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Efficiency of *Penicillium commune* to Biodegradation Crude oil

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Abstract

This study investigated the ability of Penicillium commune to degrade crude oil. This fungus was isolate from the soil contaminated with crude oil residues in the AL- Nasiriyah oil field (Al-Katea field) southern Iraq, using the dilution method, samples were collected quarterly, with one composite sample for each season, and the results were expressed quarterly, as the visits were divided into four visits and according to the number of seasons of the year. The results showed that the Penicillium commune was more frequent during this study, and the percentage appeared to reach 75% in soil contaminated with crude oil by diagnosed PCR technology, at different concentrations (1,2,3) %. The ability of Penicillium commune to degrade crude oil was studied in the presence of different carbon and nitrogen sources (glucose, maltose), (NaNo3, NH4Cl) and at different temperatures (18, 25, 42 oC) in the mineral salt's medium. The results showed a decrease in the pH value, and the PH reached (3, 5,1) respectively after 28-day incubation, as well as the metabolic products and the remaining concentration that were calculated by gas chromatography -mass spectroscopy.

Keywords: Crude oil; Degradation; Environment; Penicillium; Pollution; Soil

1. Introduction

Crude oil is a complex mixture of hydrophobic components such as n-alkanes, aromatics, resins, and asphaltenes. Some fractions of crude oil are toxic to living organisms [1]. Oil pollution is defined as the phenomenon of introducing an oil compound or one of its derivatives into the aquatic or terrestrial environment, which leads to damage to it through a change in its physical, chemical and biological properties, and this in turn leads to harm to humans in direct or indirect ways [2]. Crude oil spillage is the accidental discharge or pouring of crude oil into the environment which involves the contamination of the environment with liquid hydrocarbons. These spills endanger public health, drinking water and natural resources and disrupt the economy [3]. The leakage of crude oil into soil damages the biota residing in the soil, including microorganisms and plants, and increasingly toxic beyond a concentration of 3% [4]. *Penicillium* fungus has a high ability to remove oil pollution from areas contaminated with crude oil waste through biodegradation of petroleum hydrocarbons. It also works to reduce the level of soil contamination with crude oil. It also works to biodegrade kerosene at different concentrations [5]. The increasing levels of oil pollution and the diversity of its sources in the world in general and Iraq in particular and working to find solutions to it are among the most important reasons that led to the preparation of this study. The AL- Nasiriyah oil field in Thi-Qar Governorate was chosen because there is no study that addressed the susceptibility of fungi isolated from the soil of this field to biodegradation of crude oil waste.

2. Material and methods

2.1. Samples of Crude Oil

Crude oil was supplied by AL-Nasiriya (Al-Katea field) in Iraq. It was transferred to the laboratory in a dark glass bottle closed tightly and kept in a cold and dark place to use.

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2.2. Collection of Soil Samples

Samples of soil contaminated with crude oil waste of the Al- Nasiriyah oil field were collected from different places within the field. A combined sample of (1) kilogram was made to isolate and identify the types of fungi present in this environment. The collection period was quarterly, and with one site visit for each season. characterized by the soil of this field is a dark, blackish desert color due to the high concentrations of crude oil contained in it, a sterile laboratory spoon was used to collect soil samples. The surface layer of soil was removed to a depth of approximately 5-15 cm according to the method [6]. After that, a 1 kg amount of soil contaminated with crude oil was taken and placed in clean, sterile plastic bags. Information was recorded on an identification card for each sample, including the sample number, location and date of sample collection, and some field notes. It was then transferred to the laboratory and kept in the refrigerator until analyzes were conducted on it.

2.3. Fungal Isolation

The fungi were isolated from the soil of the AL- Nasiriyah oil field by the dilution method and using their own culture media. The soil samples were transferred to the laboratory for the purpose of isolating and diagnosing the fungi present in them, and the culture medium was used Potato Dextrose Agar (PDA) to grow the fungi according to the method [7]. Dilutions (10^{-1},10^{-2}, 10^{-4}) were made after homogenization. The pour-plate method was used, where 1 ml was withdrawn from the last dilution and emptied into Petri dishes, then added to it the culture medium (PDA) was pre-solidified after 250 mg/L of the antibacterial agent Chloramphenicol was added to it to inhibit and prevent the growth of bacteria. The dish was moved in a circular motion for the purpose of homogeneity, and the dishes were incubated after solidification at a temperature of 25 degrees Celsius for 7 days. The numbers of fungi in soil samples were estimated based on the total counting method developed by [7] using the PDA medium mentioned above, where the number of growing colonies was calculated, and results were obtained as an average number of fungal colonies in one gram of soil sample.

2.4. Molecular Identification of Fungi that Degrade Oil

That internal transcribed spacer (ITS) region was amplified and sequenced in order to perform molecular identification. Using primers (ITS 1 and ITS 4), the internal transcription space region (ITS1-5.8S-ITS2) was amplified using polymerase chain reaction (PCR) technology. The source of these primers is Macrogen, Korea. Using the genomic DNA as a template and the ITS primers of ITS1(5π- TCCGTAGGTGAACCTGCGG-3 $_R$) and ITS4 (5 $_R$ – TCCTCCGCTTATTGATATGC-3 $_R$), the ITS region was amplified using polymerase chain reaction (PCR). One microliter of isolated fungal genomic DNA, 0.5 micrograms of each primer and 50 microliters of Maxima Hot Start PCR Master Mix (Thermo) made up the PCR mixture. AL Ameen Foundation For Study used a DNA Engine Thermal Cycler to do the PCR.& Research(Najaf, Iraq) with a hot start that lasted for four minutes at 94 ^{0}C, thirty cycles of 94 ^{0}C,56 ^{0}C , and 72 ^{0}C, and a final extension that lasted for seven minutes at 72 ^{0}C.At Macrogen Company (Korea), a DNA sequencer was used for the commercial sequencing. The NCBI BLAST tool was used to match the ITS sequence against the Gen Bank database. Then, using BLASTN, sequences were matched with ITS sequences in the Gen Bank database.

2.5. Biodegradation of Crude Oil in Mineral Salts Medium

Mineral salt media for fungal growth was prepared in twelve conical flasks with a capacity of (250) ml. After that, the medium was sterilized with an autoclave at a temperature of 121°C and a pressure of 15 pounds per square ang for 20 minutes. Crude oil was added to the culture medium after the temperature was reduced. It was heated to an appropriate temperature with three concentrations of (1,2,3) %, three beakers for each concentration and three control beakers. All of the beakers except the control beakers were inoculated with the *Penicillium commune* inoculants by transferring a 5 mm diameter disk from 7-year-old fungal cultures days with a sterilized cork borer and then incubated all the flasks in a Shaker incubator with a rotation speed of 150 rpm per minute, at a temperature of (18°C, 25°C, 42°C) for a period of 28 days, with the addition of a carbon source, which is glucose once, and maltose again, each separately, at a concentration of 0.1 g/L, and adding a nitrogen source, which is NH_4CL, once. And sodium nitrate $NaNo_3$ again and each separately at a concentration of 0.1 g/L for each type of nitrogen source. This experiment was carried out with three replicates for each treatment. The mycelium was obtained by filtering the culture medium, where the filtrate was passed through Whatman No.1 filter paper in a Buechner funnel, and the filtrate was placed in a separating funnel, and 25 ml of a mixture of petroleum ether and acetone was added to it in a ratio of 1:1. Then the separating funnel was shaken well three times, and then the sample was left to settle, which led to the formation of three layers, one at the top containing petroleum hydrocarbons, and the middle and bottom layer containing Acetone and water. The middle and lower layers were separated, while the upper layer containing the petroleum hydrocarbons was transferred to a clean beaker. The extracted filtrate was passed through anhydrous sodium sulfate to remove moisture. The extracted filtrate was evaporated in a water bath at a temperature of 70°C to about 1 ml, then the filtrate was placed in a vial. Small, tightly

sealed container. After completing the extraction process, it was then examined using gas chromatography – Mass spectroscopy.

2.6. Analysis using a Gas Chromatograph-Mass Spectrometer (Gc-Ms spectro meter).

This analysis was conducted in the same manner as the previous one, where the samples were analyzed using a gas chromatograph - Mass spectroscopy (Gc-Mass spectroscopy) type Agilent 5977A MSD at the Basra Oil Company in the Research and Quality Control Department at the Nahran Bin Omar Laboratories. The biodegradation rate was calculated according to the equation [8].

$$\% \text{ Degradation} = A_s / A_o \times 100$$

- A_s: Concentration of remaining crude oil
- A_o: Standard crude oil concentration

3. Results and discussion

3.1. Isolation of fungi

Penicillium communes were isolated from the upper surface of soil contaminated with crude oil waste in the AL-Nasiriyah oil field, north of Thi- Qar governorate, southern Iraq, using the dilution method. Table 1 showed that the frequency of *Penicillium commune* reached to 75% . The reason for its high appearance is attributed to the production of reproductive units, and this is consistent with what was stated in the studies they conducted [9] [, [10] where some other genera recorded a high appearance rate, including *Alternaria*, *Penicillium*, *Rhizopus*, and *Ulcladium*, as it reached to 45,67.5, 27.5 and 20%, respectively, and the reason for their high appearance is attributed to the production of small reproductive units. They are numerous in number and form resistant to unfavorable environmental factors.

Table 1 Frequency of *Penicillium commune* isolated from the surface of Soil contaminated with crude oil

Species	Number of *Penicillium commune* appeared	Frequency%
Penicillium commune	3	75%

Number of samples studied= 4

3.2. Molecular Diagnosis of Fungi

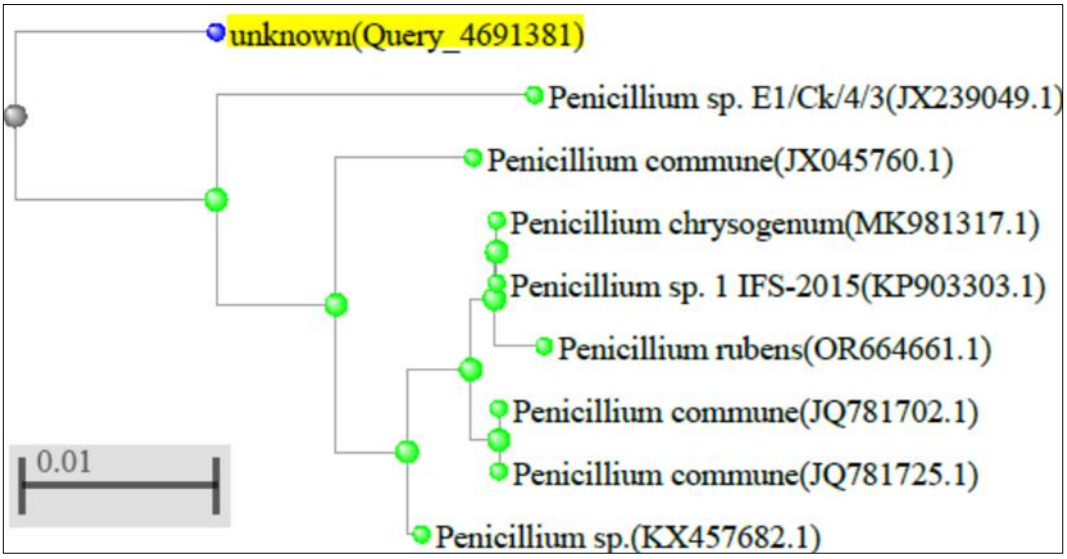

Figure 1 Phylogenetic tree of ITS sequences of the fungal isolate with the sequences from NCBI and designated as *Penicillium commune*

During this study, a number of fungi taken from the AL- Nasiriyah oil field were isolated and there was one fungal species that was the most visible in the samples, The taxonomic status of fungal isolate was defined by sequencing of ITS genes.

The morphological culture characteristic as well molecular identification based on ITS sequencing analysis for isolates was similar to *Penicillium commune* as shown in Fig (1)

3.3. Biodegradation of Crude Oil in Mineral Salts Media

The ability of *Penicillium commune* isolated from contaminated soil in the study area to biodegradation crude oil at a concentration of (1,2,3) % in the culture medium. The results of gas chromatography - Mass spectroscopy (Gc-Mass) analysis showed the ability of this fungus to biodegrade crude oil at different concentrations and different temperatures (18, 25, 42 ^{o}C) after 28 days of incubation and in the presence of carbon sources (glucose, maltose) and nitrogen sources (NH_4Cl, $NaNo_3$) separately. Figure (2), which represents the standard curve (control) for crude oil, as it represents the time of appearance of crude oil (Retention time) in GC – Mass. Figures (3), (4), (5) represent the ability of the *Penicillium commune* to biodegradation crude oil at concentrations of(1, 2, 3)% in the mineral salts medium supplemented with a carbon source of maltose and a nitrogen source of $NaNo_3$ at a temperature of 18°C. Figures(6),(7), (8) represent the ability of this fungus to biodegradation crude oil under the same conditions, while changing the carbon source to glucose and the nitrogen source to NH_4Cl. Figures (9),(10), (11) represent the ability of the *Penicillium commune* to biodegrade crude oil at concentrations of(1, 2, 3)% in the mineral salts medium supplemented with a carbon source of maltose and nitrogen $NaNo_3$ at a temperature of 25°C. While Figures (12), (13) ,(14) represent the ability of *Penicillium commune* to biodegradation crude oil under the same conditions while changing the carbon source to glucose and the nitrogen source to NH_4Cl. Figures(15), (16), (17) represent the ability of the *Penicillium commune* to biodegradation crude oil at concentrations of (1, 2, 3)% in the mineral salts medium supplemented with a carbon source of maltose and a nitrogen source of $NaNo_3$ at a temperature of 42°C. While Figures (18),(19) (20) represent the ability of this fungus to biodegradation crude oil under the same conditions while changing the carbon source to glucose and the nitrogen source to NH_4Cl. Mycelial organisms can penetrate insoluble substances such as crude oil and this increase the surface are available for microbial attack[11].The GC-MS profiles demonstrated the efficiency of the newly isolated fungal strains in remediating petroleum-contaminated soil in the microcosm systems. GC-MS was performed for the confirmation of PHCs biodegrading and identification of the volatile degradation products. The presented GC-MS profiles are similar to those reported by Al-Hawash et al. [12], using indigenous oil-degrading fungi affiliated to the genus *Penicillium*. However, the degrading compounds are mostly related to the microbial metabolic pathway. The pH was measured at the beginning of the experiment, and it was equal to PH = 6. At the end of the 28-day incubation period, the pH was measured again in the mineral salts medium to which different concentrations of crude oil were added (1,2,3%) and the pH was equal to PH = (3,5,1) respectively at a temperature of 18°C, PH=(3,1,3) at a temperature of 25°C,PH=(5,1,3) at a temperature of 42°C . The reduction of the PH in the liquid medium after 28-day incubation period confirmed chemical changes of the hydrocarbon substrates which must have been precipitated by microbial enzymes [11]. Hydrogen ion concentration is a major variable governing the activity and composition of fungi. Many species can metabolize over a wide PH range from the highly acidic to alkaline extremes. Thus, the insensitivity of the fungi to high hydrogen ion concentration and narrow PH range of most bacteria account for the sharp drop in PH. Microbial degradation of crude oil often leads to production of organic acids and other metabolic products [13]. Tuss organic acids probably produced account for the reduction in PH levels [14]. In this study, the tested fungus showed their ability to remove the aromatic compound in different proportions after 28 days .This study agrees with various studies that showed that many Ascomycota fungi, including *Penicillium*, *Aspergillu*s, *Pseudallascheria* and *Fusarium* not only spread widely in contaminated areas, but it is also even capable of removing contaminants in soil contaminated by industrial spills or gas station tanks[15]. The results agree with the findings of [16].

Table 2 Biodegradation of crude oil by *Penicillium Commune*

Species	temperature	Crude oil concentration	PH	Carbon and nitrogen sources	Biodegradation rate
Penicillium commune	18	(1,2,3) %	(3,5,1)	Maltose +$NaNo_3$	36%,35%,71%
		(1,2,3) %	(3,5,1)	Glucose +NH_4CL	49%,12%,15%
	25	(1,2,3) %	(3,1,3)	Maltose +$NaNo_3$	60%,77%,63%
		(1,2,3) %	(3,1,3)	Glucose +NH_4CL	96%,70%,79%
	42	(1,2,3) %	(5,1,3)	Maltose +$NaNo_3$	73%,21%,62%
		(1,2,3) %	(5,1,3)	Glucose +NH_4CL	76%,71%,9%

The results of Table (2) also indicate the ability of the *Penicillium commune* to biodegradation crude oil, as it shows the rate of biodegradation at different temperatures, different concentrations and different carbon and nitrogen sources after 28 days of incubation

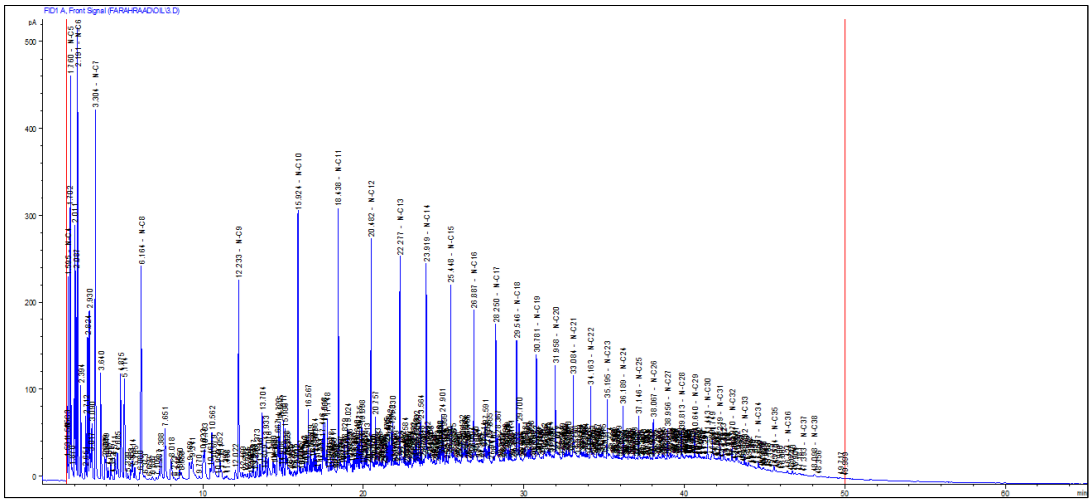

Figure 2 Standard curve (control) for crude oil using GC-Mass spectroscopy

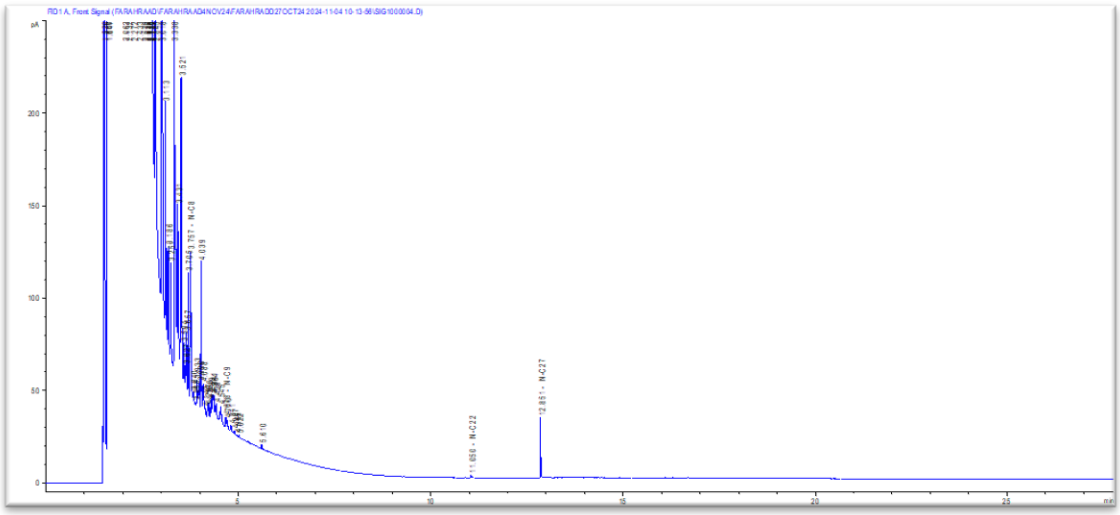

Figure 3 Biodegradation of crude oil at a concentration of 1% by the fungus *Penicillium commune* in mineral salts medium in the presence of a carbon source of maltose and nitrogen $NaNo_3$ at a temperature of 18°C using gas chromatography-Mass spectroscopy (GC-Mass)

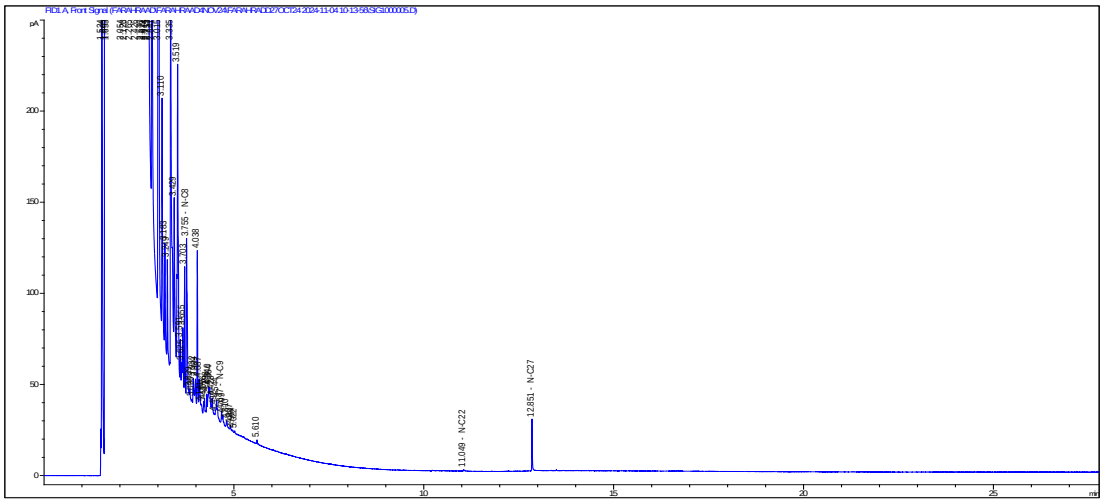

Figure 4 Biodegradation of crude oil at a concentration of 2% by the fungus *Penicillium commune* in the mineral salts medium in the presence of a carbon source of maltose and nitrogen $NaNo_3$ at a temperature of 18°C gas chromatography-Mass spectroscopy (GC-Mass)

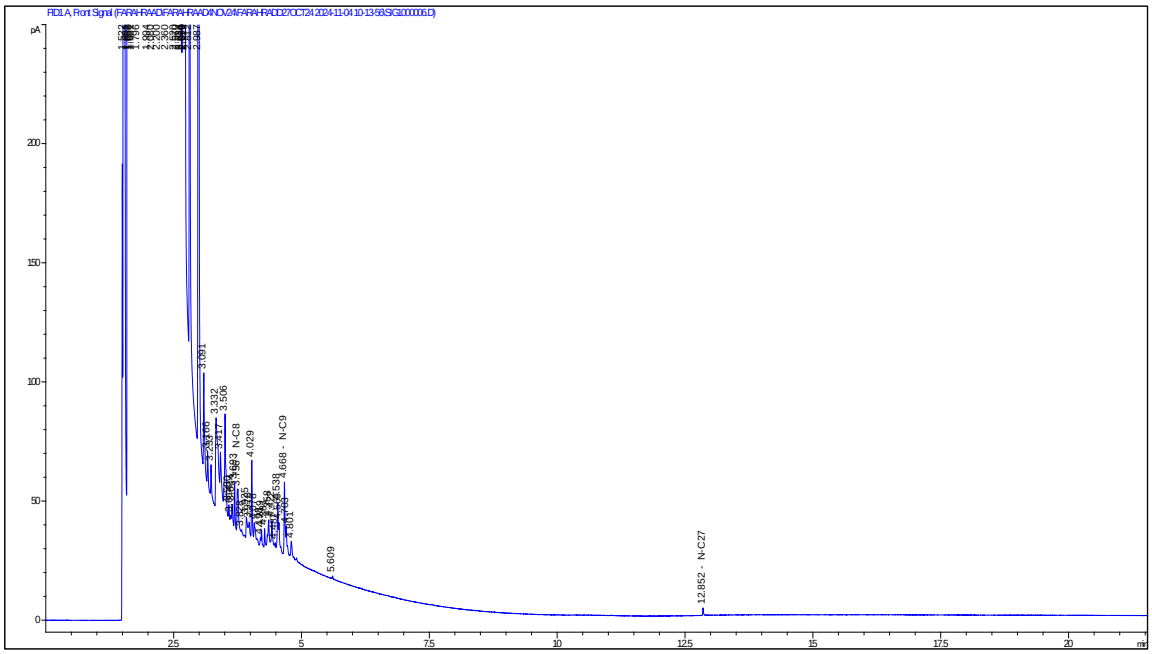

Figure 5 Biodegradation of crude oil at a concentration of 3% by the fungus *Penicillium commune* in the mineral salts medium in the presence of a carbon source of maltose and nitrogen $NaNo_3$ at a temperature of 18°C using gas chromatography-Mass spectroscopy (GC-Mass)

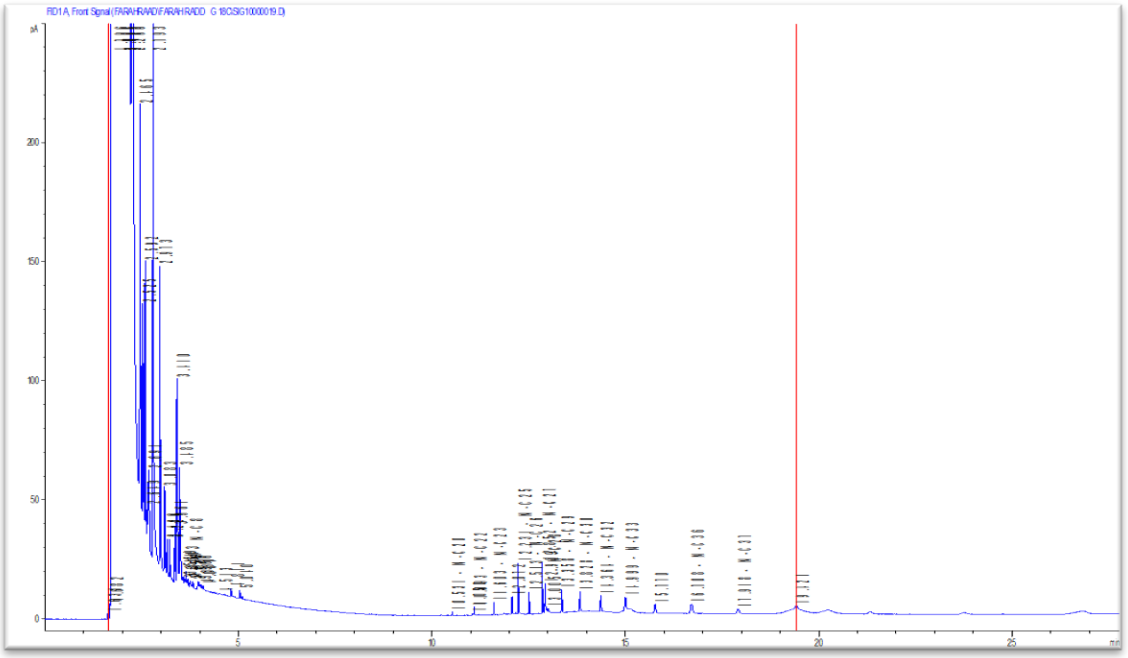

Figure 6 Biodegradation of crude oil at a concentration of 1% by the fungus *Penicillium commune* in the mineral salts medium in the presence of a carbon source of glucose and nitrogen NH_4CL at a temperature of 18°C using gas chromatography-Mass spectroscopy (GC-Mass)

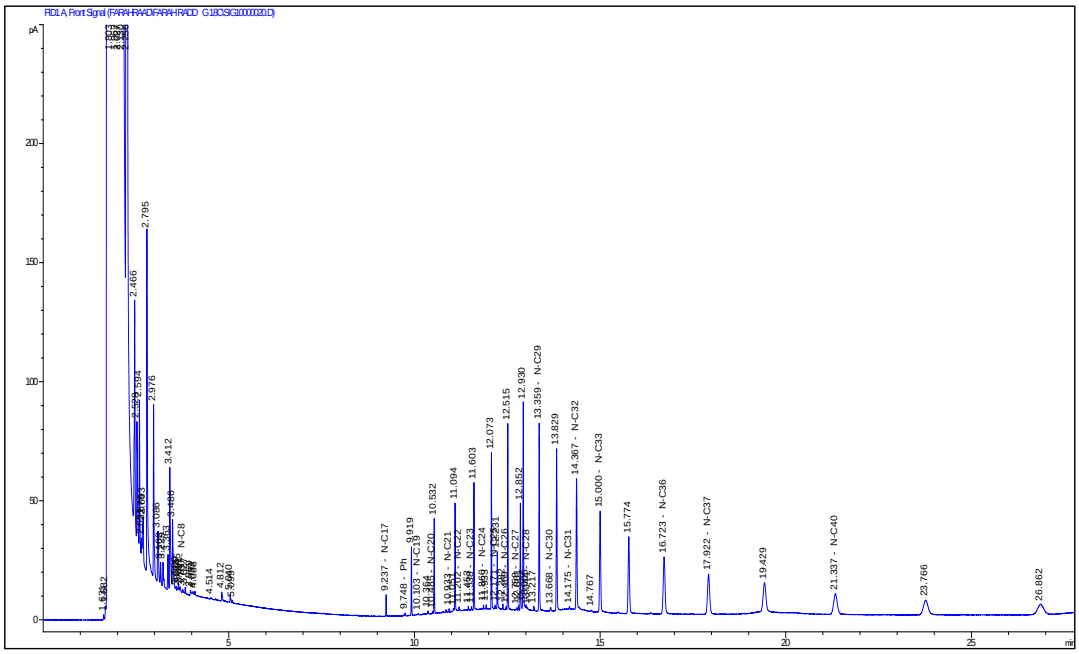

Figure 7 Biodegradation of crude oil at a concentration of 2% by the fungus *Penicillium commune* in the mineral salts medium in the presence of a carbon source of glucose and nitrogen NH_4CL at a temperature of 18°C using gas chromatography-Mass spectroscopy (GC-Mass)

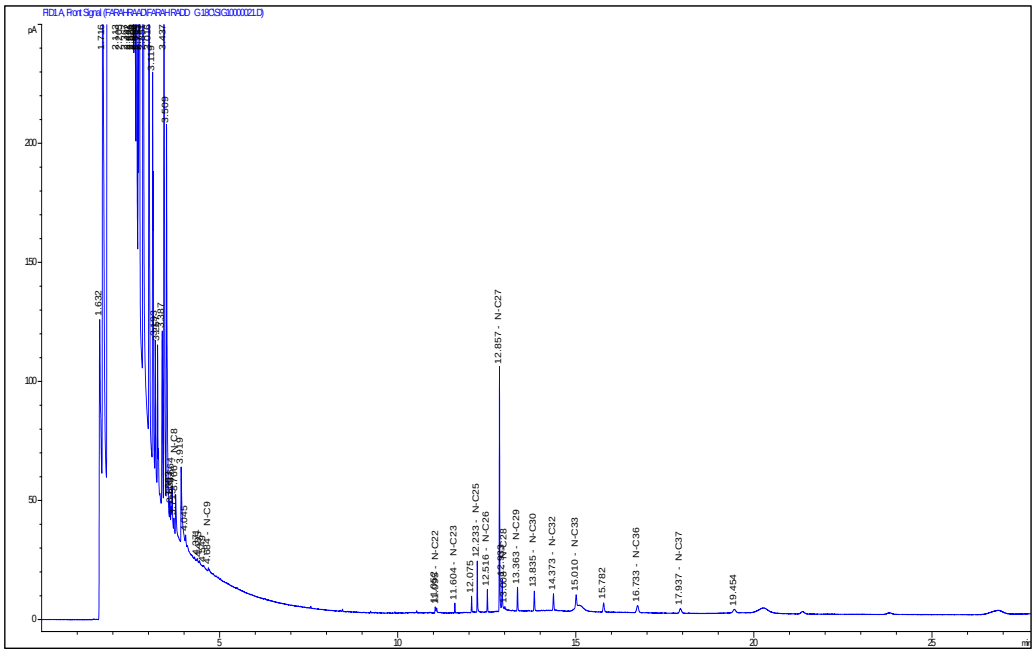

Figure 8 Biodegradation of crude oil at a concentration of 3% by the fungus *Penicillium commune* in the mineral salts medium in the presence of a carbon source of glucose and nitrogen NH_4CL at a temperature of 18°C using gas chromatography-Mass spectroscopy (GC-Mass)

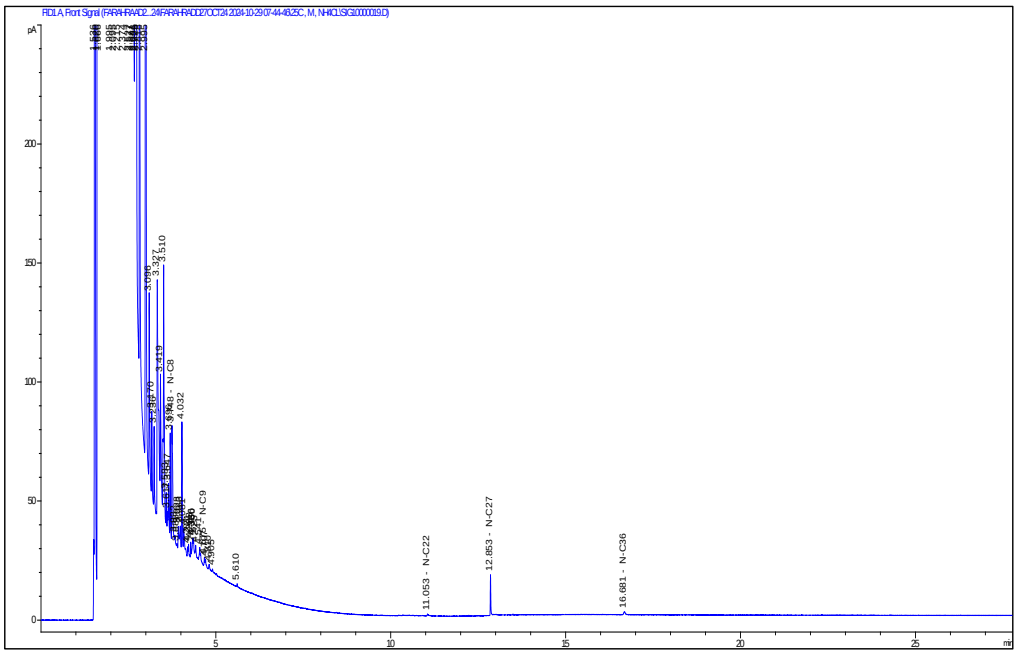

Figure 9 Biodegradation of crude oil at a concentration of 1% by the fungus *Penicillium commune* in the mineral salts medium in the presence of a carbon source of maltose and nitrogen $NaNo_3$ at a temperature of 25°C using gas chromatography-Mass spectroscopy (GC-Mass)

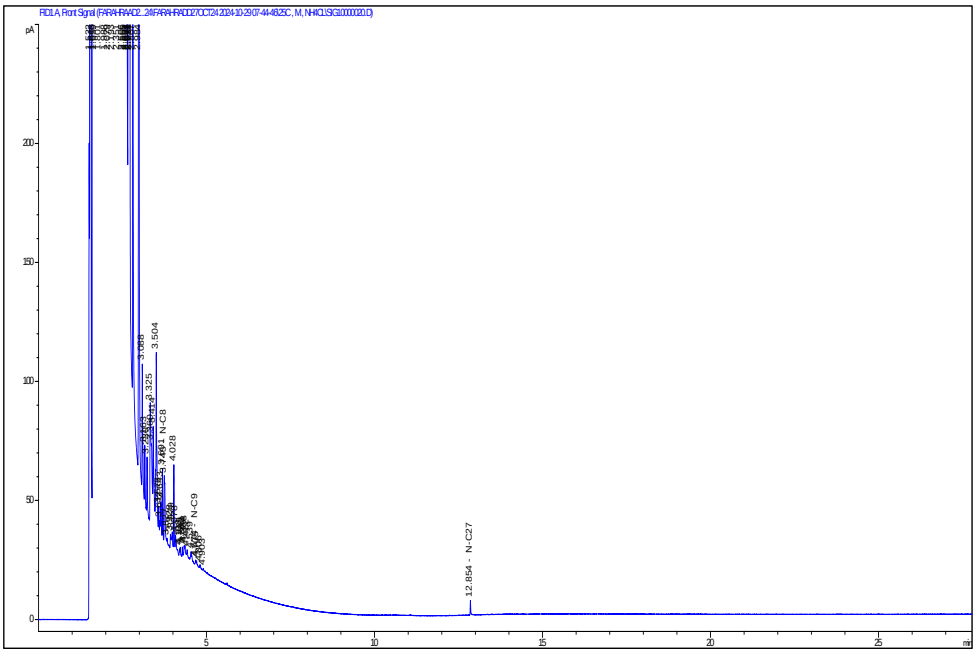

Figure 10 Biodegradation of crude oil at a concentration of 2% by the fungus *Penicillium commune* in the mineral salts medium in the presence of a carbon source of maltose and nitrogen $NaNo_3$ at a temperature of 25°C gas chromatography-Mass spectroscopy (GC-Mass)

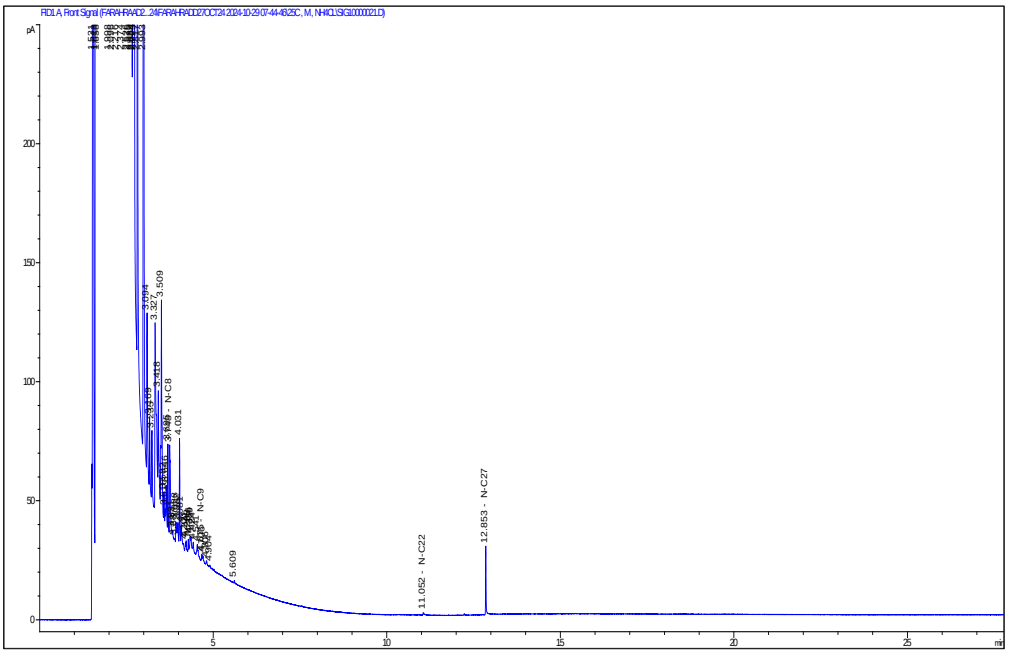

Figure 11 Biodegradation of crude oil at a concentration of 3% by the fungus *Penicillium commune* in the mineral salts medium in the presence of a carbon source of maltose and nitrogen $NaNo_3$ at a temperature of 25°C using gas chromatography-Mass spectroscopy (GC-Mass)

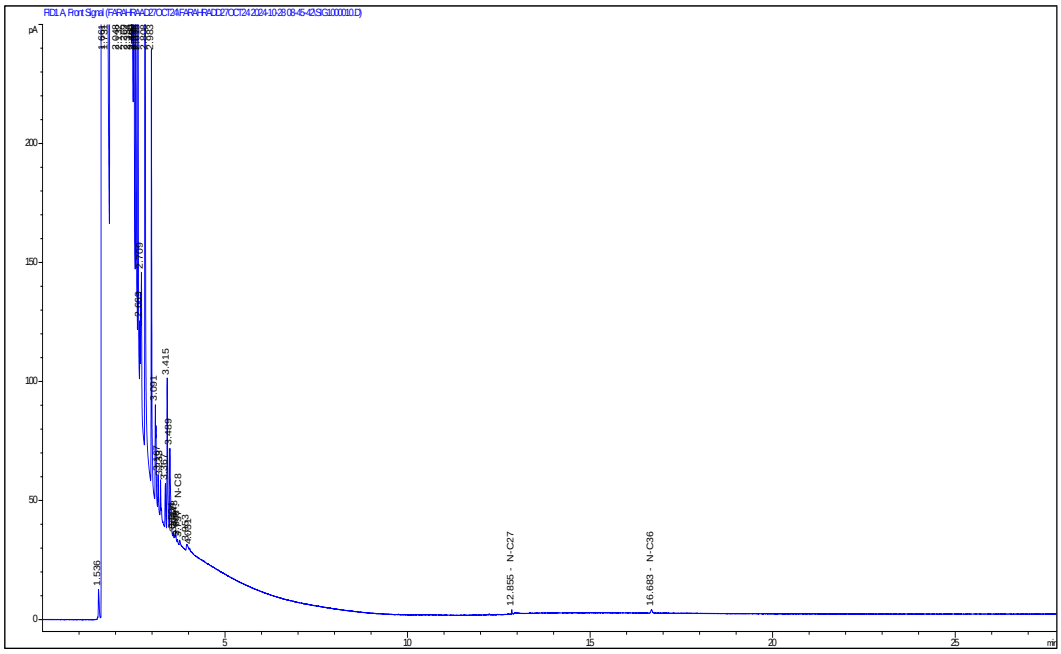

Figure 12 Biodegradation of crude oil at a concentration of 1% by the fungus *Penicillium commune* in the mineral salts medium in the presence of a carbon source of glucose and nitrogen NH_4CL at a temperature of 25°C using gas chromatography-Mass spectroscopy (GC-Mass)

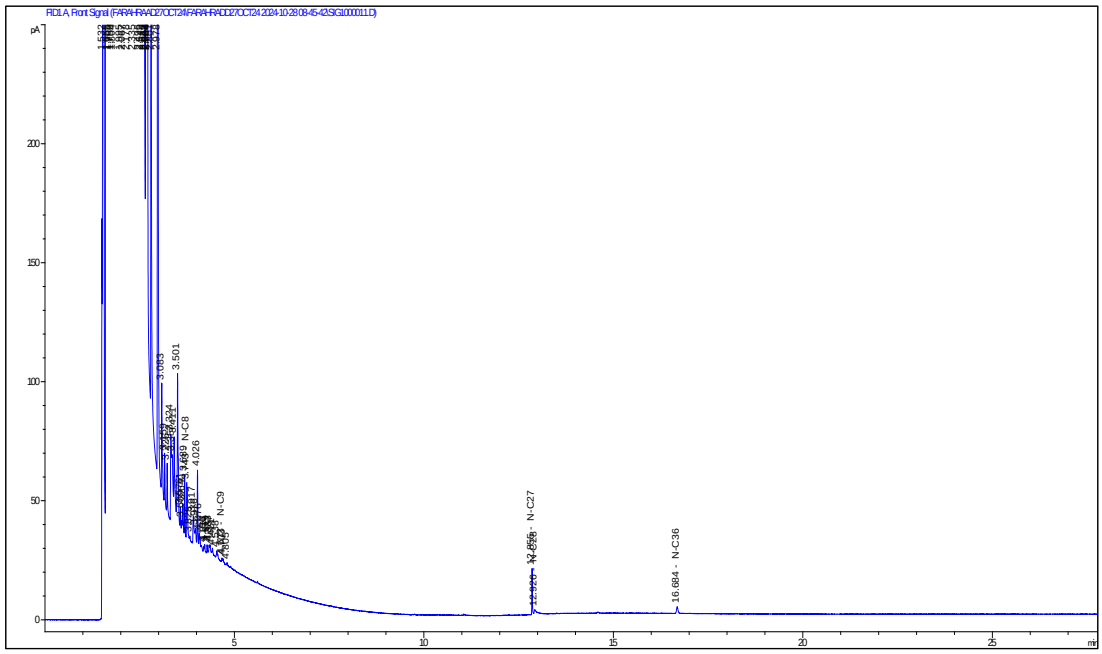

Figure 13 Biodegradation of crude oil at a concentration of 2% by the fungus *Penicillium commune* in the mineral salts medium in the presence of a carbon source of glucose and nitrogen NH_4CL at a temperature of 25°C using gas chromatography-Mass spectroscopy (GC-Mass)

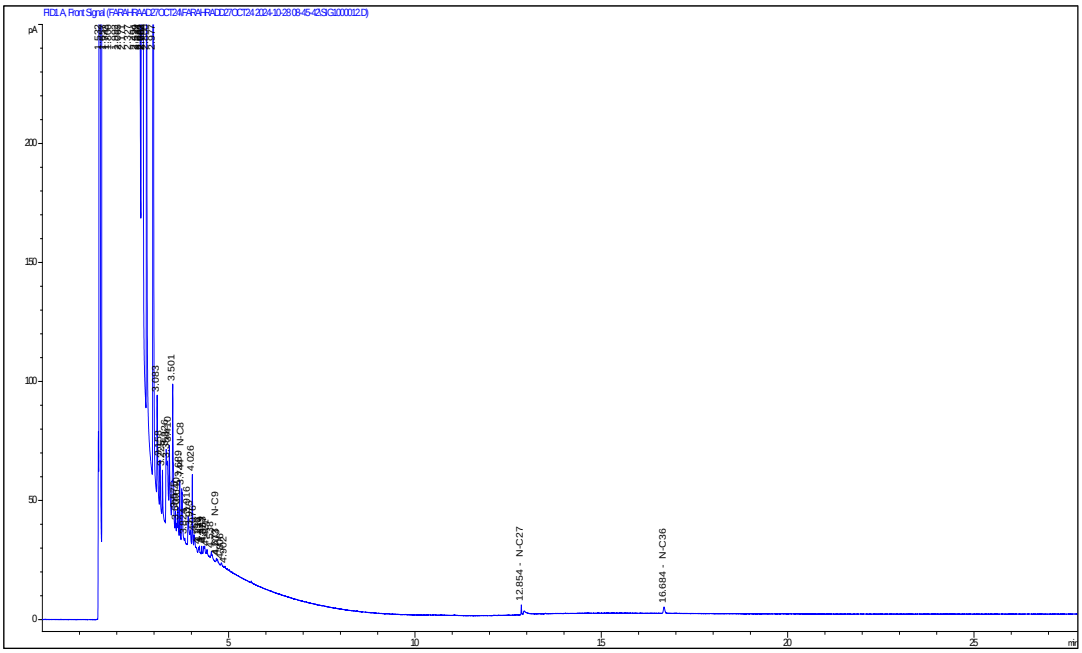

Figure 14 Biodegradation of crude oil at a concentration of 3% by the fungus *Penicillium commune* in the mineral salts medium in the presence of a carbon source of glucose and nitrogen NH_4CL at a temperature of 25°C using gas chromatography-Mass spectroscopy (GC-Mass)

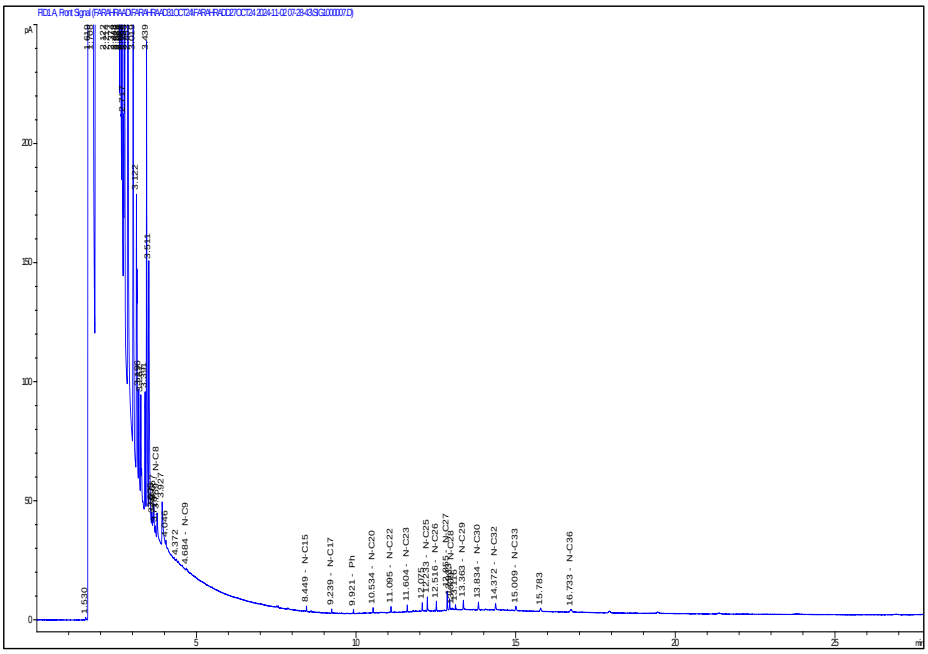

Figure 15 Biodegradation of crude oil at a concentration of 1% by the fungus *Penicillium commune* in the mineral salts medium in the presence of a carbon source of maltose and nitrogen $NaNo_3$ at a temperature of 42°C using gas chromatography-Mass spectroscopy (GC-Mass)

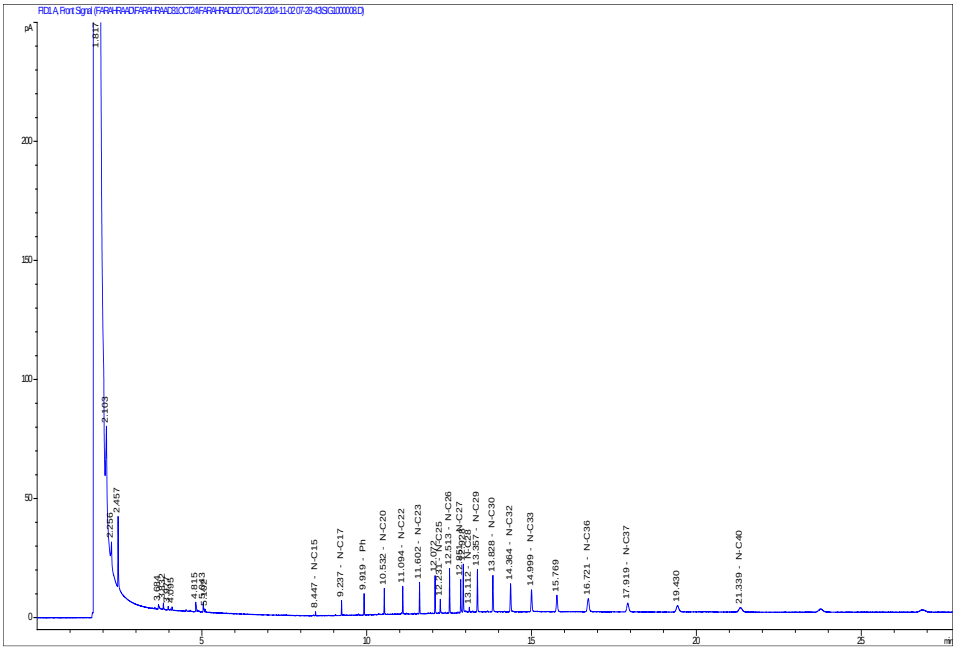

Figure 16 Biodegradation of crude oil at a concentration of 2% by the fungus *Penicillium commune* in the mineral salts medium in the presence of a carbon source of maltose and nitrogen $NaNo_3$ at a temperature of 42°C using gas chromatography-Mass spectroscopy (GC-Mass)

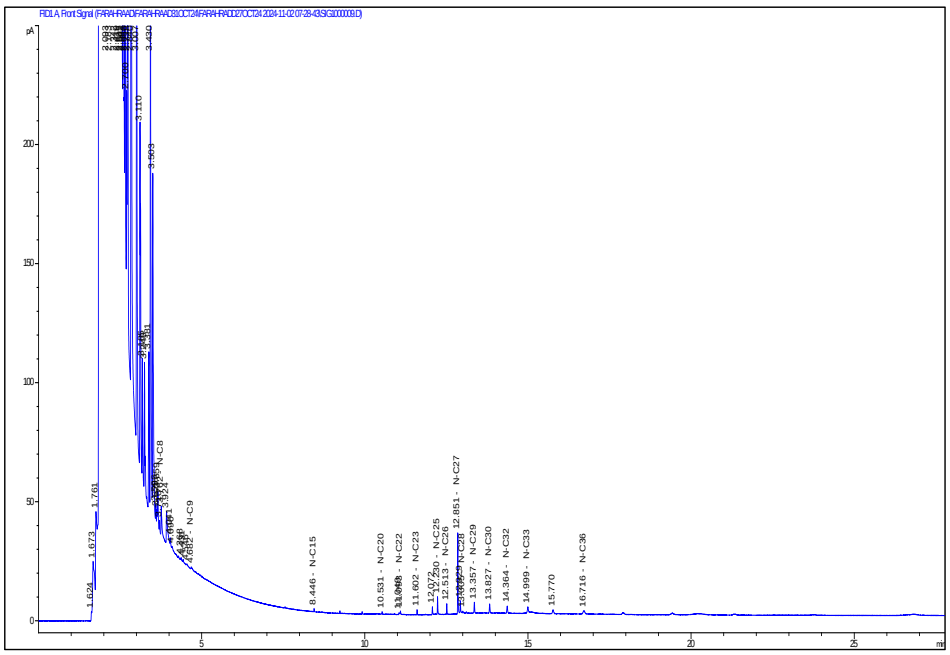

Figure 17 Biodegradation of crude oil at a concentration of 3% by the fungus *Penicillium commune* in the mineral salts medium in the presence of a carbon source of maltose and nitrogen $NaNo_3$ at a temperature of 42°C using gas chromatography-Mass spectroscopy (GC-Mass)

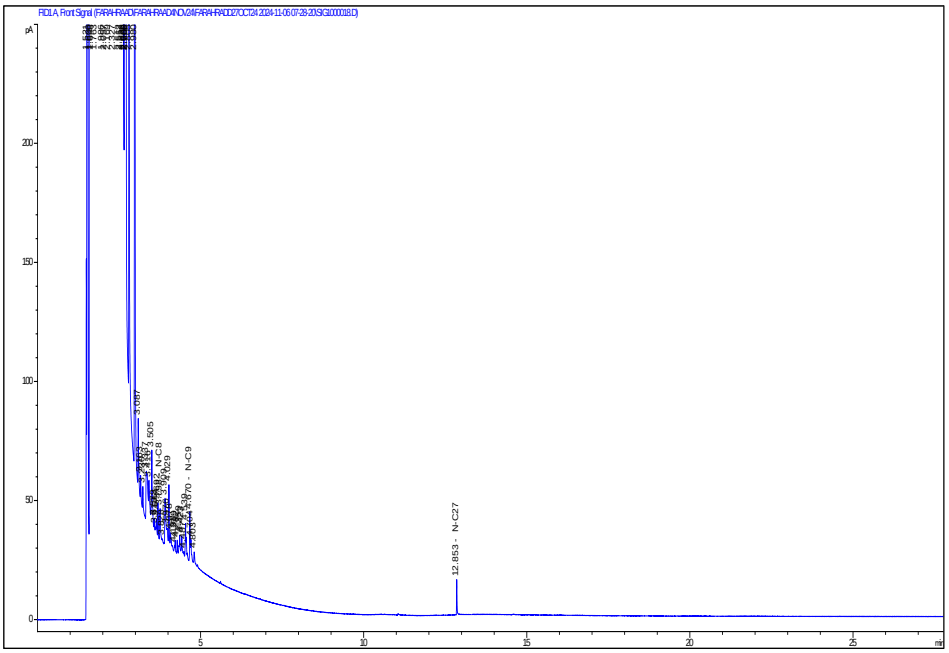

Figure 18 Biodegradation of crude oil at a concentration of 1% by the fungus *Penicillium commune* in the mineral salts medium in the presence of a carbon source of glucose and nitrogen NH_4CL at a temperature of 42°C using gas chromatography-Mass spectroscopy (GC-Mass)

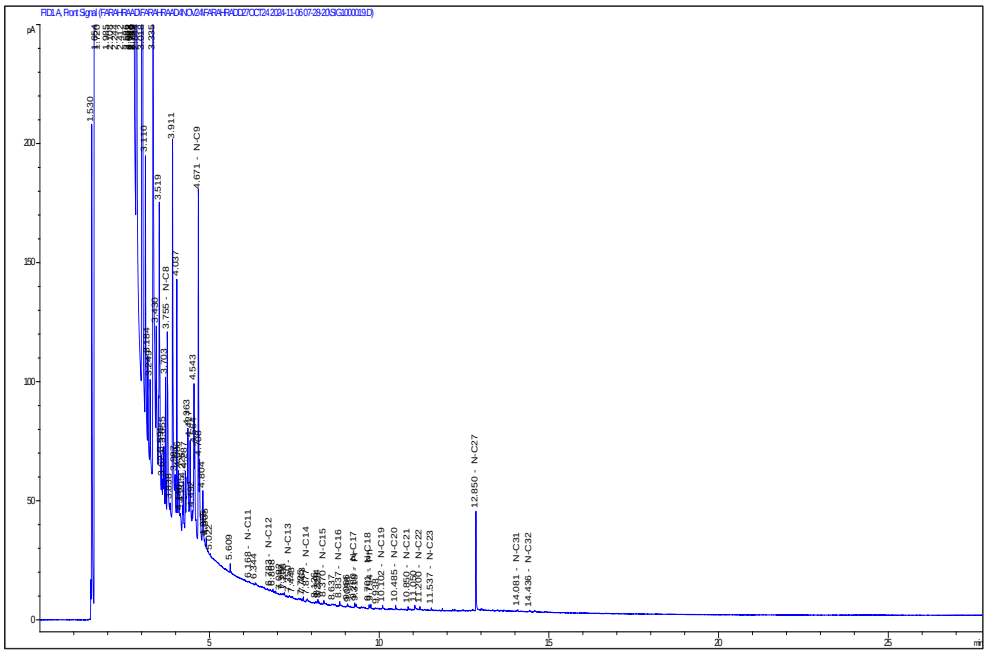

Figure 19 Biodegradation of crude oil at a concentration of 2% by the fungus *Penicillium commune* in the mineral salts medium in the presence of a carbon source of glucose and nitrogen NH_4CL at a temperature of 42°C using gas chromatography-Mass spectroscopy (GC-Mass)

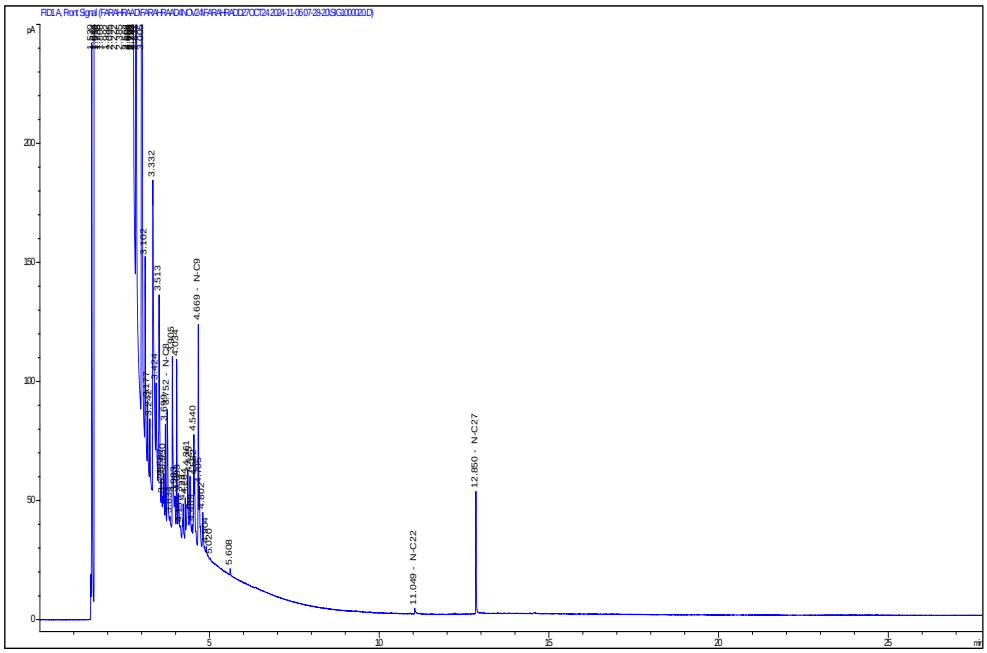

Figure 20 Biodegradation of crude oil at a concentration of 3% by the fungus *Penicillium commune* in the mineral salts medium in the presence of a carbon source of glucose and nitrogen NH_4CL at a temperature of 42°C using gas chromatography-Mass spectroscopy (GC-Mass)

4. Conclusion

This study appear that *Penicillium commune* have ability to Biodegradation of crude oil by after 28 days of incubation in mineral salts medium at different temperatures and at different concentrations of crude oil , and when adding different carbon and nitrogen sources, it reached different percentages.

Differences in temperature and different oil concentrations, as well as the different carbon and nitrogen sources, do not have a significant impact on biodegradation, as *Penicillium commune* appear different rates of biodegradation. The present study has advanced our knowledge of crude oil. The behavior of fungi in soil contaminated with crude oil, and how these fungi break down or decompose pollutants in the environment

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Costello J (1979) Morbidity and mortality study of shale oil work-ers in the United states. Environ Health perspect 30:205-208

[2] Al-Saad HT, saeed A, Salman NA (2003). Marine pollution, Hadida University pub. Yamen, 260 p.

[3] Gesinde AF, Agbo EB, Agho MO, Dike EFC (2008). Bioremediation of some Nigeria and Arabian crude oils by fungal isolated. Int. Pure Applied Sci.,2:37-44

[4] Onuoha Cl, Arinze AE, Ataga AE (2003). Evaluation of growth of some fungi in crude oil polluted environment. GlobJ Agric Sci 2: 1596-2903

[5] Lotfinasabasl S, Gunale VR, Rajurkar NS (2012). Assessment of Petroleum hydrocarbon degradation from soil and tarball by fungi, Biosci. Discov.,3(2): 186-192

[6] Letcher PM, powell M (2001). Distribution of Zoosporic fungi in forest soils of the Blue Ridge and Appalachian Mountains of Virginia. Micologia.,93:1029-1041

[7] Cappuccino JG, Sherman N (1987). Microbiology manual.Benjamine cumming publishing co. Inc.Calfornia.Amsterdam.Sydney 120 P

[8] Sukkar AF (2021). The ability of fungi to degrade some polycyclic hydrocarbon compounds isolated from sediments of the central marshes in Dhi Qar Governorate / southern Iraq. Master's thesis. College of Education for Pure Sciences, Dhi Qar University. 157 pages

[9] Watanabe T (2002). Pictorial atlas of soil and seed fungi morphologies of cultured fungi and key to species CRC Press, 486pp

[10] Okerenttugba P, Ezeronye O (2003). Petroleum degrading potentials of single and mixed microbial cultures isolated from rivers and refinery effluent in Nigeria. Afri. J Biotechnol., 2(9): 288-292

[11] Al-Jawhari IFH (2014). Biodegradtion of kerosene by Aspergillus niger and Rhizopus stolinifer.Journal of Applied and Environmental Microbiology, Vol.2,no.1(2014):31-36

[12] Al-Hawash AB, Alkoorance JT, Abbood HA, Zhang J, Sun J, Zhang X, Ma F (2018). Isolation and Characterization of two crude oil – degrading fungi strains from Rumaila oil field, Iraq. Biotechnol.Rep.,17,104-109[Cross Ref]

[13] Nwachukwu SU, Ugoji Eo. (1995). Impact of crude petroleum spills on microbial communities of tropical soil. Intern. J. Environ. Sci., 21:169-175

[14] Oboh OB, IIori MO, Akinyemi JO, Adebusoye SA (2006). Hydrocarbon degrading potential of bacteria isolated from Nigerian Bitumen (Tarsand) Deposit.Nature and Science.4(3):1-57

[15] Zafra G, Absalon A E, Cuevas MDC, Cortes-Espinosa DV (2014). Isolation and selection of a highly tolerant microbial consortium with potential for PAH biodegradation from heavy crude oil-contaminated soil. Water, Air, and Soil Pollution 225(2): 1- 18.

[16] Al-Jawhari IFH (2016). Ability of sediment fungi in biodegradation of diesel fuel. Indian Journal of Pure and Applied Biosciences 4(2):27-37