutrition and aeration.

Degradation by some species of *Trichoderma* was influenced by incubation time. As observed, the incubation time in MG-07 was similar to that of *T*. *viride* [9]. In *T*. *viride*, the proportion of degraded crude oil increased over time (~2–8 weeks), but biomass accumulation began to decrease after 4 weeks. On the other hand, biomass accumulation in MG-07 remained constant. In contrast, *T*. *atroviride* rapidly degraded crude oil during the first 2 weeks, but its biodegradation efficiency started to decline thereafter [13]. Different species appear to exhibit characteristics related to the time needed for degrading crude oil.

Salinity plays a crucial role in fungal growth and crude oil biodegradation, as high salinity can inhibit metabolic activity by inducing osmotic stress and cell lysis [3,4]. We observed that the growth of MG-07 was lower in the absence of seawater compared to its presence. Similarly, biodegradation of crude oil occurred in the medium with seawater. These findings indicate that MG-07 performs better in the presence of seawater than in non-saline environments. Fungal consortia were tested for their ability to biodegrade crude oil in salinities ranging from 2% to 8%, and the biodegradation efficiency increased with rising salinity up to 4% but decreased with a further increase in salinity [16]. MG-07 could grow at a salinity of 5% without losing its ability to degrade crude oil, exceeding the typical seawater salinity of around 3.5%. MG-07 was a strain sourced from the marine environment, which enabled it to survive and adapt to high salinity.

4. CONCLUSION

MG-07 was successfully isolated and demonstrated significant potential for degrading crude oil in saline environments. MG-07 showed significantly higher growth than other isolates when cultured in a medium containing crude oil, degrading ~80% of the aliphatic hydrocarbons within two weeks. Additionally, MG-07 demonstrated robust growth in natural seawater supplemented with glucose and Tween 80, achieving optimal growth and biodegradation efficiency at minimum concentrations of 15 g/L for glucose and 4% for Tween 80. Extended incubation times further enhanced the crude oil degradation effectiveness of MG-07. Unlike its lack of growth or crude oil degradation in non-saline conditions, MG-07 thrived and efficiently degraded crude oil in media with salinity levels from 1% to 5%. These findings may offer valuable insights for applying fungi in marine oil spill remediation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Maamar A, Lucchesi M-E, Debaets S, van Long NN, Quemener M, Coton E, et al. Highlighting the crude oil bioremediation potential of marine fungi isolated from the port of Oran (Algeria). Diversity. 2020; 12(5):196.
Available:https://doi.org/10.3390/d12050196.
2. Hazaimeh MD, Ahmed ES. Bioremediation perspectives and progress in petroleum pollution in the marine environment: A review. Environ. Sci. Pollut. Res. 2021;28: 54238-54259.
Available:https://doi.org/10.1007/s11356-021-15598-4
3. Daâssi D, Almaghribi FQ. Petroleum-contaminated soil: Environmental occurrence and remediation strategies. 3 Biotech. 2022;12:139.
Available:https://doi.org/10.1007/s13205-022-03198-z.
4. Cunyan X, Qaria MA, Qi X, Daochen Z. The role of microorganisms in petroleum degradation: Current development and prospect. Sci. Total Environ. 2023;865: 161112.
Available:https://doi.org/10.1016/j.scitotenv.2022.161112
5. Barnes NM, Damare SR, Bhatawadekar VC, Garg A, Lotlikar NP. Degradation of crude oil-associated polycyclic aromatic hydrocarbons by marine-derived fungi. 3 Biotech. 2023;13:335.

Available:https://doi.org/10.1007/s13205-023-03753-2

6. Setiani P, Ramdani F. Oil spill mapping using multi-sensor sentinel data in Balikpapan Bay, Indonesia. 4th International Symposium on Geoinformatics; 2018.
Available:https://ieeexplore.ieee.org/abstract/document/8612057
7. Stewart P, Groth CP, Huynh TB, Ng MG, Pratt GC, Arnold SF et al. Assessing exposures from the Deepwater Horizon oil spill response and clean-up. Ann. Work Expo. Health. 2022;66:i3-i22.
Available:https://doi.org/10.1093/annweh/wxab107
8. Virués-Segovia JR, Muñoz-Mira S, Durán-Patrón R, Aleu J. Marine-derived fungi as biocatalysts. Frontiers in Microbiology. 2023;14:1125639.
Available:https://doi.org/10.3389/fmicb.2023.1125639
9. Essabri AMA, Aydinlik NP, Williams NE. Bioaugmentation and Biostimulation of total petroleum hydrocarbon degradation in a petroleum-contaminated soil with fungi isolated from olive oil effluent. Water Air Soil Pollut. 2019;230:76.
Available:https://doi.org/10.1007/s11270-019-4127-8
10. Bala S, Garg D, Thirumalesh BV, Sharma M, Sridhar K, Inbaraj BS, et al. Recent strategies for bioremediation of emerging pollutants: A review for a green and sustainable environment. Toxics. 2022;10:484.
Available:https://doi.org/10.3390/toxics10080484.
11. Chuah LF, Chew KW, Bokhari A, Mubashir M, Show PL. Biodegradation of crude oil in seawater by using a consortium of symbiotic bacteria. Environ. Res. 2022;213:113721.
Available:https://doi.org/10.1016/j.envres.2022.113721
12. Li N, Xia Y, He X, Li W, Yuan L, Wu X et al. Glucose addition enhanced the advanced treatment of coking wastewater. Water: 2021;13(23): 3365.
Available:https://doi.org/10.3390/w13233365
13. Khandelwal A, Singh SB, Sharma A, Nain L, Varghese E, Singh N. Effect of surfactant on degradation of *Aspergillus* sp. and *Trichoderma* sp. mediated crude oil. Int. J. Environ. Anal. Chem. 2021;103(7):1667-1680.
Available:https://doi.org/10.1080/03067319.2021.1879800.
14. Ling H, Hou J, Du M, Zhang Y, Liu W, Christie P et al. Surfactant-enhanced bioremediation of petroleum-contaminated soil and microbial community response: A field study. Chemosphere. 2023;322:138225.
Available:https://doi.org/10.1016/j.chemosphere.2023.138225.
15. Ameen F, Moslem M, Hadi S, Al-Sabri AE. Biodegradation of diesel fuel hydrocarbons by mangrove fungi from Red Sea Coast of Saudi Arabia. Saudi J. Biol. Sci. 2016;23:211-218.
Available:https://doi.org/10.1016/j.sjbs.2015.04.005.
16. Abd El-Aziz ARM, Al-Othman MR, Hisham SM, Shehata SM. Evaluation of crude oil biodegradation using mixed fungal cultures. Plos One. 2021;26:1-19.
Available:https://doi.org/10.1371/journal.pone.0256376
17. Jain S, Choudhary DK, Varma A. Ecological Perspective of halophilic fungi and their role in bioremediation. In Parray JA, Mahmoud AHAE, Sayyed R (Eds) Soil Bioremediation: An approach towards sustainable technology. Wiley, USA. 2021;175-192.
Available:https://doi.org/10.1002/9781119547976.ch8
18. Susmiyati HR, Harjanti W, Al Hidayah R. Development of the Nusantara capital, sustainable extraction for East Kalimantan. In Warsilah H, Mulyani L, Nasution IK, Editors. Assembling Nusantara: Mimicry, friction, and resonance in the new capital development. Springer, Singapore. 2023;151-164.
Available:https://doi.org/10.1007/978-981-99-3533-8_11.
19. Afrida S, Tamai Y, Watanabe T, Osaki M. Screening of white rot fungi for biobleaching of *Acacia* oxygen-delignified kraft pulp. World J. Microbiol. Biotechnol. 2009;25:639-64.
Available:https://doi.org/10.1007/s11274-008-9932-y
20. Yanto DHY, Tachibana S. Biodegradation of petroleum hydrocarbons by newly isolated *Pestalotiopsis* sp. NG007. Int. Biodeterior. Biodegradation. 2013;85:438-450.
Available:https://doi.org/10.1016/j.ibiod.2013.09.008

21. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR Protocols: A guide to methods and applications. Academic Press, Inc, New York. 1990;315-322.
22. Horisawa S, Iwamoto K. Identification and typing of strains of wood-rotting basidiomycetes by protein profiling using MALDI-TOF MS. BioTech. 2022;11(3): 30. Available:https://doi.org/10.3390/biotech11030030.
23. Tamura K, Stecher G, Kumar S. MEGA 11: Molecular evolutionary genetics analysis version 11. Mol. Biol. Evol. 2021;38(7): 3022-3027. Available:https://doi.org/10.1093/molbev/msab120.
24. Rahman KSM, Banat IM, Thahira J, Tha. Thayumanavan, Lakshmanaperumalsamy P. Bioremediation of gasoline contaminated soil by a bacterial consortium amended with poultry litter, coir pith and rhamnolipid biosurfactant. Bioresour. Technol. 2002;81(1):25-32. Available:https://doi.org/10.1016/S0960-8524(01)00105-5.
25. Lotfinasabasl S, Gunale VR, Rajurkar NS. Assessment of petroleum hydrocarbon degradation from soil and tarball by fungi. Bioscience Discovery. 2012;3(2): 186-192.
26. Ławniczak Ł, Woźniak-Karczewska M, Loibner AP, Heipieper HJ, Chrzanowski Ł. Microbial degradation of hydrocarbons-basic principles for bioremediation: A review. Molecules. 2020;25(4):856. Available:https://doi.org/10.3390/molecules25040856.
27. Muthukumar B, Surya S, Sivakumar K, Alsalhi MS, Rao TN, Devanesan S et al. Influence of bioaugmentation in crude oil by *Pseudomonas* species on the removal of total petroleum hydrocarbon. Chemosphere. 2023;310:136826. Available:https://doi.org/10.1016/j.chemosphere.2022.136826
28. Pandolfo E, Caracciolo AB, Rolando L. Recent advances in bacterial degradation of hydrocarbons. Water. 2023;15(2):375. Available:https://doi.org/10.3390/w15020375
29. Zafra G, Cortés-Espinosa DV. Biodegradation of polycyclic aromatic hydrocarbons by *Trichoderma* species: A mini review. Environ. Sci. Pollut. Res. 2015;22: 19426-19433. Available:https://doi.org/10.1007/s11356-015-5602-4
30. Afzal I, Sabir A, Sikandar S. *Trichoderma*: Biodiversity, abundance, and biotechnology applications. In Yadav AN, Editor. Recent trends in mycological research. Springer Nature, Switzerland. 2021;293-315. Available:https://doi.org/10.1007/978-3-030-60659-6_13
31. Nazifa TH, Bin Ahmad MA, Hadibarata T, Salmiati, Aris A. Bioremediation of diesel oil spill by filamentous fungus *Trichoderma reesei* H002 in aquatic environment. J. Int. Integr. Eng. 2018;10(9): 103-107. Available:https://doi.org/10.30880/ijie.2018.10.09.019
32. Fuentes M, Quiñones RA, Gutiérrez MH, Pantoja S. Effect of temperature and glucose concentration on the growth and respiration of fungal species isolated from a highly productive coastal upwelling ecosystem. Fungal Ecol. 2015;13:135-149. Available:https://doi.org/10.1016/j.funeco.2014.09.006.
33. Atakpa EO, Zhou H, Jiang L et al. Improved degradation of petroleum hydrocarbons by co-culture of fungi and biosurfactant-producing bacteria. Chemosphere. 2022;290:133337. Available:https://doi.org/10.1016/j.chemosphere.2021.133337

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/115286