JJBS

Volume 15, Number 3,September 2022
ISSN 1995-6673
Pages 441 – 448
https://doi.org/10.54319/jjbs/150313

Jordan Journal of Biological Sciences

Potential for Crude Oil and Diesel Biodegradation by the Indigenous *Pseudomonas* sp. Strain LGMS7 Using GC-MS and GC-FID Analyses

Abdelkrim Chaida[1,*] , Farid Bensalah[1] , Belhadj Trari[2]

[1]*Laboratory of Microbial Genetics (LGM), Department of Biology, Faculty of Nature and Life Sciences, University Oran 1, 31000 Oran, Algeria;* [2]*Laboratory of Bioactive Organic Synthesis, Department of Industrial Organic Chemistry, Faculty of Chemistry, University of Sciences and Technology of Oran Mohamed Boudiaf-USTO-MB, 31003 Oran, Algeria.*

Received: August 14, 2021; Revised: December 24, 2021; Accepted: January 19, 2022

Abstract

Pollution caused by hydrocarbons in the environment is a serious global issue, especially in countries in development such as Algeria. In this context, *Pseudomonas* sp. strain LGMS7 (MT071345) previously isolated from a soil contaminated with hydrocarbons, in Algeria, was selected to evaluate its crude oil and diesel degradation capacity, individually. For this, we have used an MSM medium with 1% (v/v) of crude oil and 2% (v/v) of diesel as the sole carbon sources, individually, incubated for 27 days at room temperature (25 ± 1 °C) and 150 rpm, and analysed with GC-MS and GC-FID, respectively. Consequently, after analysing of the chromatogram, the results revealed the presence of a linear fraction of aliphatic hydrocarbons *n*-alkane (C13-C30) of average molecular weight for crude oil, with highly biodegradation efficiency of 98.50%. Nevertheless, biodegradation efficiency of 100% was recorded for the lightest molecules of the *n*-alkane fraction (C13-C30), which corresponds to the C13-C14 molecules, and for the heaviest molecules of the same *n*-alkane fraction, which corresponds to molecules C27-C30. Furthermore, biodegradation efficiency >96.00% was recorded for the C15-C26 fraction. While for diesel, biodegradation efficiency of 70% was recorded. Because of its intriguing biodegradation properties for hydrocarbons, this strain appears to be a promising bioremediation candidate for hydrocarbon-polluted soils in Algeria.

Keywords: Biodegradation; Crude oil; Diesel; GC-FID; GC-MS; Pollution

1. Introduction

Crude oil-derived hydrocarbons are the most common type of pollution in the world's environment (Ławniczak *et al,.* 2020). Their carcinogenic, mutagenic and toxic nature poses a serious problem for ecosystems and living beings (Varjani and Upasani, 2016). Algeria is a major oil producer in Africa, with a primarily fossil-fuel-based economy like oil and natural gas (Chaida *et al.,* 2021; Harrouz *et al.,* 2017). However, the consequences of such production are devastating for the environment. Regardless of the pollution source, accidental or as a result of various extraction, refining, or transport activities, it is threatening the life of living beings and endangering the purity of groundwater, a significant source for Algeria's freshwater supply, by the permeability of hydrocarbons through the soil or the sand (in the case of Sahara).

For pollution treatment, various biological and physicochemical methods can be considered. Among these methods is the microorganism-mediated bioremediation method, which is considered to be as a better bio-based approach to remove petroleum hydrocarbons from contaminated sites because it is an economical, profitable, green, and sustainable method (Elkarmi *et al.,* 2008; Logeshwaran *et al.,* 2018).

Crude oil is divided into two main categories: aliphatic and aromatic hydrocarbons, which might be pronounced recalcitrant and threatening to health (Hidayat and Tachibana, 2012). Several bacterial strains can easily degrade the major crude oil's aliphatic component. However, the polycyclic aromatic hydrocarbons (PAHs) and large branched-chain fractions, on the other hand, are difficult to degrade because of their complex structures. (Hasanuzzaman *et al.,* 2007; Khan *et al.,* 2019).

Diesel, defined as a complicated hydrocarbon pollutant, consists of an aggregate of alkanes and aromatics, which are regularly stated as soil pollutants (Gallego *et al.,* 2001). The microorganisms' natural abilities to overcome the constraints of bioavailability plague the bioremediation of those pollutants. In addition, pH, nutrients, electron acceptor availability and temperature are among the environmental factors to consider (Mukherji and Vijay, 2002).

For bioremediation of crude oil-polluted sites, several bacterial strains have been reported in the literature, belonging to the genera *"Alcaligenes*, *Brevibacillus*, *Paenibacillus*, *Stenotrophomonas*,

* Corresponding author. e-mail: chaida.abdelkrim@edu.univ-oran1.dz ; chaida.abdelkrim@gmail.com.

** **Abbreviations:** MSM: Mineral Salt Medium; NB medium: Nutrient broth medium; GC-FID: Gas chromatography-flame ionization detection; GC-MS: Gas chromatography-Mass spectrometry

Lysinibacillus, *Bacillus, Delftia*, *Achromobacter*, and *Pseudomonas"* (Roy *et al., 2014*). However, *Pseudomonas* sp., and *Achromobacter xyloxidans* have been identified as the best biodegraders of hydrocarbons. Moreover, special strategies including Gas chromatography (GC) in addition to gas chromatography-mass spectrometry (GC-MS) may be employed to evaluate crude oil's ability to degrade by comparison of abiotic and biotic control (Varjani *et al.,* 2015).

In Algeria, studies on the biodegradation of hydrocarbons are uncommon. However, a better understanding of the mechanisms involved in the degradation of hydrocarbons by indigenous microorganisms could be extremely important for future bioremediation programs at polluted sites.

In this context, the present study highlighted a promising capacity of the indigenous bacterium *Pseudomonas* sp. strain LGMS7 (MT071345) isolated from a hydrocarbon-polluted soil in western Algeria, capable to degrade crude oil and diesel at room temperature (25 ± 1 °C), using greater in-intensity studies techniques, including GC-MS and GC-FID, to assess degradation performance of every oil individually.

2. Materials and methods

2.1. Strains and chemicals

Pseudomonas sp. strain LGMS7 was previously isolated from a hydrocarbon-contaminated soil at Ain El Arbaa, Algeria, after enrichment in the nutrient broth medium (NB) (Chaida *et al.,* 2021) (Fig. 1). The sequence of 16S rRNA of the strain LGMS7 has been defined and placed in the GenBank nucleotide database under the accession number (MT071345). Regarding the chemicals, the crude oil and diesel gas were purchased from an oil refinery (NAFTEC) in Arzew and a service station in Oran, western Algeria, respectively. All additional chemicals that were used, including dichloromethane, were purchased from the Sigma-Aldrich Company.

Figure 1. Hydrocarbon-contaminated soil at Ain El Arbaa region (Aïn Temouchent city), Algeria

2.2. Media composition

The mineral salt medium (MSM) used consisted of (g/l): KH_2PO_4 (0.7), $NaNO_3$ (2), $CaCl_2.2\ H_2O$ (0.1), $FeSO_4.7\ H_2O$ (0.001), Na2HPO4 (0.9), MgSO4.7 H2O (0.4), as well as 1 ml of a solution composed of different trace element consisted of (g/l): $Na_2MoO_4.2\ H_2O$ (0.06), $CuSO_4.5\ H_2O$ (0.5), H_3BO_4 (0.26), $MnSO_4.\ H_2O$ (0.5), $ZnSO_4.7\ H_2O$ (0.7), for a litre of distilled water (Chebbi *et al.,* 2017). To achieve a pH of 7.2, solutions of (5M) NaOH and (6N) HCl were used. Afterwards, separately, it was followed by the addition of crude oil and diesel at a rate of 1 and 2%, respectively. The medium was autoclaved at 121 °C for 20 minutes to sterilize it.

2.3. Growth of the strain LGMS7 using diesel and crude oil as only carbon sources

We have studied the strain LGMS7's capacity to grow utilizing only carbon sources from diesel and crude oil. The experiment was conducted with an inoculum of 3% (v/v) in Erlenmeyer flask composed of 100 ml of the MSM supplemented with (1%, v/v) of crude oil and (2%, v/v) of diesel individually, for 27 days of incubation at room temperature (25 ± 1 °C), and 150 rpm. A 600-nm optical density measurement and colony-forming units (CFUs) were used to monitor bacterial growth. The abiotic control (without the addition of strain LGMS7) was also used with the same experimental conditions. Results are defined as the mean of two replicates experiments ± standard deviation.

2.4. Liquid-liquid extraction of crude oil and diesel

A double liquid/liquid extraction with dichloromethane (Khan *et al.,* 2005) on abiotic control (without the LGMS7 strain) and biotic control (with the LGMS7 strain) was used for the recovery of the hydrocarbons tested (Fig. 2). Centrifugation was performed individually for 20 minutes at 6000 rpm to extract the supernatant after incubation of LGMS7 strain for 27 days at room temperature (25 ± 1 °C) in MSM medium containing (1%, v/v) and (2%, v/v) crude oil and diesel, respectively. The extraction operation was conducted by inserting an equal amount of the supernatant and the dichloromethane in a separation funnel (Khan *et al.,* 2005). A series of systematic stirring followed by degassing was carried out to extract the hydrocarbons found in the supernatant and retrieve them with the extraction solvent.

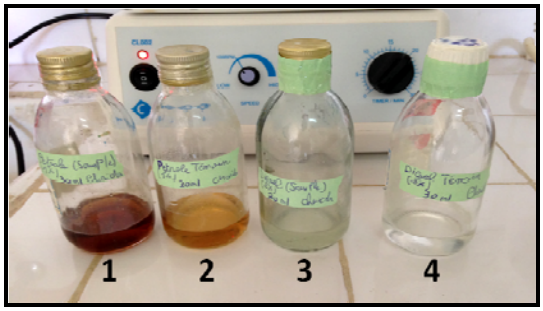

Figure 2. Extraction of crude oil and diesel with dichloromethane after incubation of the samples for 27 days at room temperature and at 150 rpm. (1) Crude oil (biotic control); (2) crude oil (abiotic control); (3) diesel (biotic control); (4) diesel (abiotic control)

2.5. GC-MS analyses

GC-MS analyses were carried out to confirm the strain LGMS7's ability to degrade crude oil using a Clarus 500 GC interfaced to a Clarus 500 MS equipped with an EI source and auto-injector. TurboMass software was used for data processing (PerkinElmer, USA). The chromatography of undifferentiated polar amines has been privileged using a special column (50% methyl-50% phenyl-silicone). The column temperature was ramped from 75ºC for 1min to 275 ºC at 10 ºC/min, during 10 min, with a total of 31

minutes per sample. Helium was the carrier gas used at a debit of 1 ml/min and was brought to a pressure of 30 Psi. Both the injector and GC-MS transfer line temperatures were 250 °C. The injector had a double-tapered liner installed during which it was set to splitless mode for two minutes following the injection (volume of injection 1ml, the division ratio of 10: 1). The electron multiplier detector and the electron ionisation energy have been set to 358 V and of 70 eV, respectively. A rate of 250 scans/sec was used to acquire spectral data with a scanning range of 30 to 600 u. The percentage of degradation (%) of crude oil was calculated as follows:

Degradation percentage (%) = 100 - (Tpb x 100 / Tpa), where Tpb: Chromatogram's total peak area (biotic control); and Tpa: Chromatogram's total peak area (abiotic control) (Varjani *et al.,* 2015).

2.6. GC-FID analyses

The biodegradation potential of diesel by the LGMS7 strain was studied using a GC Perkin Elmer Clarus 500 with HS40 "Headspace" injector coupled to FID using an HP5 30 m*0.32 ID 0.25 µm capillary column. The data acquisition and processing system was obtained using a computer equipped with Total Chrom 6.3 software. Helium (He) was used as a carrier gas, with 2 µl injection volume using a split injection mode with a 5: 1 split ratio. A 40 °C pre-oven temperature was used to achieve a higher peak resolution, with 10 min preserve period; ramp as much as 200 °C for 10 mn, with a upward push in pace of 5 °C/min. The temperatures of the detector and injector were both adjusted to 250 °C. Degradation percentage (%) of diesel was calculated as follows:

Degradation percentage (%) = 100 - (Tpb x 100 / Tpa), where Tpb: Chromatogram's total peak area (biotic control); and Tpa: Chromatogram's total peak area (abiotic control) (Varjani *et al.,* 2015).

2.7. Statistical analysis

Bacterial growth determination was performed at two replicates experiments. GraphPad Prism 6 (Trial version) was performed to calculate the means and standard deviations.

3. Results

3.1. Bacterial growth

Monitoring the growth of the strain LGMS7 during 27 days of incubation at room temperature, together with non-static conditions, has demonstrated its ability to grow individually with (1%, v/v) and (2%, v/v) crude oil and diesel, respectively in MSM medium, where maximum OD was registered at 0.52 ± 0.04 (300.10^7 CFU/ml) and 0.92 ± 0.04 (200.10^7 CFU/ml) (Fig. 3a, b). Accordingly, the stain LGMS7 is capable of metabolizing diesel and crude oil, which are used as the only carbon sources.

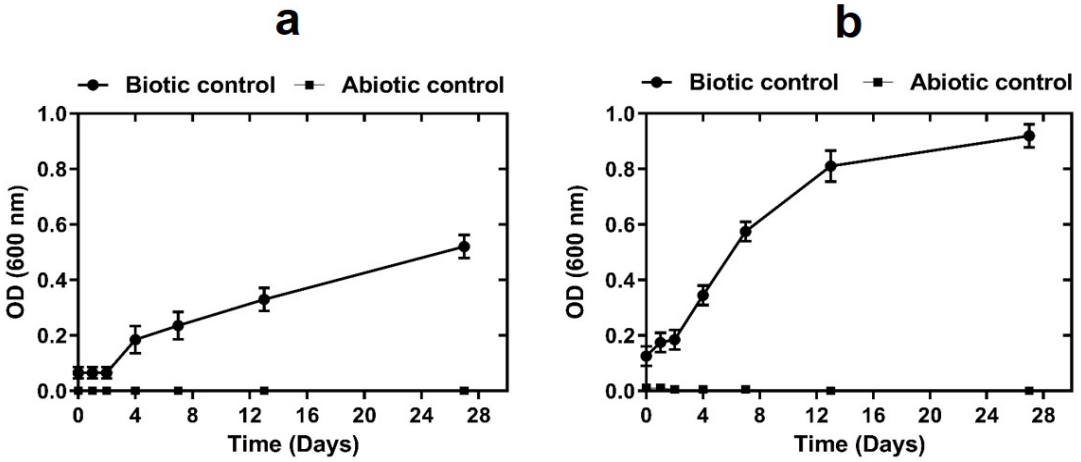

Figure 3. Growth monitoring (OD 600 nm); biotic control (●) and abiotic control (■) in the presence of (1%, v/v) of crude oil as sol carbon source **(a)**; Growth monitoring (OD 600 nm); biotic control (●) and abiotic control (■) in the presence of (2%, v/v) of diesel as sol carbon source **(b)**, of the strain LGMS7 for 27 days of incubation at room temperature, and 150 rpm

3.2. GC-MS analyses

Crude oil metabolizing capacity of strain LGMS7 was confirmed by GC-MS. However, examination of the obtained chromatograms revealed the presence of a linear fraction of aliphatic hydrocarbons *n*-alkane (C13-C30) of average molecular weight, i.e. with several carbon atoms of 13-30 (Table 1). Each chromatogram peak has a defined retention time that corresponds to a hydrocarbon molecule present in crude oil that was identified using mass spectrometry (MS) and a data library (Fig 4). Besides, through calculating the total peak area by integration, which corresponds to each retention time recorded on the chromatograms of both biotic and abiotic controls, we obtained a very interesting degradation efficiency (%) of the *n*-alkane fraction (C13-C30), equal to 98.50%, with a degradation efficiency of 100% noted for the lightest molecules of the *n*-alkane fraction (C13-C30), which corresponds to the C13-C14 molecules, and for the heaviest molecules of the same *n*-alkane fraction, which corresponds to the C27-C30 molecules. Concerning the other C15-C26 molecules, degradation efficiency > 96.00% was recorded (Table 1, Fig. 5).

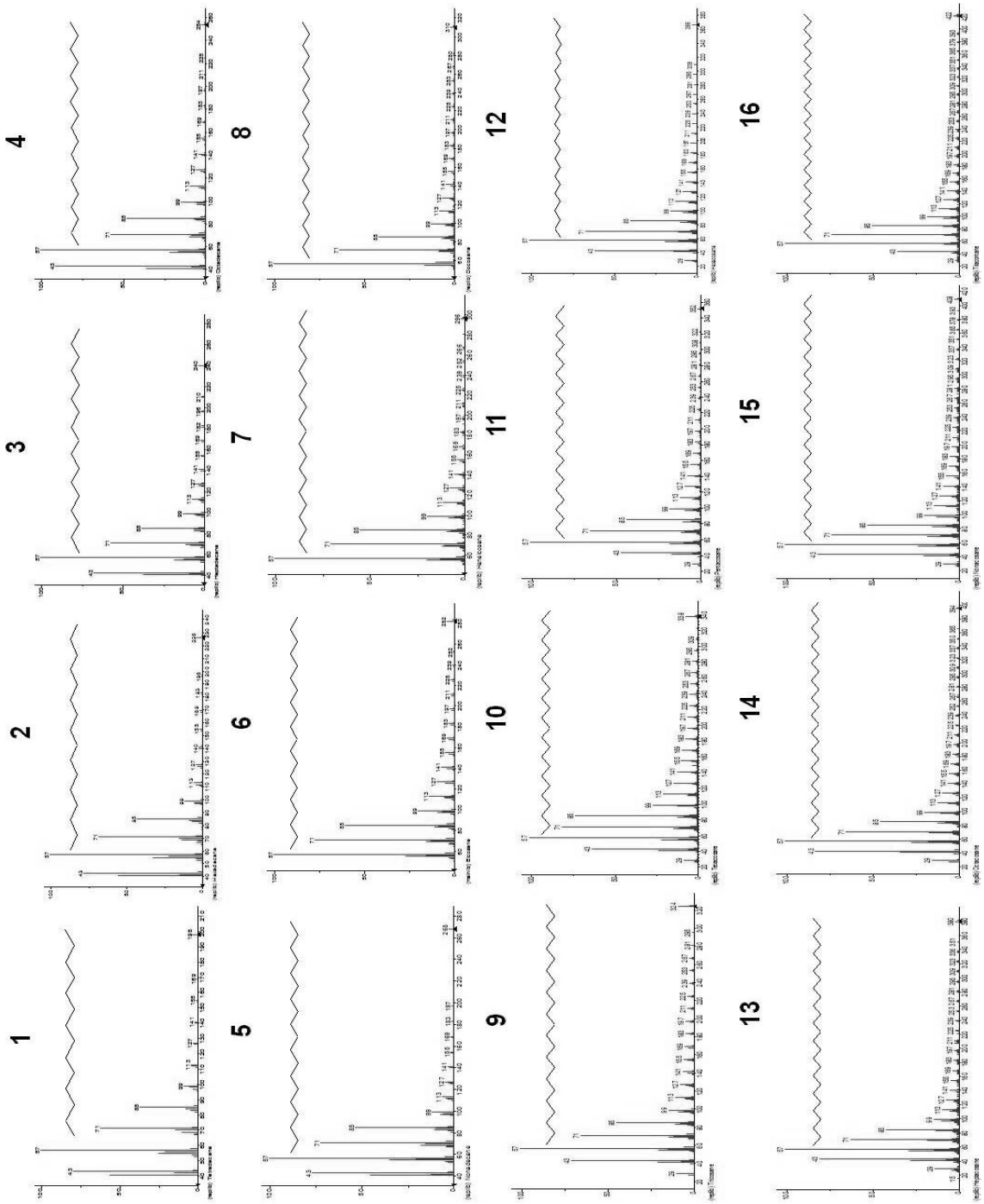

Figure 4. Mass spectrometry (MS) of the majority of components of *n*-alkanes in crude oil: Tetradecane (1), Hexadecane (2), Heptadecane (3), Octadecane (4), Nonadecane (5), Eicosane (6), Heneicosane 7), Docosane (8), Tricosane (9), Tetracosane (10), Pentacosane (11), Hexacosane (12), Heptacosane (13) Octacosane (14) Nonacosane (15), Triacontane (16)

Table 1. Degradation efficiency (%) of *n*-alkanes fraction of crude oil (1%, v/v) by *Pseudomonas* sp. strain LGMS7 after 27 days of incubation at room temperature and 150 rpm

Retention time (mn)	Molecular masse (g/mol)	Crude chemical formula	Compound	Degradation effeciency (%)
7.90	184	$C_{13}H_{28}$	Tridécane	100
9.27	198	$C_{14}H_{30}$	Tetradécane	100
10.54	212	$C_{15}H_{32}$	Pentadécane	100
11.75	226	$C_{16}H_{34}$	Hexadécane	99.074
12.90	240	$C_{17}H_{36}$	Heptadécane	97.522
13.97	254	$C_{18}H_{38}$	Octadécane	96.656
15.00	268	$C_{19}H_{40}$	Nonadécane	96.422
15.98	282	$C_{20}H_{42}$	Eicosane	96.803
16.91	296	$C_{21}H_{44}$	Heneicosane	98.125
17.82	310	$C_{22}H_{46}$	Docosane	97.985
18.68	324	$C_{23}H_{48}$	Tricosane	97.158
19.51	338	$C_{24}H_{50}$	Tetracosane	99.193
20.30	352	$C_{25}H_{52}$	Pentacosane	96.500
21.08	366	$C_{26}H_{54}$	Hexacosane	97.536
21.88	380	$C_{27}H_{56}$	Heptacosane	100
22.79	394	$C_{28}H_{58}$	Octacosane	100
23.87	408	$C_{29}H_{60}$	Nonacosane	100
25.15	422	$C_{30}H_{62}$	Triacontane	100

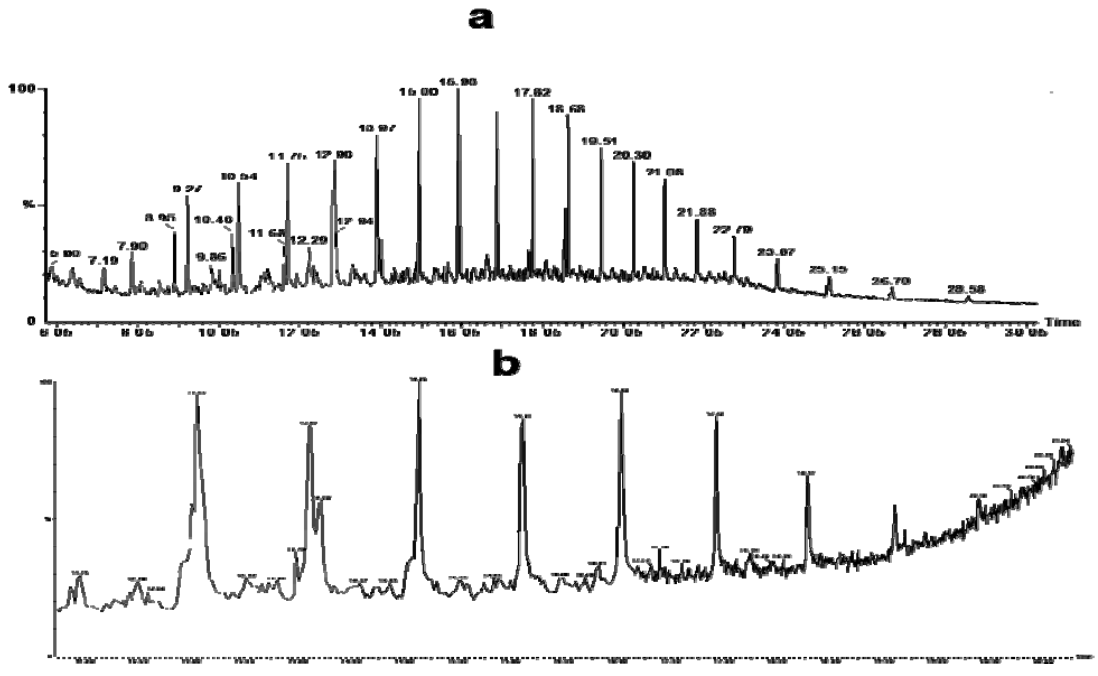

Figure 5. Crude oil (1%, v/v) analysis using GC-MS: **(a)** abiotic control; **(b)** biotic control

3.3. GC-FID analyses

GC-FID analysis confirmed strain LGMS7's ability to digest diesel oil (Fig. 6). Through calculating the total peak area by integration, we have recorded degradation efficiency (%) of 70% of the diesel fraction (Table 2). The retention times recorded on the chromatogram of the abiotic control (22.438 min, 26.233 min, 26.808 min, 29.585 min, and 32.640 min) were not recorded on the chromatogram of the biotic control. The disappearance of the peaks is the result of total degradation of the studied fraction of diesel with an efficiency of 100%. In addition, biodegradation efficiency of 17.83%, 37.83%, 2.90%, 72.91%, 68.55% was recorded for the other compounds (Table 2). The peak recorded on the chromatogram of the biotic control at a retention time of 4.84 min, which is not recorded on the abiotic chromatogram, is probably a compound of the degradation products of diesel.

Table 2. Degradation efficiency (%) of diesel (2%, v/v) by *Pseudomonas* sp. strain LGMS7 after incubation period of 27 days of at room temperature and 150 rpm

Abiotic control		**Biotic control**		
Retention time	peak area	Retention time	peak area	**Degradation efficiency (%)**
(mn)	µ V.s	(mn)	µ V.s	
2.60	23323.26	2.62	19164.75	17.83
3.31	386157.54	3.33	240086.53	37.83
4.51	8291158.41	4.55	8051391.46	2.90
no peak	/	4.84	8385.14	/
5.13	299386.5	5.16	81111.06	72.91
22.44	7945.57	no peak	/	100
26.23	31809.27	no peak	/	100
26.81	8456.81	no peak	/	100
29.59	29717.14	no peak	/	100
32.64	29316.84	no peak	/	100
35.46	16947.33	35.50	5330.96	68.55

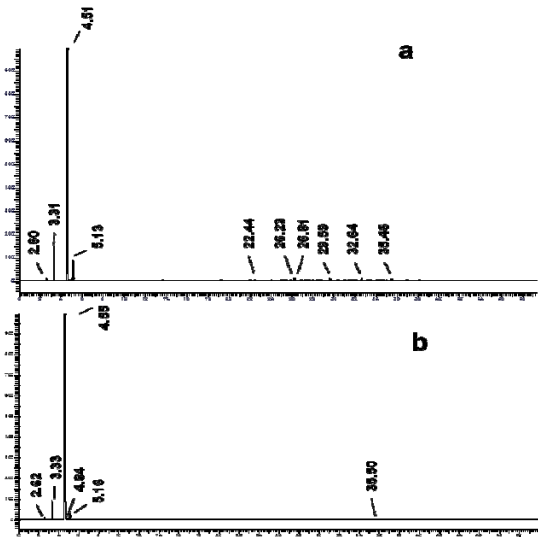

Figure 6. Diesel (2%, v/v) analysis using GC-FID: **(a)** abiotic control; **(b)** biotic control

4. Discussion

Most polluting oil appears to be used as a source of organic carbon by microbes, causing petroleum components to break down into lower molecular weight compounds (Chhatre *et al.,* 1996). Nevertheless, *n*-Alkanes are the components of aliphatic hydrocarbons that decompose the fastest, and are categorized into four molecular mass categories: gaseous alkanes, aliphatic hydrocarbons of low molecular mass (C8-C16), aliphatic hydrocarbons of medium molecular mass (C17 - C28), and finally, aliphatic hydrocarbons of high molecular mass (> C28) (Abbasian *et al.,* 2015). Numerous studies have shown the biodegradation capacity of crude oil's *n*-alkane group by several strains of bacteria, with different degradation efficiencies, in agreement with the findings of the current study.

In this study, we discovered an intriguing crude oil degradation efficiency, by strain LGMS7 compared to the majority of studies available in the literature. We've also shown that this strain is better at biodegrading petroleum hydrocarbons than other *Pseudomonas* spp. For example, Varjani *et al.* (2015) found that the fraction (C8-C35) was degraded by 83.49%, using GC-FID analysis with a higher concentration (3%, v/v) of crude oil as the only carbon source. Likewise, Varjani and Upasani (2016) published their report showing a degradation percentage of 60.63% of the fraction (C8-C36 +) of petroleum (3%, v/v) on average, for 60 days at 37 °C in non-static conditions (180 rpm). In addition, the halotolerant *Pseudomonas* sp. strain NAPH6 was tested for crude oil degradation using GC-MS analysis. It was found that it was capable of metabolizing 96.2% of crude oil aliphatic group (1%, v/v), in basic medium with NaCl concentration of 30 g/l, within 20 days of incubation at 37 °C (Hentati *et al.,* 2020). Zenati (2018) showed significant degradation of 83.68% of petroleum *n*-alkanes (1%, v/v) by the *Marinobacter hydrocaromrehtru*. Furthermore, it has been reported that *n*-alkanes (C14 - C30) degrade faster than PAHs by *Pseudomonas* sp. (Sugiura *et al.,* 1996).

On the other hand, several authors isolated and characterized bacteria from hydrocarbon-contaminated soils, capable of producing biosurfactant and degrading hydrocarbon (Chebbi *et al.,* 2017; Ebadi *et al.,* 2018). For instance, Ebadi *et al.* (2017) have proven the bioremediation efficiency of an oil-contaminted saline soil using a consortium of *Pseudomonas aeruginosa*, producer of biosurfactants. They discovered that using the consortium to treat polluted soils increased dehydrogenase activity significantly (approximately 2 times). In a lettuce seedling bioassay, the level of phytotoxicity in the soil was reduced by 30% after treatment compared to untreated soil. Treatment with a suitable bacterial consortium could help to mitigate the negative effects of salinity on petroleum microbial degradation. As a result, microbial remediation of saline petroleum-contaminated soils will be more efficient.

In our study, crude oil degradation by *Pseudomonas* sp. strain LGMS7 at room temperature and close to the average outside soil temperature is a precondition for its *in situ* application when dealing with hydrocarbon-polluted soils using bioremediation technology, as reported by the study of Varjani and Upasani (2016).

In Algeria, only a few detailed studies have been conducted on the microbial capacity of hydrocarbon biodegradation. For instance, M'rassi *et al*. (2015) investigated the bacteria's' abilities to degrade both aliphatic and aromatic hydrocarbons in the contaminated soil of a refinery, in Arzew, Algeria. These authors have reported the growth of several strains related to *Pseudomonas* and effectively the use of an *n*-branched alkane like pristane, while *Enterobacter, Pseudomonas, Acinetobacter, and Bacillus* strains were found to be capable of degrading PAHs of high molecular mass like pyrene and benzo [a] pyrene. Accordingly, the *Pseudomonas* sp. strain LGM2 had the characteristic of degradation ability of both PAHs and *n*-alkanes, indicating that it could play a crucial role in hydrocarbon-polluted areas. Moreover, numerous bacterial strains were selected that could be used to develop and implement appropriate biological strategies for contaminated soil and refinery wastewater treatment (M'rassi *et al.,* 2015).

Diesel pollution is a growing environmental problem. The most common sources of soil and groundwater pollution are diesel spills caused by numerous production storage issues, as well as transportation mishaps, and other issues related to damage at the level of the pipes (Lee *et al.,* 2006). Diesel is made up of saturated hydrocarbons such as paraffin and aromatic hydrocarbons. The ability of microorganisms to metabolize diesel and use it as an energy source for growth has been documented in numerous studies in correlation with our study. In this context, Palanisamy *et al*. (2014) demonstrated that *Acinetobacter baumannii* could degrade > 99% of diesel (4%, v/v), incubated for 5 days at 37 °C and pH 7, using GC-MS analysis. According to these authors, *A. baumannii* can be used to effectively degrade diesel from diesel-polluted industrial waste. Ameen *et al.* (2016) studied the diesel degradation ability by fungi. An oil-degrading fungus consortium was found to be highly effective, as evidenced by the chromatogram of the diesel fuel after an incubation period at 0 and 30 days. As a result, all of the hydrocarbons found in diesel were completely destroyed, yielding short-chain compounds.

Additionally, *Burkholderia cepacia* and *Exiguobacterium aurantiacum* were able to use diesel substrate as the only carbon source. For diesel degradation, the crops showed promising results for the *n*-alkane fraction (C9-C26). They have also shown a degradation capacity of pristane (Mohanty and Mukherji, 2008).

The strain LGMS7 has an advantage when it comes to growth over diesel (2%, v/v) as the only carbon source at room temperature (25 ± 1 °C), which implies its ability to adapt to variations in the temperature during its growth, allows its use *in situ* in diesel-contaminated soil where it can resist variations in outside temperatures. In this context, Michaud *et al.* (2004) investigated the temperature effect on the efficiency of diesel biodegradation by two strains of bacteria. Therefore, after 60 days of growth in mineral medium containing (1%, v/v) commercial diesel, gas chromatographic analysis revealed a degradation percentage of 55.20% and 86% for strain E28 at 4° and 20 °C, respectively. While concerning the strain E60, a degradation rate of 57.66% was recorded at 4 °C and 89.2% at 20 °C. These authors suggested the future biotechnological processes in which these two bacteria could be used, directly in the form of microorganisms released on the ground in contaminated marine environments, both cold and temperate.

Additional investigations will be performed in the future to examine the LGMS7 strain's ability to degrade recalcitrant hydrocarbons like PAHs, under various stress conditions, including pH, salinity, and temperature. However, additional research will be conducted on the genes implicated in biodegradation, to fully comprehend the strain's degradation mechanisms, and consequently develop a strategy for implementing it on hydrocarbon-contaminated sites (Saadoun et al., 2020).

5. Conclusion

In the current research, we analysed the ability of the indigenous bacterium *Pseudomonas* sp. strain LGMS7 to metabolize the crude oil and diesel fuel used as only source of carbon in MSM medium at the room temperature. Interestingly, strain LGMS7 was able to degrade 98.50% of the linear fraction *n*- alkanes (C13-C30) of aliphatic hydrocarbons of crude oil (1%, v/v) and 70% of the diesel fraction (2%, v/v). Other studies will have to be carried out to assess the capacity of this strain to degrade recalcitrant hydrocarbons such as long branched-chain hydrocarbons and PAHs. This newly isolated strain, which has previously been studied for its ability to reduce surface tension and produce rhamnolipid biosurfactants, represents a promising path for the treatment of hydrocarbon-polluted sites in Algeria.

Acknowledgments

We are grateful to the chemistry department of the scientific police of the Oran police station for the GC-MS and GC-FID analyses.

References

Abbasian F, Lockington R, Mallavarapu M, Naidu R .2015. A comprehensive review of aliphatic hydrocarbon biodegradation by bacteria. *Appl. Biochem. Biotechno*, **176**: 670-699.

Ameen F, Moslem M, Hadi S, Al-Sabri AE .2016. Biodegradation of diesel fuel hydrocarbons by mangrove fungi from Red Sea Coast of Saudi Arabia. *Saudi J. Biol*, Sci, **23**: 211-218.

Chaida A, Chebbi A, Bensalah F, Franzetti A .2021. Isolation and characterization of a novel rhamnolipid producer *Pseudomonas* sp. LGMS7 from a highly contaminated site in Ain El Arbaa region of Ain Temouchent, Algeria*. 3 Biotech,* **11:** 1-15.

Chebbi A, Hentati D, Zaghden H, Baccar N, Rezgui F, Chalbi M, Sayadi S, Chamkha M .2017. Polycyclic aromatic hydrocarbon degradation and biosurfactant production by a newly isolated *Pseudomonas* sp. strain from used motor oil-contaminated soil. *Int. Biodeterior. Biodegrad*, **122**: 128-140.

Chhatre S, Purohit H, Shanker R, Khanna P .1996. Bacterial consortia for crude oil spill remediation. *Water Sci. Technol*, **34**: 187-193.

Ebadi A, Olamaee M, Khoshkholgh Sima NA et al .2018. Isolation and Characterization of Biosurfactant Producing and Crude Oil Degrading Bacteria from Oil Contaminated Soils. *Iran J Sci Technol Trans Sci,* **42**: 1149–1156

Ebadi A, Sima NAK, Olamaee M, Hashemi M, Nasrabadi RG .2017. Effective bioremediation of a petroleum-polluted saline soil by a surfactant-producing *Pseudomonas aeruginosa* consortium. *J. Adv. Res,* **8**: 627-633.

Elkarmi A, Abu-Elteen K, Khader M .2008. Modeling the biodegradation efficiency and growth of *Pseudomonas alcaligenes* utilizing 2, 4-dichlorophenol as a carbon source Pre- and Post-exposure to UV radiation. *Jordan J. Biol. Sci*, **1** : 7-11

Gallego JL, Loredo J, Llamas JF, Vázquez F, Sánchez J .2001. Bioremediation of diesel-contaminated soils: evaluation of potential in situ techniques by study of bacterial degradation. *Biodegradation,* **12**: 325-335.

Harrouz A, Abbes M, Colak I, Kayisli K .2017. Smart grid and renewable energy in Algeria. Paper presented at the 2017 IEEE 6th International Conference on Renewable Energy Research and Applications (ICRERA).

Hasanuzzaman M, Ueno A, Ito H, Ito Y, Yamamoto Y, Yumoto I, Okuyama H .2007. Degradation of long-chain n-alkanes (C36 and C40) by *Pseudomonas aeruginosa* strain WatG. *Int. Biodeterior. Biodegrad,* **59**: 40-43.

Hentati D, Chebbi A, Mahmoudi A, Hadrich F, Cheffi M, Frikha I, Sayadi S, Chamkha M .2020. Biodegradation of hydrocarbons and biosurfactants production by a newly halotolerant *Pseudomonas* sp. strain isolated from contaminated seawater. *Biochem. Eng. J,* **166** : 107861.

Hidayat A, Tachibana S .2012. Biodegradation of aliphatic hydrocarbon in three types of crude oil by *Fusarium* sp. F 092 under stress with artificial sea water. *J. Environ. Sci. Technol,* **5**: 64-73.

Khan AHA, Ayaz M, Arshad M et al .2019. Biogeochemical Cycle, Occurrence and Biological Treatments of Polycyclic Aromatic Hydrocarbons (PAHs). *Iran J Sci Technol Trans Sci,* **43**: 1393–1410

Khan Z, Troquet J, Vachelard C .2005. Sample preparation and analytical techniques for determination of polyaromatic hydrocarbons in soils. *Int. J. Environ. Sci. Technol,* **2**: 275-286.

Ławniczak Ł, Woźniak-Karczewska M, Loibner AP, Heipieper HJ, Chrzanowski Ł .2020. Microbial degradation of hydrocarbons-basic principles for bioremediation: a review. *Molecules,* **25**: 856.

Lee M, Kim M, Singleton I, Goodfellow M, Lee ST .2006. Enhanced biodegradation of diesel oil by a newly identified *Rhodococcus baikonurensis* EN3 in the presence of mycolic acid. *J. Appl. Microbiol,* **100**: 325-333.

Logeshwaran P, Megharaj M, Chadalavada S, Bowman M, Naidu R .2018. Petroleum hydrocarbons (PH) in groundwater aquifers: An overview of environmental fate, toxicity, microbial degradation and risk-based remediation approaches. *Environ. Technol. Innovation,* **10**: 175-193.

M'rassi AG, Bensalah F, Gury J, Duran R .2015. Isolation and characterization of different bacterial strains for bioremediation of n-alkanes and polycyclic aromatic hydrocarbons. Environ. *Sci. Pollut. Res,* **22**: 15332-15346.

Michaud L, Giudice AL, Saitta M, De Domenico M, Bruni V .2004. The biodegradation efficiency on diesel oil by two psychrotrophic Antarctic marine bacteria during a two-month-long experiment. *Mar. Pollut. Bull,* **49**: 405-409.

Mohanty G, Mukherji S .2008. Biodegradation rate of diesel range n-alkanes by bacterial cultures *Exiguobacterium aurantiacum* and *Burkholderia cepacia*. *Int. Biodeterior. Biodegrad,* **61**: 240-250.

Mukherji S, Vijay A .2002. Critical issues in bioremediation of oil and tar contaminated sites. Paper presented at the ACE 2002*:* International Conference on Advances in Civil Engineering.

Palanisamy N, Ramya J, Kumar S, Vasanthi N, Chandran P, Khan S .2014. Diesel biodegradation capacities of indigenous bacterial species isolated from diesel contaminated soil. *J. Environ. Health Sci. Eng,* **12**: 1-8.

Roy AS, Baruah R, Borah M, Singh AK, Boruah HPD, Saikia N, Deka M, Dutta N, Bora TC .2014. Bioremediation potential of native hydrocarbon degrading bacterial strains in crude oil contaminated soil under microcosm study. *Int. Biodeterior. Biodegrad,* **94**: 79-89.

Saadoun I, Alawawdeh M, Jaradat Z, Ababneh Q .2020. *Streptomyces* flora in chronically fuel oil-polluted soils and analysis of their alkane hydroxylase (alkB) gene by PCR. *Jordan J. Biol. Sci*, **13**: 731-734

Sugiura K, Ishihara M, Shimauchi T, Harayama S .1996. Physicochemical properties and biodegradability of crude oil. *Environ. Sci. Technol,* **31**: 45-51.

Varjani SJ, Rana DP, Jain AK, Bateja S, Upasani VN .2015. Synergistic ex-situ biodegradation of crude oil by halotolerant bacterial consortium of indigenous strains isolated from on shore sites of Gujarat, India. *Int. Biodeterior. Biodegrad,* **103**: 116-124.

Varjani SJ, Upasani VN .2016. Biodegradation of petroleum hydrocarbons by oleophilic strain of *Pseudomonas aeruginosa* NCIM 5514. *Bioresour. Technol,* **222:** 195-201.

Zenati B .2018. Biodegradation of petroleum hydrocarbons by marine bacteria: Application in the treatment of petroleum-contaminated seawater. PhD thesis, Saad Dahlab Blida 1 University, Algeria.