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Assessment of Docosene Surfactant Mediated Crude Oil Remediation by Marine *Plectosphaerella cucumerina* Strain HBKB

Hasnaa E-B. Ghonam[1], Khouloud M. Barakat[1]*, Ahmed M. Gad[1]
[1]National Institute of Oceanography & Fisheries (NIOF), Egypt
***Corresponding Author: kh2m2@yahoo.com**

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ABSTRACT

The goal of the current work was to assess biosurfactant (BS) produced by marine fungal isolated from the contaminated site of El-max Bay, Egypt, for bioremediation of oil contaminated sea water. In this regard, the four biosurfactants assessment tests oil displacement (OD), emulsification index (E24), hemolysis tests, and surface tension (ST) were used to screen selected marine fungal isolates. By measuring 1.5cm oil displacement, E24 57%, hemolytic activity, and the highest surface tension 37.1mN/ m, *Plectosphaerella cucumerina* strain HBKB was considered as the most potent isolate for producing a highly active biosurfactant. To assess the factors influencing biosurfactant production from minimal salt medium (MSM), Plackett-Burman design was used to get the maximum biosurfactants yield. Out of the seven variables that were examined, three (NH_4NO_3, KH_2PO_4 and yeast extract) were conducted for response surface methodology (RSM). These key effective elements were tested as independent variables and the surface tension (Mn/ m^2) as a dependent variable. It was observed that the correlation coefficient (R^2) showed a good fitness with the ‘‘Adjustive R-Squared” (with deference less than 0.1), thus the experimental and predicted values of the model agreed. The 3D curves of the quadratic model indicated the interactions between both NH_4NO_3 (4g/ L) and yeast extract (0.8g/ L) concentrations increased the surface tension level by 32mN/ m^2. GC- MS was used to identify ethyl acetate biosurfactant extract. The largest greatest peak indicated the presence of the ionic surfactant fatty acid 1-Docosene (1-Nonadecene). This biosurfactant enhanced the bioremediation technique that is used to treat contaminated water containing petroleum oil residues. Moreover, the unsterilized- unamended supplied by 1-docosene extracted biosurfactant showed a higher oil remediation represented by E24 (55%), OD (2cm), and ST (34.5mN/ m^2). Therefore, the breakdown, deterioration, and remediation of contaminated oil from aquatic habitats could be accomplished by *P. cucumerina* strain HBKB that produce the docosene surfactant.

INTRODUCTION

Accidental oil leaks and major oil spills from recreational boating and untreated waste disposal factories polluted our ecosystems **(Ugboma, 2014)**. Biological remediation is an efficient, secure, and environmentally benign method to clean up this oil spill. Due to the long-time persistence of these hydrocarbons in the environment, oil biodegradation

revealed a restricted factor of low solubility of these hydrocarbons in water **(Wei *et al.*, 2005)**. The wide quest for environmentally friendly biosurfactants rather than their chemically equivalents is encouraged by a real need as surface-active chemicals from microbial origin **(Mnif *et al.*, 2015)**. In the fields of biotechnology and microbiology, marine microbial biosurfactants have also been considered unique **(Dhasayan *et al.*, 2015)**.

Many anamorphic ascomycetes produced asexual spores (conidia) on branched structures, are known to create biosurfactants **(da Silva et *al.*, 2021)**. They are isolated from areas that have been polluted by hydrocarbon, oil, and effluent (**Rodrigues *et al.*, 2014; Dos-Reis *et al.*, 2018)**. Glycolipid and lipopeptide are biosurfactants secreted by fungal strains throughout minerals media fermentations using olive oil as a carbon source represented by a higher emulsification activity **(Ferreira *et al.*, 2016; Gmoser *et al.*, 2017)**. Ascomycetes which produce biosurfactants are being reviewed **(Sanches *et al.*, 2021)**.

In this regard, *Penicillium*, *Aspergillus*, and *Trichoderma* were the more prevalent genera in the water samples obtained by **Bovio *et al.* (2017)**, and tested to grow on crude oil as the only source of carbon. In addition, the ability of four fungal strains (*Lulworthaiales* sp., *Trichoderma harzianum*, *Penicillium* sp., and *Aspergillus terreus*) to break down oil in liquid cultures was further evaluated **(Varjani & Upasani, 2017)**. Twelve filamentous marine fungal strains are grown on the Arabian light oil and identified as BS producing strains **(Maamar *et al.*, 2020)**. The biomineralization process was intensively studied using *Plectosphaerella cucumerina* **(Carles *et al.*, 2018; Pasquale *et al.*, 2019)**.

Carbon and nitrogen sources, different inorganic salts, and trace elements are some of the medium components that have a significant impact on the growth and accumulation of metabolic products by fungal growth **(Padmavathi, 2015)**. For this reason, medium optimization was a crucial requirement for the creation of BS. Due to its arduous nature, the factorial combination of medium optimization, which involves one variable at a time while maintaining the others at a fixed level, fails. Given their significant impact on growth rate, statistical approaches such as the Plackett-Burman design for nutrient modifications and culture conditions serve as helpful tools for nutrient screening. This helps to understand the interactions among the process parameters at different levels **(Swetha *et al.*, 2014)**. Recently, there has been a great deal of interest in the application of statistical experimental design in medium optimization **(Darwesh *et al.*, 2021)**. A number of publications have also been published in the literature detailing the use of these techniques for the synthesis of different biomolecules **(Zhou *et al.*, 2023)**.

In this study, different marine fungal strains were isolated from oil spill cleanup locations. According to their capacity to create biosurfactant, fungal isolates were screened. Novel marine *P. cucumerina* strain HBKB was recognized as the powerful isolate. Placket-Burmen design and RSM were used in this work to maximize the

production of biosurfactant. Finally, the effectiveness of biosurfactants in removing oil spills under various situations was assessed.

MATERIALS AND METHODS

1. Sampling, isolation, and purification

In order to isolate marine fungi able to produce biosurfactants, sediment samples were collected from El-max Bay in Egypt (latitude of 29˚ 47.1 to 29˚ 50.4 and longitude 31˚ 7.5 to 31˚ 9 N), which is believed to be contaminated by oil wastes from the nearby petroleum factories (Fig. 1). Under septic conditions, 1g of sediment samples was added to 30mL of sterile sea water. Unsettled samples were combined with 1mL of potato dextrose agar (PDA) medium using pour plat method. Then, the plates were incubated at 30°C for 2 weeks. A carbon source by mean 1% (v/ v) motor oil was supplied to mineral salt medium broth (MSM). Each 100mL of MSM was then inoculated with marine fungal isolates which they had been picked up and purified. The MSM composition, according to **Kiran *et al*. (2009)**, was as follows (g/ L): NH_4NO_3, 3.0; K_2HPO_4, 0.5; KH_2PO_4, 1.14; $FeSO_4.7H_2O$, 0.04; NaCl, 0.1; $MgSO_4.7H_2O$, 0.20; $CaCl_2$ and 0.5 of yeast extract. Incubation time was carried out at 30^{o}C, pH of 7, and 120rpm for 2 weeks.

Fig. 1. Map showing the sampling stations in sectors of El-max Bay

2. Screening of the biosurfactant producing fungi

A variety of screening approaches, such as oil displacement (OD), emulsification index (E24), hemolytic assay (HA), and surface tension (ST) test method were carried out and used to evaluate the capability of marine fungal isolates for biosurfactants production **(Astuti *et al*., 2019)**.

3. Identification of the most active biosurfactant-producing fungi

The most active fungus for biosurfactant synthesis was identified through macroscopic features and microscopical analysis, and the taxonomic arrangement identified in the 2nd Edition Identification of Fungi. To verify identification methods, molecular analysis of the collected microorganisms was performed. After the genetic material was extracted, purified, and sequenced finally with the DNA STAR SeqMan, the

18srRNA gene was amplified with the previously reported primers. The amplification's primers were ITS1 (**5`** TCCGTAGGTGAACCTGCGG **3`**), ITS4 (**5`** TCCTCCGCTTATTGATATGC **3`**) **(White *et al*., 1990)**. The resulting sequences were entered into the GenBank database. The sequence analyses were acquired by conducting a BLASTN similarity search on the web page (http://www.ncbi.nlm.nih.gov/BLAST), and the phylogenetic tree was built using the MEGA_X_10.1.6 program, through the maximum likelihood method.

4. Optimization of the biosurfactant production using experimental designs

The Plackett-Burman design based on the first order model was used to screen and evaluate the important media components that influence the biosurfactant production. All the experiments were carried out according to designed matrix (Table 1) using the equation: $Y= \beta o +\Sigma \beta i Xi$ (i= l-k), where, Y is the estimated target function-available biosurfactant production represented by the surface tension assay, βo is a model intercept/constant, and βi is the regression co-efficient. X is the independent variable and k is the number of variables **(Padmavathi, 2015)**. A group of nine tests were built for seven variables, including the medium ingredients and the culture conditions: (1) NH_4NO_3, (2) KH_2PO_4, (3) K_2HPO_4, (4) inoculum size, (5) pH, (6) temperature, and (7) yeast extract. The primary effect of each factor was evaluated, and the statistical t-values for the two tests were determined. Each variable used was assessed for significance using the student's t-test. The least squares method was used to find the regression coefficient. The interactions between the variables were not considered in this approach, though. Response surface methodology (RSM) is one statistical and mathematical optimization strategy that may be used to optimize the variables screened by Plackett- Burman design.

Table 1. Plackett- Burmen design for biosurfactant production by marine fungal strain

Design: 2(7-4) design**							
Run	**NH_4NO_3**	**KH_2PO_4**	**K_2HPO_4**	**Yeast extract**	**pH**	**Temperature**	**Inoculum size (mL)**
1	4	2.28	1	1	6	30	2
2	4	2.28	0.25	1	5	25	0.5
3	4	0.57	1	0.25	6	25	0.5
4	4	0.57	0.25	0.25	5	30	2
5	2	2.28	1	0.25	5	30	0.5
6	2	2.28	0.25	0.25	6	25	2
7	2	0.57	1	1	5	25	2
8	2	0.57	0.25	1	6	30	0.5
9 (cont.)	3	1.14	0.5	0.5	7	35	1

5. Design of response surface methodology

Three elements from the previous design with the highest main effect were used for a central composite design (CCD) of RSM in order to produce the best biosurfactant production using this approach **(Isaie & Padmavathi, 2015)**. The three elements were considered as independent variables, and the surface tension was considered as a dependent variable (Table 2). The media components selected for the production of biosurfactant include in g/ L: NH_4NO_3, K_2HPO_4, and yeast extract.

6. Production, extraction, and purification of biosurfactants

One liter of the improved MSM medium was used to cultivate the most potent strain HBKB. Filtration was used to remove the fungal biomass at room temperature. Ethyl acetate was employed to extract lipopeptides from the entire volume of the supernatant. The culture supernatant, ethyl acetate and NaCl (30g/ L) were combined at a volume ratio of 1: 1.1, followed by a magnetic mixer for two hours. In a rotary evaporator, the ethyl acetate fraction was recovered and fully dried, then re-suspended in methanol, and filtered using a 0.45m Durapore filter from Milipore (Billerica, USA).

Table 2. Experimental design and results of the central composite design

Run	X1	NH_4NO_3 (g/ L)	X2	KH_2PO_4 (g/ L)	X3	Yeast extract (g/ L)
1	-1	2	-1	1.14	0	0.5
2	-1	2	0	0.57	0	0.5
3	-1	2	+1	2.28	0	0.5
4	0	3	-1	1.14	0	0.5
5	0	3	0	0.57	0	0.5
6	0	3	+1	2.28	0	0.5
7	+1	4	-1	1.14	0	0.5
8	+1	4	0	0.57	0	0.5
9	+1	4	+1	2.28	0	0.5
10	-1	2	-1	1.14	-1	0.25
11	-1	2	0	0.57	-1	0.25
12	-1	2	+1	2.28	-1	0.25
13	0	3	-1	1.14	-1	0.25
14	0	3	0	0.57	-1	0.25
15	0	3	+1	2.28	-1	0.25
16	+1	4	-1	1.14	-1	0.25
17	+1	4	0	0.57	-1	0.25
18	+1	4	+1	2.28	-1	0.25
19	-1	2	-1	1.14	+1	1
20	-1	2	0	0.57	+1	1
21	-1	2	+1	2.28	+1	1
22	0	3	-1	1.14	+1	1
23	0	3	0	0.57	+1	1
24	0	3	+1	2.28	+1	1
25	+1	4	-1	1.14	+1	1
26	+1	4	0	0.57	+1	1
27	+1	4	+1	2.28	+1	1

7. Gas chromatography–mass spectrometry (GC-MS) analysis

The chemical composition of the ethyl acetate extract was determined by the GC-MS (Thermo Scientific GC- TSQ mass spectrometer, Austin, USA). The column (TG-5MS 30m × 0.250mm × 0.250µm, Agilent, USA) was used as a separation column. The components were identified by comparing their mass spectra to those in the WILEY 9 and NIST 14 mass spectral databases **(Abd El-Kareem *et al*., 2016)**.

8. Bioremediation of contaminated wastewater

Samples of wastewater-containing oil residues were employed in this section without being sterilized to explore how the acquired *P. cucumerina* strain HBKB interacted with the native microflora that naturally exists in the samples that were collected. Wastewater samples were kept in a flask without any additional nutrients. Nutrients (a medium ingredient) were placed in two flasks, either sterilized or not. *P. cucumerina* strain HBKB was inoculated in all treated flasks. The flasks were incubated at 30°C while being shaken (120rpm). The oil degradation was discovered and verified using an OD, E24%, and ST analysis was carried out.

9. Statistical analysis

The graphical and regression analysis of the tested data was performed using Microsoft Excel software, and the response surface and contour plots were examined using STATISTICA 10 (STA999K347150-W, USA).

RESULTS

Out of five selected marine fungal isolates, the most potent isolate 1 from station no.2 was based on its capacity to produce high biosurfactant activity using various screening techniques, including the emulsification index (E24), oil displacement test (OD), hemolytic activity (HA), and surface tension (mN/ m^2) after two weeks of incubation (Table 3). The highest E24% was noted to be 83%, followed by clear zone of 1.5cm oil considered as the highest oil displacement **(Fig. 2).** The isolates'-hemolytic activity was observed when they were streaked on blood agar plate media. Furthermore, potent fungal strain has the highest surface tension of 37.1mN/ m^2.

Table 3. Screening methods for biosurfactant production from the five marine fungal isolates

Isolate code	E24 (%)	ODT (cm)	HA (Haemolysis)	Surface tension(mN/ m)
1	83	1.5	++	37.1
2	67	1.2	+	38.3
3	19	0.3	N	50.1
4	55	0.7	N	41.4
5	53	0.6	N	44.2

N: Negative

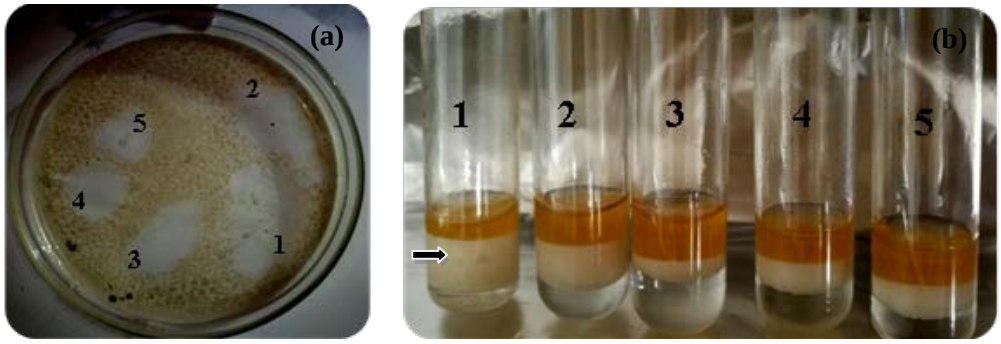

Fig. 2. (a) Oil displacement technique and **(b)** Emulsification test of the selected fungal isolates producing biosurfactant

1. Identification of the potent fungal isolate producing biosurfactant

The most potent biosurfactant producer was identified as a marine *P. cucumerina* strain HBKB. Using a light microscope, the colonies' morphology of this isolate was studied after 12 days at 30°C, the cultural characteristics of the colony on Sabouraud dextrose agar SDA reached 7072mm diameter, floccose, circumfer ence flat, whole margin white, reverse yellow (Fig. 3a, b). Mycelium is made up of 1.4µm wide hyphae that was laterally branched, septated, hyalined, smoothed, and thin-walled. Conidiophores are formed by unbranched submerged hyphae with cell walls that are typically thicker than those of vegetative hyphae. Phialides are thin, terminal and smooth-walled, 44µm long, 1.5µm wide at the base. Conidia are slimy heads with cylindrical, spherical ends that contain one-celled, hyalined, thin, smooth-walled conidia. Chlamydospores are smooth-walled, globuled with a truncate base (Fig. 3c, d).

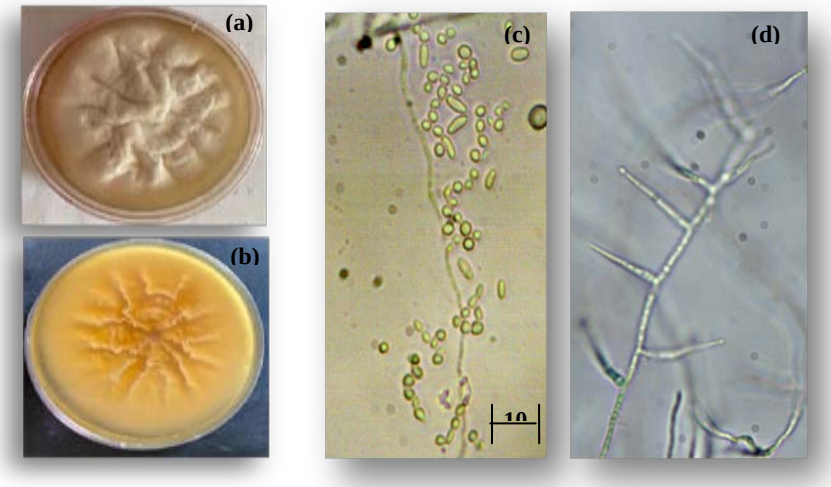

Fig. 3. *P. cucumerina* strain HBKB. Front and reverse of colony on **(a, b)** Sabouraud dextrose agar (SDA) after 12 days at 30°C, **(c)** Conidia and **(d)** Conidiophores

Additionally, molecular biology techniques were used to validate identification of the potent isolate. The 18srRNA amplicon was acquired after genomic DNA extraction, and then it was sequenced. The sequence was submitted to Gen-Bank with the accession

number OQ999399.1. The obtained sequence was 98% identical to *P. cucumerina* strain HBKB (Fig. 4). As shown, the neighbor joining approach was also used for the phylogenetic reconstruction.

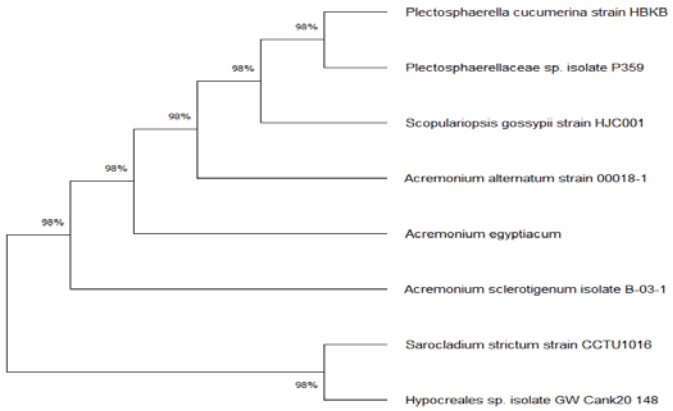

Fig. 4. *Plectosphaerella cucumerina* strain HBKB (OQ804634.1:1-547). Phylogenetic tree represented by 98% similarity

2. Placket-Burman experimental design and RSM for biosurfactant production

Plackett-Burman design was employed to identify significant factors among many representative components, and only the main effects were calculated. ANOVA was crucial to determine their significance and estimating their main effects. Surface tension was tested and used in this experimental research. The regression coefficients, *t*-values and *P*-values were estimated for each independent variable on biosurfactant production, as shown in Table (4). The results revealed that the presence of high levels of NH_4NO_3, K_2HPO_4, and yeast extract presented in the upper portion and then progressed down to the lower effect (Fig. 5).

Table 4. Regression coefficient results from Plackett-Burman data

Variable	Coefficient	Standard error	t -value	*P*-value	Note
Constant	34.473	14.029	2.457	0.246	NS
NH_4NO_3	2.338	1.949	1.199	0.442	Significant
KH_2PO_4	2.834	2.266	1.250	0.429	Significant
K_2HPO_4	-4.606	5.167	-0.891	0.537	NS
Yeast extract	4.727	5.167	0.915	0.528	Significant
pH	-0.394	0.975	-0.404	0.756	NS
Temp.	-0.103	0.258	-0.399	0.758	NS
Inoculum size (ml)	0.730	2.584	0.283	0.825	NS

NS: Non-significant.

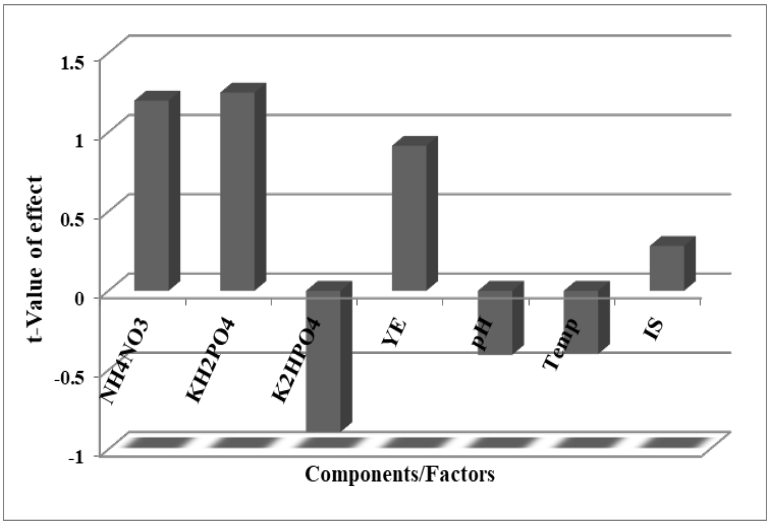

Fig. 5. Main effect for biosurfactant production by *P. cucumerina* strain HBKB using surface tension (mN/ m^2)

A central composite design was studied to show the interactions between these significant factors. It was also used to determine the optimal levels of these three factors. The equation explaining the relationship of the three variables for biosurfactant production is given below:

$$Y=532.683-245.8X_1-7.790X_2-3.029X_3+41.291X_1X_2-3.258X_1X_3+6.742X_2X_3+24.483X_1^2-0.032X_2^2+2.471X_3^2.$$

Where, X_1 is NH_4NO_3, X_2 is K_2HPO_4, and X_3 is yeast extract.

The results indicated a good agreement between the experimental and predicted values of the correlation coefficient (R^2) on biosurfactant production, thus the "Adjustive R-Squared" of 0.8531, related to R^2 0.9039, indicated a good fitness of the model. In order to ensure the highest levels of biosurfactant yield, the interaction effects of the three variables were used by plotting 3D surface curves against the surface tension independent variable while keeping other variable at their central (0) level. The 3D curves and contour plots of the quadratic model from the interactions between the three variables of the calculated response are shown in Fig. (6). It was observed that, at high NH_4NO_3 concentration, the surface tension increased with a decrease in KH_2PO_4 concentration, while the increase in both NH_4NO_3 (4g/ L) and yeast extract (0.8g/ L) concentrations were detected when surface tension increased. The increase in KH_2PO_4 levels with decreasing yeast extract concentrations showed a higher production of biosurfactant related to the surface tension reduction values (32mN/ m^2).

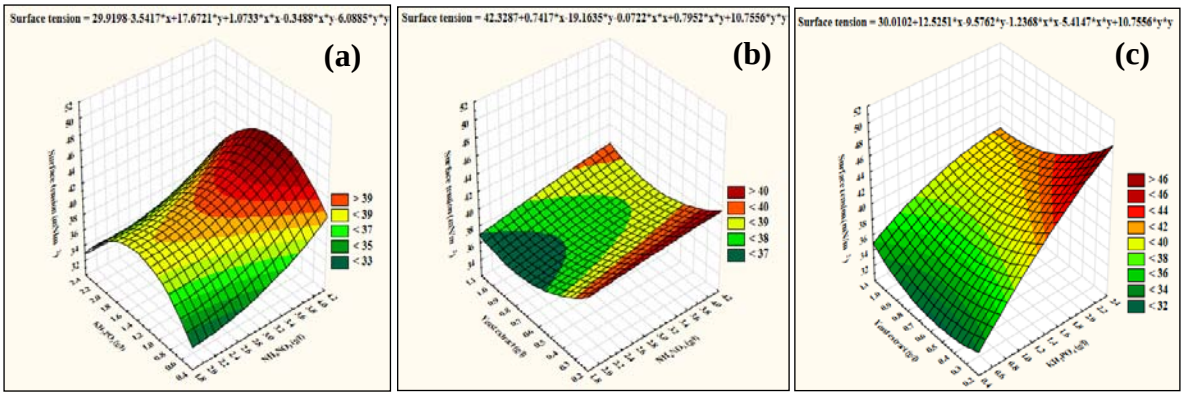

Fig. 6. Response surface methodology for maximum biosurfactant yield by *P. ucumerina* strain HBKB using surface tension (mN/ m^2) assessment test

3. Characterization of the active purified agents via GC- MS profile of biosurfactant

The agents of biosurfactant were produced with optimal conditions, followed by the extraction process implemented depending on ethyl acetate extraction. The extracted samples were examined by GC- MS technology to identify the compounds produced. The profile of GC-MS analyses revealed 21 compounds at various retention times (RT) (Fig. 7). The major highest peak with associated compounds were listed as 1-docosene and was recorded at 27.60min. The ionic surfactant fatty acid 1-docosene, also called 1-nonadecene, has a long alkyl tail $C_{22}H_{44}$ and a molecular weight of 308g mol^{-1}.

RT	Compound name	Area%	MF	Molecular formula	Molecular weight (g mol^{-1})
27.60	1-docosene	36.49	958	$C_{22}H_{44}$	308

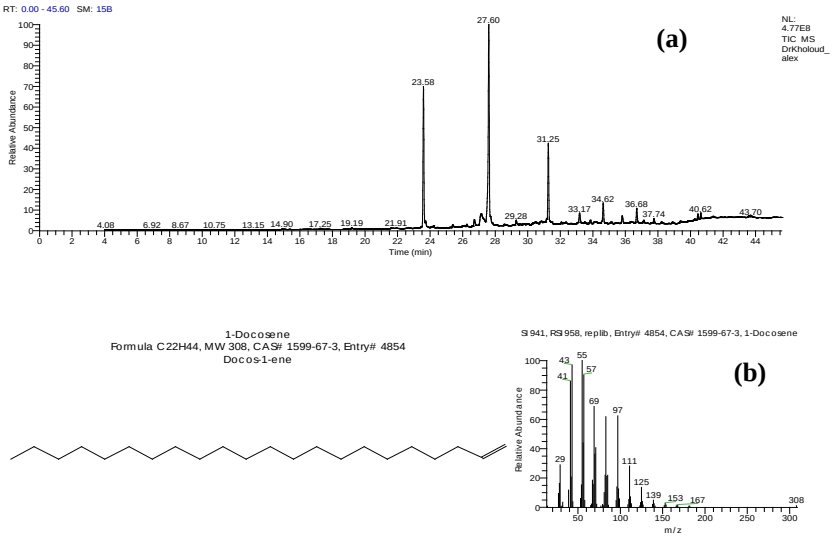

Fig. 7. GC- profile for the biosurfactants produced by **(a)** *P. cucumerina* strain HBKB and **(b)** MS of 1- docosene fatty acid profile

4. Bioremediation of oil spill contaminated water

Our study revealed four trials, in which 1- docosene was used to improve the microbial community of oil-contaminated wastewater. From Table (5), it was estimated that, 1- docosene exhibits optimal growth and biosurfactant production in unsterilized, unamended cultures, with E24 (%), ODT, and ST values reaching 55%, 2cm, and 34.5 mN/ m2, respectively.

Table 5. Petroleum oil bioremediation in polluted wastewater using ethyl acetate extractable 1- docosene

Culture condition	E24 (%)	ODT (cm)	Surface tension (mN/ m^2)
Unsterilized- unamended	55± 2.75	2± 0.1	34.5± 1.73
Unsterilized amended	45± 2.25	1.8± 0.09	40.3± 2.02
Sterilized unamended	20± 1	0.5± 0.025	47.2± 2.36
Sterilized amended	15± 0.75	0	53.1± 2.66

DISCUSSION

According to a previously published review by **Nikolova and Gutierrez (2020)**, 60- 70 percent of the petroleum's crude oils were discovered around oil tanks following predicted oil recovery methods. These oils were also dispersed near oil stations and water/ sewage parties and caused negative environmental issues. It is crucial to clean up polluted locations utilizing low-cost technology in order to make available non-traditional water resources and a cleaner environment. According to reports, microbial decomposition of oil-contaminated locations is a clean, low-cost solution. The primary obstacle to the widespread use of oil-degrading microorganisms is the inability to locate surface-active substances (biosurfactants) in contaminated areas **(Karlapudi *et al.*, 2018)**. In cases where different microorganism communities are confined in contaminated areas and enhanced by suitable conditions, the oil-degrading process is bioaugmented **(Ławniczak *et al.*, 2020)**.

Isolate 1 from station no.2 was selected based on its capacity to produce high biosurfactant activity, through oil degradation, using various screening techniques: E24 at 83% and oil displacement of 1.5cm. This method is employed since it is quick, simple, and requires little equipment and only a tiny amount of sample **(Antoniou *et al.*, 2015)**. In addition, selected fungal strain has the highest surface tension of 37.1mN/ m^2. According to **Karlapudi *et al.* (2018)**, reduction of the interfacial tensions, indicating that microbial surfactant can eliminate oil from contaminated locations. After 14 days of incubation, *Aspergillus niger* and *Rhodotorula* sp. produced biosurfactant, noted oil displacement and emulsification activity of 0.133 and 0.167cm, 48.067 and 51.133%, respectively **(Nweze *et al.*, 2021)**. According to **Piegza *et al.* (2021)**, the initial surface tension of three *Trichoderma citrinoviride* B3, HL, and C1 strains ranged from 32- 38mN/ m^2. **Menses *et***

al. **(2017)** isolated *Aureobasidium* sp. strain LB01 that is capable of producing biosurfactant from olive oil mill waste products, which can reduce the surface tensions by 31.20mN/ m^2. On the other hand, a single *Phoma* sp. strain was selected and examined in relation with biosurfactants production showed a surface tension of the post-culture extract 51.03mN/ m **(Lima *et al.*, 2016)**. According to the authors, a decrease in the potential below 50mN/ m qualifies microbes to undergo additional testing, whereas a level of 40mN/ m indicates a potential worth further investigation, and the most effective biosurfactants producers decrease surface tension to 30mN/ m or lesser **(Aparna *et al.*, 2012)**. The lower these values, the greater the bio-producer potential **(Darwesh *et al.*, 2021)**.

The statistical analysis of the main components of culture media was represented by Plackett-Burman design and ANOVA test to determine their significance and estimating their main effects indicating that the high levels of NH_4NO_3, K_2HPO_4, and yeast extract are presented in the upper portion. The effect of different medium components on the solubilization of phosphate by *Aspergillus niger* was determined using the Packett-Burman design. It was observed that glucose and ammonium sulphate had significant effect on phosphate solubilization **(Padmavathi, 2015)**. To optimize biosurfactant production by *Fusarium fujikuroi*, a Plackett-Burman design and a central composite rotational design assessed temperature (T), agitation (A), and incubation time (I) as the main effective variables which demonstrated statistical significance ($P< 0.1$) resulting for the surface tension reduction in fungal culture medium **(Dos Reis *et al.*, 2018)**. A recent report by **El-Shahed *et al.* (2022)** showed the Placket–Burman design of biosurfactant-producing *Candida* strain with a maximum production of biosurfactant obtained under optimal conditions of culture medium supplemented with 30g/ L of carbon source and 1.5g/ L of nitrogen source, and incubation at 42°C for 15 days.

The 3D surface curves were plotted against the surface tension independent variable, indicating the highest levels of biosurfactant yield using the interaction effects of the three variables. At a high NH_4NO_3 concentration, the surface tension increased with the decrease in KH_2PO_4 concentration, while the increase in both NH_4NO_3 (4g/ L) and yeast extract (0.8g/ L) concentrations were detected when surface tension increased. Moreover, a gradual increase of KH_2PO_4 levels with decreasing yeast extract concentrations showed surface tension reduction values of 32mN/ m^2. In the main concept, *Fusaruim fujikuroi* reduced surface tension from 72 to 20smN/ m^2 under the optimized conditions of pH 5.0, 37°C, and 7 days of incubation with 190rpm agitation **(Dos Reis *et al.*, 2018)**. The analysis from RSM revealed that the optimum values for the tested variables were glucose at 2g/ 50mL, ammonium sulphate at 0.2g/ 50mL, and tricalcium phosphate at 1g/ 50mL. Phosphate solubilization of 3.64mg/ mL was observed as comparison to original level of 1.88mg/ mL, which a 1.93-fold increase was obtained **(Padmavathi, 2015)**.

Using GC-MS analyses, the major highest peak was identified as 1- docosene and was recorded at 27.60min considering ionic surfactant fatty acid. *Pichia sorbitophila* WG1 yeast that produces glycolipid biosurfactants was structurally characterized by GC- MS **(Bhatia & Saharan, 2016)**. The characterization of the sophorolipid biosurfactant was primarily focused on a novel yeast strain called *Rhodotorula babjevae* YS3 **(Sen *et al*., 2017)**. According to GC- MS analysis, the biosurfactant that causes *Candida parapsilosis* contains a molecule of the 13- docosenamide type with a molecular weight of 337.5g/ mol **(Garg *et al*., 2018)**. Docosene was considered as effective bioactive compounds created by many marine macro and micro-organisms **(Dhevika & Balaraman, 2018; Hassan & Shobier, 2018; Albrattya *et al*., 2021)**.

Four trials were conducted using 1- docosene extracted material to enhance the microbial community of oil-contaminated wastewater. Among these trials, unsterilized, unamended cultures exhibited the highest values for E24 (%), ODT, and ST, reaching 55%, 2cm, and 34.5mN/ m2, respectively. The active fungi biomass established with a biological augmentation induced changes in the microbial populations, leading to a selective and sustained growth and biological degradation activity, which clarifies the success of the experimentation method used in the current research, even though the culturable isolated strain may represent the majority of active fungal diversity **(Medaura *et al*., 2021)**. Similar, earlier bioaugmentation researches studied native, saprophytic ascomycetes with a lengthy history of decontamination since they were thought to have the highest chance of surviving and metabolizing hydrocarbons **(Atagana *et al*., 2006; Mancera-López *et al*., 2008; Fayeulle *et al*., 2019)**.

CONCLUSION

Five marine fungal isolates were subjected for biosurfactants screening methods, where *P. cucumerina* strain HBKB was chosen, utilizing qualitative and quantitative experimentations, as a highly active biosurfactant producer. Plackett-Burman design was applied to assess the factors influencing the biosurfactant synthesis utilizing minimal salt medium (MSM). Three of the seven variables were studied using the response surface methodology (RSM) method, which offered the highest level of biosurfactant output. An ethyl acetate-based biosurfactant extract was identified using GC- MS. Oil-contaminated locations can be cleaned up using *P. cucumerina* strain HBKB extractable matter1(-decanoic acid). Further study into the determined biosurfactant from *P. cucumerina* strain HBKB is possible, especially in strongly suggested areas for biological control of pests and antibacterial activities.

CONFLICT OF INTEREST DISCLOSURE

The above-mentioned manuscript has not been published before and is not under consideration for publication anywhere else. The publication of this article was approved by all authors, as well as by the responsible authorities.

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