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Study the ability of *Rhodotorula mucilaginosa* S6 to degradation hydrocarbon compounds

Sarah I Hayes, Badia A Malla Obaida*
Department of Biology, College of Science, University of Mosul, Iraq

Abstract
To reduce or stop the threats to the ecosystems, the local isolate *Rhodotorula mucilaginosa* S6 was exploited in the analysis of hydrocarbon compounds (both heavy and light crude oil as well as diesel fuel). The results of using Bushnel Haas Medium equipped with 15% hydrocarbon compounds showed a high ability of yeast to decompose and emulsify hydrocarbon compounds in a record time of 10-20 days for both types of crude oil and 7-10 days for biofuel (diesel). The layer of crude oil of both types, heavy, light, and diesel, starting from the fifth day of incubation, as indicated by the change of the color of the entire medium to the dark brown color of oil and honey of diesel, and this indicates the start of the emulsification process, and with the continuation of incubation for 15 days, it was observed that a ring appeared with light bubbles floating on the surface of the nutrient medium. The decomposition outputs using Gas chromatography–mass spectrometry (GC-MS) showed the most important chemical compounds resulting from the decomposition of crude oil. It was found that the heavy crude oil sample treated with yeast suspension consisted of 34 chemical compounds and the light crude oil sample treated in a yeast suspension consisting of 19 compounds, as for biofuels, the results showed that the treatment sample contained 47 compounds, and the compounds appeared at different retention times. The disappearance of some compounds and the appearance of different secondary metabolites in the treatment samples is due to the yeast's ability to analyze them into simple secondary compounds. They can use it as a carbon source of energy.

Keywords: *Rhodotorula mucilaginosa*, decomposition of crude, hydrocarbon compounds

Introduction
Crude oil is one of the most important resources in the world, especially for its important role in all aspects of industrial, economic, agricultural, and other life (Sari *et al*., 2019) [17]. It consists of a homogeneous natural mixture of alphatic and aromatic compounds, which are characterized by containing many carbon bonds, to which other compounds can bind to form more complex structures such as alkanes, alkenes, and polycyclic aromatic compounds (Maamar *et al*., 2020) [12]. Crude oil also contains other compounds such as nitrogen, sulfur, oxygen, and other elements such as iron, nickel, zinc, and palladium (AL-Dahgan *et al*., 2019). Recently, with the progress of the oil industry and its refining, a polluting factor has been added to the environment that has raised concern and controversy among scientists and researchers because of its risks, especially the carcinogenic and mutagenic effects. Complex hydrocarbon compounds are difficult to decompose, and therefore recovery from the environmental problems that result from it takes several years, which required the adoption of an ideal strategy, Abd El-Aziz *et al*., 2021) [1] such as low-cost "environmentally friendly" physical, chemical and biological technologies that avoid secondary pollution in cleaning up crude oil spills (Asemoloye *et al*., 2020; Mahmud *et al*., 2022) [3, 13].
The decomposition process of petroleum compounds occurs in three basic ways, including the occurrence of simple and small changes in organic compounds, the fragmentation of hydrocarbon chains into parts while keeping the chemical composition before fragmentation, the change of the composition of the organic parts into inorganic parts with the mineralization of the organic parts (Eman, 2012). Many microorganisms have been used to break down hydrocarbon compounds, such as bacteria, fungi, and yeasts (Benmessa *et al*., 2022). Previous studies have indicated the advantages of fungi, including yeasts, compared to bacteria in the biodegradation of hydrocarbons in contaminated soil, as fungi are characterized by the secretion of many specific enzymes as well as their ability to Added to this is the ability of fungi to form often hydrophobic fungal networks that may cover several hectares of soil, enhancing access to hydrocarbon pollutants (Peidro-Guzmán *et al*., 2020). Yeasts are among the best organisms used in this field due to their ability to use alkanes as a unique source of carbon and energy. Several types of yeasts have been recorded that have the ability to break down hydrocarbon compounds such as *Candida lipolytica*, *Tricosoron mucoides*, *Geotricum* spp., *Yarrowia lipolytica* (Benmessaud *et al*. 2022) [5] *R. mucilagenosa*, *C. tropicalis* (Dhabaan, 2021) [2], *Rhodosporium toruloides* and *Moniliella spathulata* (Mikolasch *et al*., 2020). Therefore, the current research aimed to use the yeast *R. mucilagenosa* in the biological treatment of hydrocarbon compounds.

Materials and methods
Isolation
The pre-diagnosed isolate *R. mucilaginosa* S6 was used in a previous study conducted in the laboratories of the Department of Biology /College of Science/ Mosul University.

An investigation Test on the Ability of *R. mucilaginosa* S6 Yeast to Consume Heavy and Light Crude Oil and Diesel
In this test, 500 ml sterilized glass beakers were used containing 60 ml of Bushnel Haas Medium, consisting of 1 g KH_2PO_4, 1 g K_2HPO_4, 1 g NH_4NO_3, 0.2 g $MgSO_4.7H_2O$ and 0.02 g $CaCl_2.2H_2O$ dissolved in 1000 ml distilled water.

Sterilized and to which 15 mL of crude oil (heavy, light and diesel fuel) was added as the only source of energy and carbon per flask. The flasks were inoculated with 25 ml of the yeast suspension at a rate of 3 flasks/treatment, in addition to the control sample containing the medium not inoculated with the yeast suspension. The flasks were incubated in the shaking incubator at a speed of 120 r/min and a temperature of 28 ± 2˚C for 15 days. The decanters were monitored and the results recorded, as it was observed that a black ring appeared for both heavy and light crude oil, and a light brown ring for diesel fuel accumulated and floated on the surfaces of the glass decanters.

Extract the Remaining hydrocarbon compounds (Heavy and Light Cude Oil and Diesel)

It was extracted by the method described by (Udeme and Antai, 1988) [19] and as follows:

1. Each sample was extracted twice by adding Diethyle ether in a volume similar to the size of the medium using a separating funnel with a capacity of (500) ml, as the medium was discarded, then the extracts were collected.
2.The extracts were passed through Whatman No.1 filter paper placed on anhydrous sodium sulfate Na2So4. After the filtration was completed, the light and heavy crude oil and diesel remaining on the surface of the filter papers were washed with a little Diethyle ether.
3.The extracts were placed in an oven at a temperature of 40 °C to get rid of Diethyl ether.
4.Extract the crude oil and diesel from the control decanters in the same way.

Gas chromatography–mass spectrometry (GC-MS)

Gas phase chromatography analyzes were carried out on a GC-MS -QP2010 Plus device using a FID flame detector, with a flow rate of 1.69 ml/min. The temperature was set to 210 ºC using helium as a vector gas, pressure 100 kPa, split ratio 10:1, injected sample volume 1.0 µl. A quartz capillary column of 30 m (L) x 0.25 mm (ID) x 0.25 µm (thickness) was used. Compounds were identified by comparison between them and substances with known structure by comparing them to the spectrum database of known compounds stored in the GC-MS library and looking at the retention index for each compound (Jiang *et al.*, 2007).

Results and discussion

Investigation of the ability of the yeast *R. mucilaginosa* S6 to consume heavy and light crude oil and diesel fuel as an energy and carbon source The results of using Bushnel Haas Medium mineral salts medium equipped with hydrocarbon compounds (heavy and light crude oil and diesel fuel) at a rate of 15% showed a high capacity for yeast *R. mucilaginosa* S6 was able to decompose and emulsify hydrocarbon compounds in a record time of 10-20 days for both types of crude oil and 7-10 days for biofuels (diesel). Apparent changes were observed in the layer of crude oil of both heavy and light types, as the observations appeared clear and evident in the nutritional medium starting from the fifth day of incubation, as the growth of yeast led to a quality mixing of crude oil with the components of the medium of mineral salts, as indicated by the change of the color of the entire medium to dark brown. This indicates the start of an emulsification of the oil, and with the continuation of the incubation for 15 days, the appearance of a brown to black ring gathered on the surface of the nutrient medium and homogeneous with it was observed with the appearance of light bubbles floating on the surface, and this indicates a total emulsification of the oil compared to the comparison sample that was not treated
with yeast, which did not appear There are no changes to Figure (1)

As for the yeast's ability to break down diesel fuel, the results of the observations showed a great ability to break it down, as indicated by the change of the color of the nutrient medium to the off-white color on the fifth day of incubation. The surface of the ring and after the end of the incubation period, it was noted that the color of the ring changed to orange, which is the color of yeast, which indicates the complete emulsification of the biofuel (Figure 2). This is consistent with the results of a previous study, which confirms that the main reason for the benefit of yeast *Rhodotorula* from hydrocarbon compounds is due to its secretion of the capsule in large quantities, which leads to the secretion of secondary metabolites such as liposaccharides and lipoproteins, which are considered types of surfactants that have high enzymatic efficiency that breaks down petroleum compounds (Shailubhali *et al.*, 1985), in addition to that, many studies confirmed that there are different types of yeasts, including *Rhodotorula* sp. and *Candida* sp. It has a high enzymatic ability to break down petroleum compounds and their derivatives (Das and Chandran, 2011; Al-Dhabaan, 2021; Benmessaoud *et al.*, 2022) [2, 5].

Comparison

Fifteenth day of incubation

Fig 1: The different phenotypic changes of the layer of heavy and light crude oil due to the yeast R. mucilaginosa S6 grown on heavy crude oil for 15 days.

Comparison **Fifteenth day of incubation**

Fig 2: Different phenotypic changes of the biofuel layer (diesel) by yeast *R. mucilaginosa* S6 grown on diesel for 15 days

Extraction of remaining hydrocarbon compounds (heavy and light crude oil and diesel Fuel)

The results of the extraction of hydrocarbon compounds decomposed by the action of yeast *R. mucilaginosa* S6 using both ether and acetone solvents are consistent with the studies of(Udeme and Antai, 1988) [19] and (Aziz, 2012) [4] where they indicated the advantage of using both ether and acetone solvents in the extraction of hydrocarbon compounds, including heavy and light crude oil and diesel fuel. Other studies indicated that Efficient separation of most common hydrocarbons is when using alcohol (Fenrández *et al*., 2015) and dichloromethane (Benmessaoud *et al*., 2022) [5].

Detection of the decomposition of crude oil and its derivatives using the gas chromatograph-mass spectrometer

The results showed that the control sample of heavy crude oil not treated with yeast suspension *R. mucilaginosa* S6 consisted of 43 chemical compounds, depending on the number of peaks that appeared in the gas chromatography after injecting the sample into the GC device. While the results showed that the sample treated with yeast suspension consisted of 34 chemical compounds, with the presence of a compound that a device could not

The GC can be identified and diagnosed in Table (1), Scheme (1-A). And that all the compounds in the comparison sample were destroyed except for the two compounds Benzene,1,2,4-trimethyl- and Benzene,1-ethyl-2,3-dimethyl- which the yeast could not destroy as it appeared in the control and treatment samples. The peaks of the compounds appeared for different retention times compared to the comparison sample.

The results also showed that the light crude oil sample treated with the yeast suspension consisted of 19 chemical compounds, compared to the comparison sample, which was found to be composed of 47 chemical compounds. 6,10-trimethyl-, Naphthalene, 2,6-dimethyl- and Octacosane, which remained the same in the comparison sample. It was also noted that the number of compounds produced in the treatment sample decreased, and the appearance of secondary metabolites that differ from the compounds in the comparison sample with a different retention time. Table (2) and chart (2-B)

As for biofuels, the results of Table (3) showed that the comparison sample contained 46 chemical compounds and the treatment sample contained 42 chemical compounds. The compounds appeared at different retention times. It was found that only five compounds were not destroyed in the treatment sample, which are 1-Octadecanesulphonyl chloride, 2-Methyloctacosane, Naphthalene, 2,6-dimethyl-, Nonahexacontanoic acid.

The planned Tetracosane (3-C). The results indicate that the secondary metabolite compounds produced in the samples treated with the yeast suspension, which were not present in the control samples, are due to the ability of the yeast to break the hydrocarbon bonds that link the compounds, which led to a change in the composition and nature of the substance and thus benefiting from it as a carbon source of energy, and this explains the reason The disappearance of some compounds in the treatment samples, and this was supported by the results of many previous studies in this field, as they indicated the emergence of secondary compounds resulting from the inoculation of hydrocarbon compounds with decomposing organisms and with different retention times due to the ability of the organism to analyze them into secondary compounds that it can benefit from as a carbon source of energy (El Hanafy *et al*., Dasgupta *et al*., 2013; Palanisamy *et al*., 2014; Kridi *et al*., 2015) [7, 15,].

Table 1: Identification of compounds forming heavy crude oil by GC-MS technique after treatment with the local isolate of *R. mucilaginosa* S6

Peaks Numbers	Name of Compound	Retention Time
1	Benzene, 1,2,4-trimethyl-	1.28 C
2	Oxalic acid, 2-ethylhexyl	4.93 C
3	Benzene, 2-ethyl-1,4-dimethyl-	1.16 C
4	Benzene, 1,3-diethyl-5-methyl-	1.76 C
5	Benzene, 1-ethyl-2,3-dimethyl-	2.44 C
6	Benzene, 1-methyl-4-(1-methylpropyl-	0.92 C
7	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.alpha.,5.alpha.)-	4.19 C
8	Benzene, 1-methyl-4-(1-methyl-2-pr	0.92 C
9	Benzene, 1-ethyl-2,4,5-trimethyl-	1.70 C

10	Pentadecane,1-bromo-	1.07 C
11	Pentafluoro propionic acid, tetrade cylester	3.92 C
12	Oxalic acid, 2-ethylhexyl octyl ester	1.84 C
13	6,7-Dimethyl-3H-isobenzofuran-1-on	0.91 C
14	Triallylmethylsilane	4.46 C
15	Cyclohexasiloxane, dodecamethyl-	1.10 C
16	Naphthalene, 1-methyl-	1.05 C
17	Dodecane, 2,6,10-trimethyl-	1.03 C
18	Naphthalene, 2,6-dimethyl-	2.16 C
19	Naphthalene, 1,3-dimethyl-	2.38 C
20	Pent-1-yn-3-ene, 4-methyl-3-phenyl	2.32 C
21	Cycloheptasiloxane, tetradecamethy	1.18 C
22	Ethanone, 1-(1-Naphthalenyl)-	1.12 C
23	Cyclooctasiloxane, hexadecamethyl-	2.01 C
24	Octacosane	1.30c
25	Hexadecane, 2,6,11,15-tetramethyl-	1.28 C
26	Cyclononasiloxane, octadecamethyl-	4.93 C
27	Hexadecane, 2,6,10,14-tetramethyl-	1.16 C
28	Cyclodecasiloxane, eicosamethyl-	1.76 C
29	2-[4-Acetamidophenylsulfonyl]-1,4- Naphthoquinone	2.44 C
30	Oleic Acid	0.92 C
31	Cyclononasiloxane, octadecamethyl-	4.19 C
32	Cyclononasiloxane, octadecamethyl-	0.92 C
33	Phenol, 2,2'-methylenebis [6-(1,1-d imethylethyl)-4-methyl-	1.70 C
34	Cyclodecasiloxane, eicosamethyl-	1.07 C

*The compounds indicated in red indicate their presence in the comparison and treatment samples

Table 2: Identification of light crude oil-forming compounds by GC-MS after treatment with the local isolate of *R. mucilaginosa* S6

Peaks Numbers	Name of Compound	Retention Time
1	Cyclohexanol,5-methyl-2-(1-methylethyl)-, (1alpha.,2. beta.5.alpha)-(.+/-.)-	3.40 C
2	Undecane, 2,6-dimethyl-	2.51 C
3	Dodecane, 4,6-dimethyl-	2.42 C
4	Cyclohexasiloxane, dodecamethyl-	5.60 C
5	Dodecane, 2,6,10-trimethyl-	2.68 C
6	Naphthalene, 2,6-dimethyl-	2.41 C
7	Naphthalene, 1,3-dimethyl-	1.95 C
8	1-Decanol, 2-octyl-	2.75 C
9	Cycloheptasiloxane, tetradecamethy	12.88 C
10	Ethanone, 1-(1-Naphthalenyl)-	2.25 C
11	Carbonic acid, octadecyl 2,2,2-tri chloroethyl ester	2.08 C
12	Cyclooctasiloxane, hexadecamethyl-	9.33 C
13	Octacosane	3.04 C
14	Pentadecane, 2,6,10,14-tetramethyl	2.69 C
15	Heptadecane, 2,3-dimethyl-	3.48 C
16	Hexasiloxane, tetradecamethyl-	6.76 C
17	Cyclotetradecane, 1,7,11-trimethyl -4-(1-methylethyl)-	2.71 C
18	Cyclononasiloxane, octadecamethyl-	4.42 C
19	Cyclononasiloxane, octadecamethyl-	6.22 C

*The compounds indicated in red indicate their presence in the comparison and treatment samples.

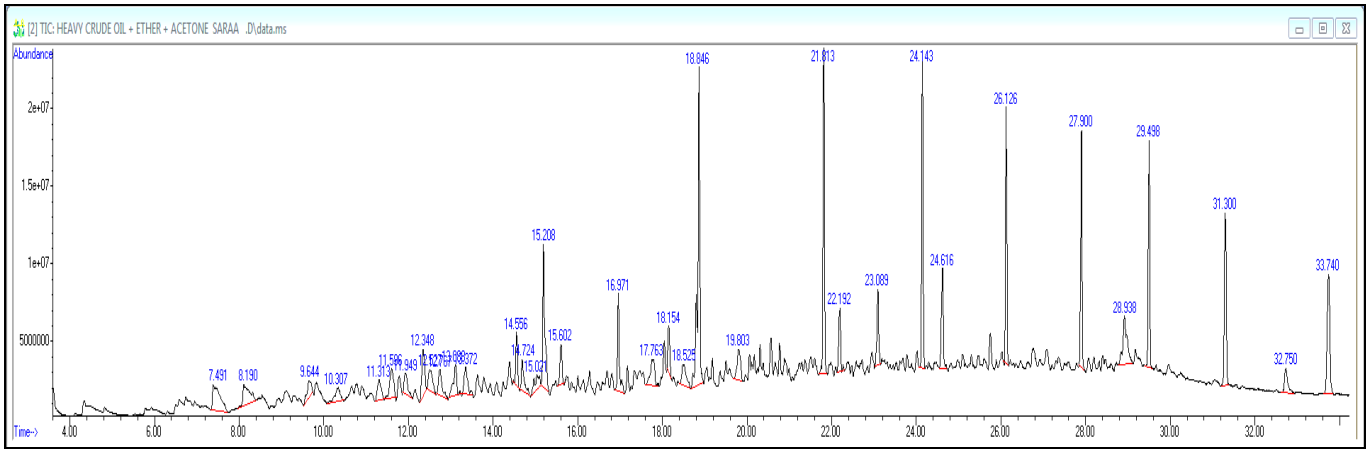
Table 3: Identification of biofuel compounds (diesel by GC-MS) after treatment with a local isolate of *R. mucilaginosa* S6

Peaks Numbers	Name of Compound	Retention Time
1	Decane, 4-methyl-	1.75 C
2	Oxalic acid, cyclobutyl tetradecyl ester	0.79 C
3	Benzene, 1,4-diethyl-	1.11 C
4	Benzene, 4-ethyl-1,2-dimethyl-	0.89 C
5	Undecane	2.77 C
6	Benzene, 1,3-diethyl-5-methyl-	1.14 C
7	Benzene, 1-ethyl-2,3-dimethyl-	2.35 C
8	p-Toluic acid, 5-tridecyl ester	0.95 C
9	Cyclohexanol, 1-methyl-4-(1-methyl ethyl)-	1.30 C
10	1H-Indene, 2,3-dihydro-4,7-dimethyl-	1.28 C
11	Octadecane, 1-chloro-	4.03 C

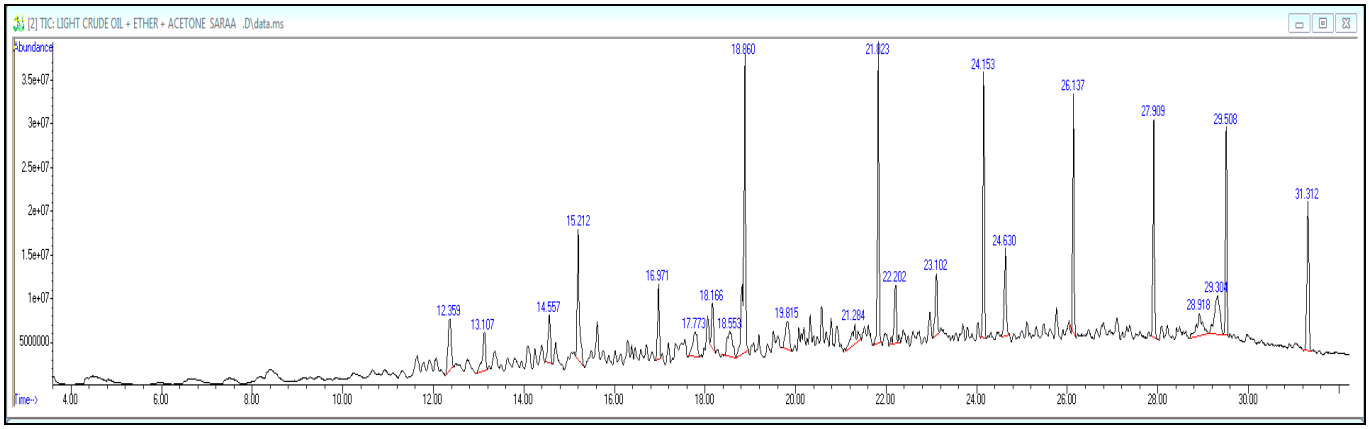
12	Undecane, 2,6-dimethyl-	2.03 C
13	4-Heptafluorobutyroxy tetradecane	1.42 C
14	Dodecane, 4-methyl-	0.86 C
15	1-Octadecanesulphonyl chloride	1.31 C
16	Hexadecane, 2,6,11,15-tetramethyl-	2.36 C
17	3,4-Nonadien-6-yne, 5-ethyl-3-methyl-	1.03 C
18	Eicosane	5.29 C
19	Naphthalene, 1-methyl-	1.17 C
20	1-Tridecene	0.91 C
21	10-Methylnonadecane	0.93 C
22	2-methyloctacosane	0.84 C
23	Dodecane, 2,6,10-trimethyl-	1.62 C
24	Hexacosane	5.13 C
25	Naphthalene, 2,6-dimethyl-	1.54 C
26	Naphthalene, 2,3-dimethyl-	0.87 C
27	Naphthalene, 1,3-dimethyl-	1.09 C
28	Tridecane, 4,8-dimethyl-	2.11 C
29	13-Methylhentriacontane	4.79 C
30	Tetradecane, 3-methyl-	0.92 C
31	Nonadecane	2.69 C
32	Naphthalene, 1,4,6-trimethyl-	1.22c
33	Heptadecyl heptafluorobutyrate	1.31 C
34	Tridecane, 2-methyl-	1.27 C
35	1-Decanol, 2-octyl-	3.58 C
36	Cyclooctasiloxane, hexadecamethyl-	1.96 C
37	2-Bromo dodecane	1.95 C
38	Cyclohexane, (1-butylhexadecyl)-	1.05 C
39	Hexadecane, 2,6,11,15-tetramethyl-	4.44 C
40	Nonahexacontanoic acid	1.04 C
41	Cyclononasiloxane, octadecamethyl-	1.88 C
42	Ethanol, 2-(octadecyloxy)-	3.32 C
43	Tetracosane	0.92 C
44	Trisiloxane, 1,1,1,5,5,5-hexamethy	1.62 C
45	18-Methyl-nonadecane-1,2-dio, trimethylsilyl ether	1.36c
46	Cyclononasiloxane, octadecamethyl-	3.50c

The compounds indicated in red indicate their presence in the comparison and treatment samples.

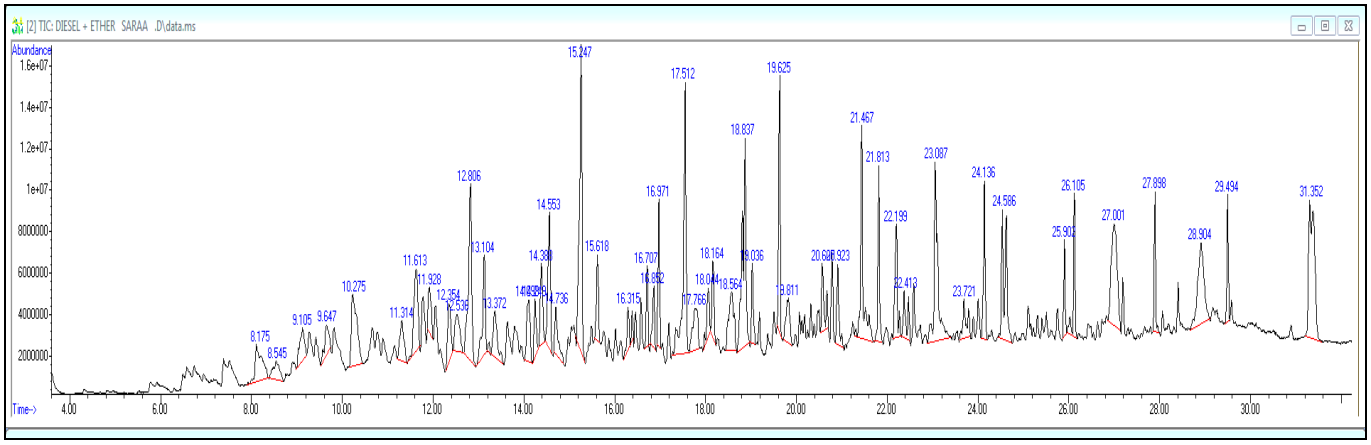
Scheme (1): Curves and peaks of the chemical compounds separated from heavy crude oil after treatment with the local isolate of
R. mucilaginosa S6 by GC-MS technique
A. Treatment with yeast *R. mucilaginosa* S6



Scheme (2): Curves and peaks of the chemical compounds separated from light crude oil after treatment with the local isolate of
R. mucilaginosa S6 by GC-MS technique
B. Treatment with yeast *R. mucilaginosa* S6



Scheme (3): Curves and peaks of chemical compounds isolated from biofuel (diesel) after treatment with the local isolate of
R. mucilaginosa S6 by GC-MS technique
C. Treatment with yeast *R. mucilaginosa S6*



Below are the most important conclusions of the current study

- The results showed that the local isolate R. mucilaginosa S6, isolated from the soil of oil fields, has the ability to decompose heavy and light crude oil and biofuel (diese).
- It is possible to benefit from this yeast in the field of biological treatment and environmental cleaning

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