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Mycodegradation of Crude Petroleum Oil by Locally Marine *Candida tropicalis*

Khouloud M. I. Barakat[1*]

[1]Microbiological Laboratory, Marine Environment Division, National Institute of Oceanography and Fisheries (NIOF), Kayet Bay, El-Anfoushy, Alexandria 21556, Egypt.

Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Aims: A great demand for crude oil as a source of energy has resulted in an increase in its production, which in turn a gross pollution of the environment. In this study, isolation of local marine yeast with a high potency in petroleum oil biodegradation was the target.
Place and Duration of Study: Samples were collected from oil-spilled seawater in Shalateen, Red Sea, Egypt.
Methodology: Redox indicator 2, 6-dichlorophenolindophenol (DCPIP) and oil extract dichloromethane were used for biodegradable crude oil verification. GC-MS analysis of the residual degraded oil was carried out during the biodegradation process. Biotoxicity of residual oil extracts was performed at interval time (0, 6th and 12th day). Bioaugmentation process was accomplished to imitate application on polluted site and determine biodegradable oil activities for both amended and non- amended polluted water samples (either under sterile or non-sterile conditions).
Results: Local marine *Candida tropicalis* strain was isolated and identified using partial sequence of 18sRNA gene. *C. tropicalis was* significantly ($p < 0.05$) capable to remove 98.63±0.73% crude oil (50% v/v) after 12 days of incubation at 25°C. Using GC-MS analysis, marine yeast isolate was capable of degrading the aliphatic fractions in crude oil, where almost hydrocarbons peaks were

**Corresponding author: E-mail: kh2m2@yahoo.com;*

consumed during the degradation process. Using *Artemia salina* as a biomarker to test the toxic effect of residual oil extracts at interval time (0, 6th and 12th day), compared with the blank and control test, revealed that no-toxic intermediates or end products were detected. Non-sterile non-amended culture significantly ($p < 0.05$) showed 94.23±0.93% oil removal followed by non-sterile amended culture 65.5±7.7%.

Conclusion: These data demonstrated that the prospect in protecting the environment from oil pollution achieves after the application of marine *C. tropicalis* isolate.

Keywords: Petroleum oil; Candida tropicalis; biodegradation; chemical analysis; biotoxicity; bioaugmentation.

1. INTRODUCTION

Oil pollution resulting from petrochemical plants, petroleum production refinery and transportation has posed a great hazard and disturbances in components of marine ecosystems during the seepage of oily materials [1]. For this reason, there is a great awareness to manage water oily hydrocarbon pollution in marine environment [2]. The traditional physical treatments of oily wastewater using floating booms, adsorption by natural or synthetic materials, etc., cannot effectively resolve this problem [3]. Nature's recycler microbes that have the ability to utilize hydrocarbons as sole carbon sources and energy for metabolic activities, are used to clean up the environment considered as one of the safe, effective and inexpensive biological methods for oil contamination [4,5].

In the aquatic ecosystems, microorganisms specially fungi have a higher tolerance to the toxicity of hydrocarbons due to their physiology and adaptation to such variations in the environment and have the mechanism for the elimination of spilled oil [6] and production of environmentally safe by products [7]. Although, there are modest information that yeasts is better crude oil degraders than bacteria [8], many researchers studied the importance of yeast in biodegradation of pollutants, where, the most common species which have been recorded as oil bio-degraders belongs to: *Candida tropicalis*, *Candida albicans*, *Yarrowia lipolytica* and *Debaryomyces hansenii* [9,10,11,12,13]. The alkane-utilizing yeast degrades very efficiently hydrophobic substrates such as triglycerides, alkanes, and fatty acids [14]. In addition, from a view to develop environmental applications, researches were focused on the factors affecting petroleum oil biodegradation rates by yeasts including: nutrients, physical state of the oil, temperature and time consumed for bioremediation [15,12,16].

The aim of the present study is to isolate crude oil - degrading yeast from oil polluted sites. This is followed by examination of the biodegradable activity of the potent marine yeast strain to explore the factors affecting the crude oil biodegradation. Toxicity level of the safe by-products obtained as result of the biodegradation was determined. Bioaugmentation process was carried out to simulate natural conditions of an environmental friendly way for cleaning up oil-polluted areas using marine yeast strain.

2. METHODOLOGY

2.1 Yeast Strain and Growth Conditions

Marine yeast was isolated from marine sediment samples (5 m depth) collected from Shalateen, Red Sea, Egypt (Lat.: 23.153611; Long.: 35.611944; Salinity: 39.29 ‰; pH: 8.12), that received many industrial waste effluents containing petroleum oily compounds. Five hundred µL of water samples were applied on filtered sea water nutrient agar using pour plate method. One percent (v/v) of the sterile filtered crude oil was added as the sole carbon source. Plates were incubated for a period of 5 to 10 days at 30°C. White creamy flat colony of yeast isolate with a distinctive odor which produced clear zone of crude oil around colony was picked up and purified. Stock culture of the purified colony was maintained at 4°C on potato dextrose agar (PDA) (Cat.1261.00, Conda S.A., Spain) until use for the biodegradation liquid medium.

2.2 Identification of the Marine Yeast Isolate

DNA extraction was carried out using a protocol of GeneJet genomic DNA purification Kit (Thermo) followed by PCR analysis using Maxima Hot Start PCR Master Mix (Thermo). The PCR mixture contained 25 µl Master Mix (2X), 1 µl (20 µM) of each primer, 5 µl of

Template DNA and 18 µl Water, nuclease-free in 50 µl of polymerase buffer as total volume. The PCR thermocycler (Eppendorf) was programmed as follow: 95°C for 10 min for initial denaturation (one cycle), 35 cycles at 95°C for 30 s (annealing), 65°C for 1 min and 72°C for 1.5 min and a final extension at 72°C for 10 min (one cycle). Four microliters of the obtained PCR product were analyzed on 1% agarose gel electrophoresis against 1Kb plus ladder (Fermentas) [17] and were visualized on UV transilluminator. PCR clean-up was carried out using GeneJET™ PCR Purification Kit (Thermo). Finally, PCR product sequencing on GATC Company using ABI 3730xl DNA sequencer applying forward and reverse universal primers [15].

18SF149:5'-GGAAGGG(G/A)TGTAT TATTAG-3'and
18SR701: 5'-GTAAAAGTCCTGGTTCCC-3',

To affiliate the yeast isolate, BLAST sequence analysis https://blast.ncbi.nlm.nih.gov/Blast.cgi was carried out.

2.3 Biodegradation Procedure

About 50 ml of potato dextrose broth (PDB) media was prepared in 250 ml Elementary flask. Oil biodegradation was verified using redox indicator 2, 6-dichlorophenolindophenol (DCPIP) [18]. Five days mother yeast culture was used to inoculate PDB containing 1% (v/v) crude oil and 0.008 mg/50 ml of redox indicator. The flasks were incubated at 30°C for 7 days and shaken at 120 rpm. The experiment was done in triplicates. Changing in color of inoculated media in the flasks from deep blue to colorless indicates the ability of fungus to degrade crude oil. Removal of color was measured spectrophotometry at 620 nm. Control flask without yeast inoculum was prepared under the same cultural conditions.

2.4 Residual Oil Extraction and Evaluation

For extraction of residual crude oil, yeast activities were stopped by adding 1% 1N HCl. Cultures supernatants were collected in separating funnel then dichloromethane organic solvent added and shaken carefully; this step repeated three times for fully extraction. Dichloromethane containing extracted oil was passed through anhydrous sodium sulphate to remove moisture. The solvent was vaporized overnight. This procedure was carried out under the same condition for the control flask [19]. The percentage degradation of the crude oil was then calculated gravimetrically described by Ijah and Ukpe [20].

> [(Weight of crude oil initial - Weight of crude oil after treatment) / Weight of crude oil initial]*100

2.5 Determination of Yeast Dry Weight

Yeast culture was harvested by centrifugation at 6000 g for 25 min. Harvested biomass was washed twice with 5 ml distilled water then dried at 80ºC to constant mass. Dry weight was estimated into g/L [21].

2.6 Optimum Biodegradable Conditions

The ability of the marine yeast isolate to utilize crude oil as the sole carbon source was firstly tested at different crude oil concentrations (10, 50, 100, 500, 1000, 2000, 5000 mg/L) using the previous biodegradation conditions. This is followed by testing the effect of different incubation times (2, 5, 10, 12, 14 days) using the optimum crude oil concentration. Finally, different incubation temperatures (4, 25, 37, 45, 60°C) were examined to determine the optimum conditions for oil biodegradation process. The biodegradation assays (redox indicator verification, percentage of the crude oil degradation and yeast dry weight) were carried out using 250 ml Erlenmeyer flasks containing 50 ml of the PDB medium and 10% of acclimated inoculum.

2.7 Analysis of Mycodegradation Product

The chemical analysis of the resulted degraded oil by-products from optimized conditions were carried out after dichloromethane extraction at 6^{th} and 12^{th} day of incubation using the initial crude petroleum oil as control. The aqueous phase sample was discarded and the crude oil containing solvent was concentrated to approximately 0.1 ml using a rotary evaporator under reduced pressure in a water bath. Afterwards, it was dissolved in equal volume of dichloromethane (1:1) and analyzed by GC/MS (Agilent Technologies 7890A GC System with a flame ionization detector, a 5975C inert XL MSD Triple-Axis Mass Detector and Agilent 19,091S-433 Trace Analysis column). GC-conditions began by injection volume of 1 µL, operating in

the split less mode with an evaporation temperature of 250°C, 1.8 bar, 2.5 mL / min. Helium carrier gas temperature was gradient 50°C/1 min, 40°C/min gradient 300°C/min, 300°C/5 min. The highest resolution chromatographic peaks were scanned to find their corresponding mass fragmentation profile. Compounds were characterized based on similarities between their mass spectrum and those of the authentic data presented by Wiley Library. Quantitative data were obtained by the peak normalization technique using integrated FID response.

2.8 Biotoxicity

The toxicity bioassay of the dichloromethane – extracts from the oil biodegradable products was evaluated using *Artemia salina* lethality test [22]. Encycted eggs of the brine shrimp were incubated in sterile brackish water and exposed to light, pH 8-9. After 48 h, ten *A. salina* nauplii were transferred to each set of vial containing: medium ingredients only (blank), uninoculated oil containing medium (control) and the inoculated medium, where the intermediates formed during the crude oil degradation process were collected at intervals time (0, 6^{th} and 12^{th} day). The number of the viable biomarker was counted after 24 h of application.

% Death = [(Test-Control)/10]*100

2.9 Bioaugmentation

Bioaugmentation refers to introduce the selected yeast isolate as wild type or genetically engineering microbial community in an effort to enhance its ability to oppose and respond the process fluctuations or degradation certain compounds resulting in improving treatment [23].

In this section of study, the Alexandria petroleum company discharged waste water samples were used to test the ability of marine yeast isolate for crude oil degradation in the presence of the indigenous microflora within this polluted environment. Four different examined flasks with amended and non- amended waste water samples (either sterile or non-sterile conditions) were prepared. Amended waste water medium was prepared by dissolving PDB medium instead of the filtered sea water and distributed into 250 ml Erlenmeyer flasks, then the medium was sterilized by autoclaving. This step was repeated without sterilization. In case of non-amended waste water, the prepared flasks were free from medium for both sterilized and non-sterilized waste water. Control flask contains filtered sea water PDB medium. The flasks were inoculated by 10% of acclimated and harvested biomass which was washed twice and poured slowly into the tested flasks under septic condition. The experiment was done in triplicates using 500 mg/L crude oil for all flasks then incubated under shaking conditions 120 rpm at 25°C. Depending on incubation times, removal of redox indicator color, the degradation and dry biomass were estimated.

2.10 Statistical Analysis

Data were statistically performed by analysis of variance using SPSS software (SPSS version 16.0, SPSS, Michigan Avenue, Chicago, IL, USA) and subjected to a one-way ANOVA. Duncan's multiple range test was used to compare differences between treatment means when significance F values, at $P<0.05$ level [24].

3. RESULTS AND DISCUSSION

Major drawback that has slowed the performance of microbial enhanced oil recovery, has been the difficulty in isolating microorganisms can survive the harsh environment of the oil reservoir [15]. Microorganisms isolated from environments with a history of pollution by oil petroleum hydrocarbons have higher ability to degrade such pollutants [25]. Therefore, in this study, marine yeast isolate pronounced crude petroleum oil biodegradation ability isolated from oil contaminated sites. This isolate was molecularly identified based on 18srRNA sequencing and found to be in a close relation to *Candida tropicalis* (accession number: EF392687.1) with a 95% identity.

3.1 Oil Biodegradation Behavior of *C. tropicalis*

Three steps of verification lead to the ability of marine isolate *C. tropicalis* in the biodegradation of crude oil mixture to its simple components: (1) changes in indicator color in culture media, (2) disappearance of crude oil from the medium and (3) developing a mass of fungal growth in the bottom of the culture medium (Fig. 1). *C. tropicalis* marine isolate has the ability to degrade crude oil by incorporating an electron acceptor of redox indicator (DCPIP) to the culture medium, it is possible to ascertain the ability of the marine yeast strain to utilize the substrate by

observing the color change of DCPIP from blue (oxidized) to colorless (reduced) [18]. This result agree with those obtained by Miranda et al. [26] who tested the biodegradation of diesel oil by yeasts isolated *Rhodotorula aurantiaca* and *Candida ernobii* using the same color change indicator. Also, the same method was studied by Al-Nasrawi [27] who observed the ability of some fungal isolates, associated with contaminated oil spill, for crude oil biodegradation using DCPIP redox indicators. A local marine yeast isolate C*. tropicalis from* polluted area of Abou-Qir gulf (Alexandria, Egypt), showed a high potency in petroleum oil biodegradation as well some hydrocarbons [15]. Based on the hydrocarbonoclastic property, two marine yeast potential strains were segregated as *Candida tropicalis* and *Pichia guilliermondii* isolated from Arabian Sea of Bengal [28]. The mechanism of hydrocarbons-degrading yeasts and substrate assimilation by two yeast strains, *Candida* and *Trichosporon*, isolated from industrial refinery waste water, has been studied by Gargouri *et al*. [12]. Recently, Benard and Tuah [16] were assessed the biodegradation of Sabah Light crude oil by locally isolated *C. tropicalis* in simulated seawater condition.

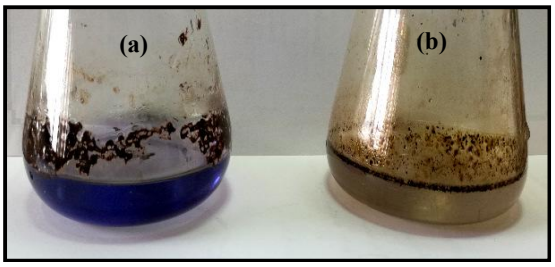

Fig. 1. Biodegradation ability of crude oil by marine yeast strain *C. tropicalis*, control (a) and test (b).

3.2 Optimized Conditions for Crude Oil Degradation by *C. tropicalis*

3.2.1 Degradation of different crude oil concentrations

To evaluate the degradation potency of *C. tropicalis* for crude oil, the PDB medium was amended with different concentrations ranging from 10 to 5000 mg/L. Results showed that removal efficiency of color indicator and crude oil degradation % was significantly (p < 0.05) decreased by increasing concentration till reached 5.93±3.30 and 22.60±13.00%, respectively, at 5000 mg/L crude oil, as well, a significant decrease in biomass. While, at the moderate concentration (500 mg/L), the strain able to degrade 79.83±3.62% after 7 days of incubation at 30°C (Table 1). Similar results presented by Ayu et al. [29] showed a general decreased trend of crude oil biodegradation % with an increased initial concentration till 5000 ppm using the white rot fungi *Polyporus sp*. S133. Among the tested concentrations, 500 mg/L was used for further experiments using *C. tropicalis* crude oil degradation improvement.

3.2.2 Degradation during different incubation times

The biodegradation efficiency of crude oil removal by *C. tropicalis* after 12 days of incubation was significantly (p < 0.05) exhibited the highest percentage 98.33±0.64 % and maximum dry weight 4.60±0.44 g/L using 500 mg/L total crude oil degradation at 30°C (Table 2). Biodegradation percentage has a linear relation with the microbial growth across the 12 days of incubation period. Sepahi et al. [30] suggested that the utilization of petroleum hydrocarbons as a sole carbon source by microbial strains is evident by the increase in the microbial density. The biodegradation assays with 12% of diesel oil was carried out using *C. ernobii* UFPEDA 862 over a period of 15 days [26]. Gargouri et al. [12] reported that *C. tropicalis* could efficiently degrade total petroleum hydrocarbon by 97% over a period of 20 days. Benard and Tuah [16] showed that *C. tropicalis RETL*-Cr1 able to remove only 40% crude oil after 4 weeks of incubation. Both *C. glabrata* and *C. krusei* showed convergent biodegradable value amounted 60 and 61%, respectively, after 7 days of biodegraded crude oil with maximum biomass 3.5 and 3.8 g/L [13]. At the end of 28 days, 49.29% of crude oil degradation by *Saccharomyces cerevisiae* was recorded [31].

3.2.3 Degradation using different incubation temperatures

C. tropicalis marine strain was screened for crude oil degradation using different temperatures at 500 mg/L crude oil for 12 days of incubation. Maximum degradation by *C. tropicalis* marine strain was 98% from 25-37°C. Temperature from 45 - 50°C showed a significant (p < 0.05) half degradation percentage, however, lower degradation % was observed in case of fridge (Table 3). Similarly, Kutty et al. [28] showed that marine *C. tropicalis* and *Pichia guilliermondii isolates* preferred 20 - 40°C for

their growth on hydrocarbon degradation medium. Flasks inoculated by *C. krusei* and *C. glabrata* were incubated in a temperature regulated chamber at 30°C for myco-degradation of crude oil [13]. Related works studied hydrocarbon and crude oil degradation by different yeast isolates within different temperatures [26,15,31].

3.3 Analysis of the Biodegradable Crude Oil

The biodegradable product(s) resulted from the previous optimized conditions were extracted and subjected for chemical analysis. The degradation of the alkane and aromatic hydrocarbons in crude oil by *C. tropicalis* was analyzed by gas chromatography - mass spectrometry (GC/MS). From Fig. 2, the marine yeast strain depicted efficient utilization of the crude oil compared to 0 time inoculated flask. Analysis by GC/MS revealed that *C. tropicalis* marine isolate was capable of degrading the aliphatic fractions, where all the detectable hydrocarbons peaks were completely utilized at 6th and 12th days compared with the initial crude oil (Fig. 3). Yeast strain isolate have the ability to degrade the entire range of long chain of n-alkanes (tridecane (C13) to nonadecane (C19)); some organosiliocon (Si-O-Si) and fatty acid traces (oleic (C18), docosanoic acids (C22)). The hydrocarbon utilization pattern of yeast strains indicated by a partially removal of some alkane's long chain (Nona-, hepta-, octa- and tridecane) after 1 week of degradation, however, fatty acid and organosilicons were found in small amount. The majority n- alkane (except hexadecane), fatty acid, and silicon organo-compounds were completely removed at the end of the oil bioremediation process (12th day). This observation may be explained by a previous study where fungi co-metabolically oxidize or mineralize some aliphatic compounds to less toxic products [32]. Due to the fact, a very efficient degradable enzyme system belongs to marine yeast strains to encourage their degradation process for short and long chain hydrocarbons discharged from the industrial waste water refinery plants [12]. Rather than aromatic or naphthenic compounds, the n-alkanes are the most biodegradable petroleum hydrocarbons and easily attacked by more microbial species. Regarding to the oxidation reaction of alkanes which was more profound than aromatic hydrocarbons [33].

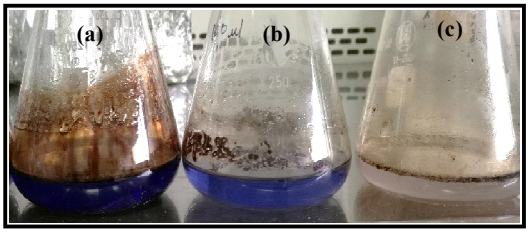

Fig. 2. Biodegradation efficiency of crude oil by *C. tropicalis* marine strain during 12 days of incubations; from left 0 time inoculated flask (a), after 6th day (b), after 12th day of degradation (c)

3.4 Biotoxicity of the By-products

The toxicity of the biodegrdable by-products against *A. salina* was obtained during the crude oil degradation process (Table 4). It was noticed that at zero time of incubation the crude oil showed a toxicity level of 79% mortality close to control (88%). After 6th day of crude oil degradation, the toxicity level was reduced to 47%. Further reduction in mortality (10%) was observed after the 12th day of degradation. This indicated that the degradation process yielded almost non-toxic intermediates or end products. Similarly, over a 1-hour period, a reduction of 63% survival in *Artemia* exposed to an untreated simulated oil spill (SOS), compared to natural conditions, while treated SOS reached approximately 80% of live *Artemia* at the end of one hour [34].

Table 1. Biodegradation of different crude oil concentrations by *C. tropicalis* after 7 days of incubation at 30°C

Crude oil concentrations (mg/L)	10	50	100	500	1000	2000	5000
Absorbance (% color removal)	90.90±0.32^{a}	89.03±0.90^{a}	75.50±5.30^{b}	78.60±1.44^{b}	50.03±1.60^{c}	44.90±5.20^{c}	5.93±3.30^{d}
% Oil degradation	94.63±2.11^{a}	89.00±0.90^{a}	82.60±2.50^{a}	79.83±3.62^{a}	37.60±9.60^{b}	32.20±16.41^{b}	22.60±13.00^{b}
Dry wt. (g l^{-1})	3.53±0.55^{a}	3.50±0.73^{a}	2.03±0.24^{b}	1.70±0.53b^{c}	0.50±0.10^{cd}	0.33±0.10^{d}	0.01±0.00^{d}

(Mean ± standard error)

Table 2. Effect of incubation times on 500 mg/L crude oil degradation by *C. tropicalis* at 30°C

Days	2	5	10	12	14
Absorbance (% removal of color)	29.70±10.90^{c}	61.33±11.60^{b}	85.53±0.83^{a}	98.50±0.66^{a}	100±0.00^{a}
% Oil degradation	22.50±6.94^{c}	82.50±2.00^{b}	91.73±1.33^{ab}	98.33±0.64^{a}	100±0.00^{a}
Dry wt. (g l^{-1})	0.03±0.00^{c}	2.83±0.50^{b}	3.53±0.55^{ab}	4.60±0.44^{a}	3.83±0.18^{ab}

(Mean ± standard error)

Table 3. Effect of different incubation temperatures on 500 mg/L crude oil degradation by *C. tropicalis* for 12 days of incubation

Temperatures (°C)	Fridge	25	37	45	60
Absorbance (% removal of color)	23.50±2.60^{c}	96.30±0.44^{a}	92.90±2.6^{a}	44.63±7.3^{b}	53.33±3.6^{b}
% Oil degradation	21.1±8.3^{c}	98.63±0.73^{a}	98.2±0.12^{a}	49.5±3.64^{b}	40.53±3.0^{b}
Dry wt. (g l^{-1})	0.13±0.10^{b}	3.20±0.30^{a}	3.53±0.54^{a}	0.60±0.04^{b}	0.38±0.02^{b}

(Mean ± standard error)

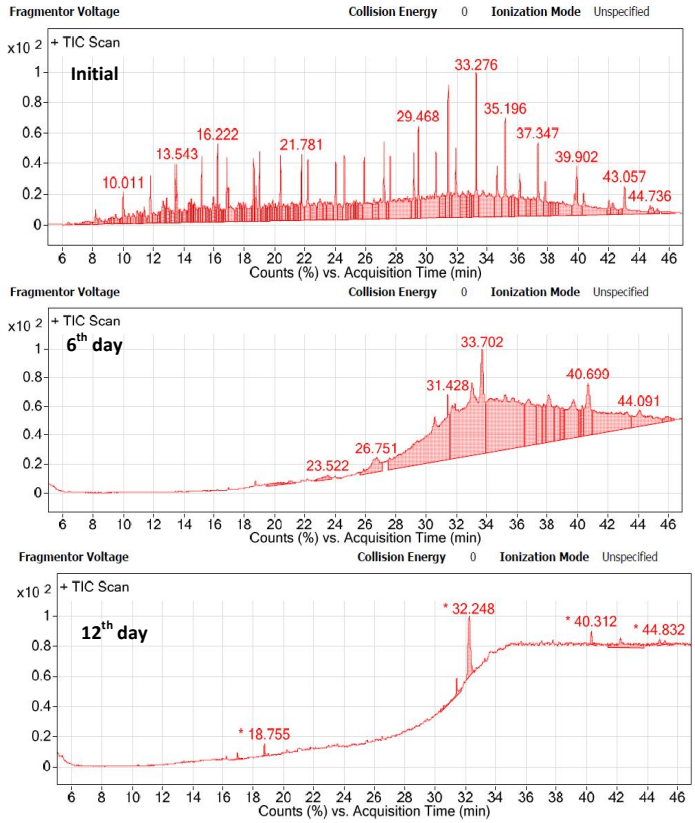

Fig. 3. GC/MS analysis shows the residual crude oil chromatogram at initial, 6th and 12th days using 500 mg/L crude oil at 25°C during the biodegradation process by marine *C. tropicalis*

Table 4. The biotoxicity test of the residual by-products at 0, 6th and 12th day during the degradation process of crude oil by *C. tropicalis* using *A. salina* as a biomarker

Intermediate sample at different interval times	Mortality (%) of *A. salina* after 24 hr.
Blank	3
Control	88
0 time	79
6th day	47
12th day	10

Table 5. Bioremediation of crude oil in contaminated water using *C. tropicalis* in amended and non-amended medium ingredients

Conditions	Control	Non-sterilized-non amended	Sterilized non amended	Sterilized amended	Non-sterilized-amended
Absorbance (% removal of color)	97.9±0.22[a]	96.2±0.5[a]	10.93±9.0[d]	56.43±4.8[c]	75.83±3.4[b]
% Oil degradation	98.3±0.6[a]	94.23±0.93[a]	10.3±7.0[d]	40.5±7.1[c]	65.5±7.7[b]
Dry wt. (g l^{-1})	3.53±0.2[a]	2.9±0.12[b]	0.004±0.01[d]	0.41±0.02[c]	0.6±0.04[c]

(Mean ± standard error)

3.5 Bioaugmentation of Crude Oil

The results obtained in the laboratory are scaled up to the natural environment for *C. tropicalis* behavior analysis in bioaugmentation experiment to determine how it may act in the natural environment for the bioremediation of hydrocarbon-polluted waste water. As recorded in Table 5, the bioaugmentation was achieved when non-sterilized-non amended conditions gave the highest significant ($p < 0.05$) degradation percentage 94.23±0.93% after 15 days of incubation, close to the control flask (98.3±0.6%), where this microbial consortium enhanced biodegradation process. While, lower degradation % was observed for both amended non-sterilized and amended sterilized media at maximum degradation ability 65.5±7.7% and 40.5±7.1 after 30 and 65 days of incubation, respectively. Least degradation % was constantly recorded using the sterilized non amended flasks (10.3±7.0%), concluding suppressed crude oil biodegradation. Sterilization process kill indigenous microflora that accelerate the desired biological processes and achieve more consistent results. Similarly, the bioaugmentation was carried out using *C. tropicalis* SK21 isolate from petroleum-contaminated soil to remove 61% of total petroleum hydrocarbons in association with the indigenous microbes [35]. Efficient bioaugmentation strategy for a soil heavily contaminated with crude oil conducted over 180 days inoculated by *Acremonium* sp. showed significantly removal of total petroleum hydrocarbons ranged from 60.1±2.0 to 74.2±2.7% considering enhancement of soil bioremediation [36]. Bioaugmentation of crude oil using efficient microcosms to simulate bioremediation treatments were previously discussed [37,38,39,40].

4. CONCLUSION

Using microbial remediation process is successful and safe way to enhance environmental health in particular with low cost, technique and high public acceptance to clean up aquatic ecosystems from oil spills. Thus, the data described in this study show that crude oil degradation efficiency of marine yeast *C. tropicalis* was 98% after 12 days of incubation at 25°C. Residual degraded oil showed that marine yeast isolate was capable of degrading the aliphatic fractions and produced non-toxic by-products. Application process by bioaugmentation was studied to mimic oil biodegradation on polluted sites. Higher potency of *C. tropicalis* to become the petroleum hydrocarbon degrader in the marine environment and a good candidate fungus in the bioremediation of crude oil contaminated sites because of its environmental friendliness.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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