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ORIGINAL ARTICLE

Biochemical, Molecular and Ecological characterization of Petroleum Biodegradable Bacteria in Misan Province/Iraq

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KEYWORDS

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ABSTRACT: Oil-biodegradable bacterial strains were isolated, identified and characterized from oil contaminated soil samples from three oil refineries in Misan Province. Four bacterial isolates were characterized at biochemical levels by ordinary and standard bacteriological tests. The isolates were further identified by the Vitek-2 system. Three bacterial isolates (*Sphingomonas paucimobilis, Novosphingobium subterraneum and Bacillus subtilis*) were identified by universal primers and one bacterial isolate (*Pseudomonas putida*) by *Pseudomonas* sp. primer. The four bacterial isolates were grown on mineral salt media (MSM) supplemented with 0.5% crude oil and incubated at three incubation periods (7, 14 and 21) days were showed positive biodegradation of crude oil. The results revealed that the higher percentageof the degradation rate of n-alkanes by *Pseudomonas putida* (84.21%) followed by *Bacillus subtilis* (83.39%), *Sphingomonas paucimobilis (*79.63%) and *Novosphingobium subterraneum* (78.18%), while the higher percentage of the degradation rate of PAHs occurred by *Sphingomonas paucimobilis (*93.39%) followed by *Novosphingobium subterraneum* (92%), *Pseudomonas putida* (88. 84%) and *Bacillus subtilis* (85.99%) at the end of the incubation periods (21 days).

INTRODUCTION

The increasing environmental pollution with toxic and dangerous materials has become a global problem, as the spontaneous and routine spills of petroleum-derived compounds pollute the groundwater, seas, rivers, air and soil [1, 2]. One of the most common environmental pollutants in the soil is petroleum hydrocarbons due to the increased use of petroleum as a source of energy in society, especially in the form of crude oil [3].To removal petroleum hydrocarbons from the soil, biological treatment techniques were developed using strategies to provide nutrients, ventilation and moisture to improve bacterial activity in pollutant degradation [4, 5]. Many types of bacteria were analyzed and isolated that capable of degrading petroleum hydrocarbons biologically, in order to build vital treatment system and use it as a source of energy and carbon [6]. Crude oil degradation processes are impacted by several factors such as Crude oil bioavailability, bacteria species, temperature, nutrients availability, oxygen availability, salinity and pH, will be very useful to achieve a high removal efficiency and the optimum condition for bacteria are highly correlated with these factors for live in the contaminated environment [7,6].However, the proper and correct performance of bacteria in degradation of hydrocarbons biologically is mainly dependent on the availability of appropriate environmental factors [8]. In recent year, attention to biodegradation of petroleum pollutants has increased using appropriate and sustainable methods as human kind strives to clean polluted environments [9]. Most environmentally safe means of disposal of petroleum pollutants soil is the application of biological techniques

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compared to other physical, chemical and mechanical methods [10].This study was amied to biochemical and molecular characterization of oil biodegradable bacteria; determine the ability of bacteria to remove petroleum compounds from soil and estimation the degree of bacterial activity in biodegradation of crude oil

MATERIALS AND METHODS

Sample collection

In this study, oil contaminated soil samples were collected from three different regions in Misan province during November (2019), these regions were as follows: Site "A":Misan Oil Company / Bazerkan Refinery, Site "B": South Oil Company Refinery / Misan and Site "C":Petro China Compony / Al-Kahlaa Oil Fields. The soil samples taken from a depth of 5-20 cm. Crude oil samples were obtained from Bazerkan Refinery station

Measuring the temperature and pH of soil

The temperature of oil contaminated soil was measured during the time of sampling by using the thermometer, while soil pH was measured in the laboratory after samples were brought from the above mentioned sites by using a pH meter, and the method of [11] was performed to measuring the soil pH by mixing 10g of soil with 100ml distilled water.

Preparationand extraction of hydrocarbons from soil samples

The soil samples were dried by leaving them exposed to air. A metal sieve of size (63µm) was used to remove the coarse materials, then the powder was placed in a clean glass vials to be ready for analysis [12].The procedure of [13] was used for the extraction of hydrocarbon compounds from the soil samples as follow: The Soxhlet apparetus was used, the thimble containing the soil sample put in it, 200 ml of methanol: benzene (1: 1 ratio) was added for 24-36 hours. At the end of the period, the saponified of the extract was made by using KOH (4 N) for two hours. The unsaponified layer was placed in a chromatographic column containing a layer of glass wool, a layer of silica and a layer of alumina. Fifty millilter of n-hexane was added to isolate the aliphatic fraction, and then 30 ml of benzene added to the column to isolate the aromatic fraction. The aliphatic and aromatic fractions were placed separately in the dark vials to be ready for analysis by Gas Chromatography.

Isolation and enumeration of bacteria

The procedure of [14, 15] was used for the Isolation of bacteria from soil samples. Serial dilutions were made by weigh1 gram for each soil samples and suspended in 100 ml of sterile distilled water. Then the mixture was serially diluted in 9 ml of distilled water and the serial dilutions were carried out by transferring 1 ml of the suspension to the first tube 10^{-1} , and after mixing, one milliliter was taken from the same tube (10^{-1}) and thus the transfer process continued sequentially until reaching dilution 10^{-7}, the values of colony forming unit per ml (CFU/ml) were determined for each sample by plating 0.1 ml of10^{-7}dilution on nutrient agar plates and enumerating the bacterial colonies.

Morphological characterizations of bacteria

Morphological characterizations of bacterial isolates were performed according to color, size, colony characteristics (margin, form, and elevation), when bacterial strains were grown on ordinary enrichment, selective and differential media such as Nutrient agar, Blood agar and Mac Conkey agar(Himedia/India) , in addation to gram staining and string test (3% KOH).

Biochemical and molecular identification

The Vitek 2 system (Biomerieux/France) was used in order to confirm the diagnosis of bacterial isolates from soil samples according to [16]. Amplification of some genes of bacteria isolated from soil samples by Polymerase Chain Reaction (PCR) assay was performed. Genomic DNA Mini Bacteria Kit was used to extract Genomic DNA from bacteria according to company's instructions (Geneaid / Taiwa). Theprimers (Bioneer / South Korea) and thermal cycler conditions used in the present study was conducted with some modifications according to [17, 18] for universal primer and [19] for *Pseudomonas* sp. As showed in Tables1-3.

Table 1. The primers which used in the present study.

NO.	Bacteria	Primers target sequence	Primer sequence (5'→3')
1	**All bacteria (universal)**	16S rDNA gene sequence	27F 5'-AGAGTTTGATCCTGGCT CAG-3' 1492R 5'-TACGGGTACCTTGTTACGA CTT-3'
2	***Pseudomonas* sp.**	16S rDNA gene sequence	F 5'-CTACGGGAGGCAGC AGTGG-3' R 5'-TCGGTAACGTCAAAACAGCAA AGT-3'

Table 2. Thermal cycler conditions of Universal primer.

PCR step	Temperature (°C)	Time	Repeat
Inatial denaturation	95	5 min	1
Denaturation	95	30 s	
Annealing	52	45 s	30 cycle
Extension	72	1.5 min	
Final extention	72	10 min	1
Hold	4		-

Table 3. Thermal cycler conditions of *Pseudomonas* sp. prime.

PCR step	Temperature (°C)	Time (min)	Repeat
Inatial denaturation	95	5 min	1
Denaturation	94	1 min	
Annealing	55	1 min	25 cycle
Extension	72	1 min	
Final extention	72	10 min	1
Hold	4		-

Measurement the ability of bacterial species to degrade crude oil

The procedure of [20, 21] was used to measurethe ability of bacterial species to degrade crude oil. One milliliter of single bacterial colony for dilution $10^{-1}$was added to 100ml MSM medium ((1g) KH_2PO_4, (1g) $(NH_4)_2SO_4$, (1g) KNO_3, (0.2g) $MgSO_4$, (0.02g) $CaCl_2$ and (0.05g) $FeCl_3$) and then 0.5 ml of crude oil was added. The entire flask was incubated in a shaker incubator at 37°C in different intervals including 7, 14 and 21 days for 121 rpm.

Extraction petroleum hydrocarbons from MSM broth

Petroleum hydrocarbons were extracted from MSM broth following the procedure of [22] with some modifications by [23] as follow: After each incubation period 100 ml of chloroform (Alpha Chemika/India) was added to the flask containing MSM medium, bacteria and crude oil. The mixture was put in a separating funnel,the lower layer was isolated. The extract was passed through column chromatography provided with glass wool and anhydrous sodium sulphate. After evaporation, 50 ml of n-hexane was added to the clean beaker which contains the sample and passed through the column chromatography provided with glass wool, silica gel and aluminato isolate the aliphatic fraction, then 30 ml of benzene were added to isolate the aromatic fraction these fractions were reduced to a suitable volume prior to analysis.

RESULTS AND DISCUSSION

Chemical and Physical properties of soil

The soil used in the current study, which collected from three sites from oil refineries in Misan Province, characterized by some properties that were observed and

diagnosed such as color, temperature and pH as show in Table 4. The current results showed varying degrees in color among soil samples where it graded from black, dark brown and brown for the three sites of the South Oil Company, Bazerkan refinery and Petro China Company respectively. From previous studies, increasing the concentration of petroleum hydrocarbons in the soil could alter the morphological properties of soil [24, 25]. The temperature measurement values were recorded, 28^{o}C for the site of Bazerkan and Petro China and 30^{o}C for the South Oil Company. Increasing the temperature above the optimum will decrease growth; however, decreasing the temperature will not kill the organisms, but only slow down growth [26]. pH values ranged from neutral to slightly alkaline (7.43, 7.95 and 7.98) for South Oil company, Bazerkan refinery and Petro China Company respectively. In agreement with our results,[27]observed range of pH for soil contaminated with oil from neutral to slightly alkaline because the high acidity or alkalinity leads to toxic effects on living organisms in the soil in addition to influence impact effect the soil balance, as well as has an effect on the activity of bacteria.

Table 4. Chemical and Physical properties of soils used in the present study.

Characteristic of soil	Site A	Site B	Site C
Color	Dark brown	Black	Brown
Temperature	28 ^{0}C	30 ^{0}C	28 ^{0}C
pH	7.95	7.43	7.98

Concentration of n-alkanes and PAHs in soil samples

The results of extraction of hydrocarbons components by the GC showed that the oil contaminated soil contain two types of petroleum hydrocarbons which are n-alkanes and PAHs as showed in Tables 5and 6. The highest concentration rate was recorded in the soil of the South Oil Company was 66644.43 µg gm^{-1} dry weight for n-alkanes and 4106.503 µg gm^{-1} dry weight for PAHs followed by a Bazerkan refinery, which were 38445.39 µg gm^{-1} dry weight for n-alkanes and 2953.512 µg gm^{-1} dry weight for PAHs, As for the soil of Petro China Company, they have less concentration for n-alkanes hydrocarbons (14180.85 µg gm^{-1} dry weight) and PAHs hydrocarbons (1361.24 µg gm^{-1} dry weight).

The reason for the difference in the concentration of petroleum hydrocarbons in the soil is that pollution with crude oil leads to a significant increase in the total organic carbon contents due to the high concentration of total petroleum hydrocarbons resulting from the crude oil spill [28]. Also, there are another reasons for the difference in the concentration of organic hydrocarbon materials in the soil is due to weathering factors and the ability of bacteria to biodegrade hydrocarbons and use them as a source of carbon and energy [29].

Table 5. n-alkanes components concentration in soil samples.

Carbon numbers	Concentrations in site A (µg gm^{-1} dry weigh)	Concentrations in site B (µg gm^{-1} dry weigh)	Concentrations in site C (µg gm^{-1} dry weigh)
C7	0	0	0
C8	0	0	0
C9	0	0	0
C10	0	0	0
C11	0	0	0
C12	0	0	0
C13	14.06133	214.4547	0
C14	174.1133	1432.239	0
C15	773.024	3056.68	44.45807
C16	1548.398	4777.736	218.5553
C17	2894.128	4360.285	311.2558

C18	2424.324	3926.586	500.8723
C19	2205.91	3499.995	458.1413
C20	2850.761	5685.864	696.5796
C21	2244.232	2745.352	764.4968
C22	4315.973	3256.114	1931.294
C23	2943.681	3119.55	1202.698
C24	3545.554	2815.744	1237.947
C25	3049.516	3883.384	1411.766
C26	1828.538	4263.081	1247.588
C27	1609.502	3511.922	713.3418
C28	870.1335	2085.607	411.844
C29	1404.636	2678.358	728.2184
C30	957.3721	1886.302	448.5532
C31	818.4926	2213.16	446.5361
C32	931.7454	1299.549	369.4892
C33	618.6222	1455.95	434.3088
C34	158.6868	1186.821	119.4467
C35	52.41275	1203.378	288.3977
C36	65.47155	1066.303	103.0522
C37	88.73602	557.3349	53.59104
C38	57.36659	440.5857	38.42067
C39	0	22.09262	0
C40	0	0	0
TPHΣ	38445.39	66644.43	14180.85

Table 6. PAHs components concentration in soil samples.

Compound name	Concentrations in site A (µg gm^{-1} dry weigh)	Concentrations in site B (µg gm^{-1} dry weigh)	Concentrations in site C (µg gm^{-1} dry weigh)
Naphthalene	0	0	0
2-Methylnaphtha	0	0	0
1-Methylnaphtha	0	0	0
Acenaphthylene	84.05682	188.7809	0
Acenaphthene	64.08016	99.3141	16.24057
Flourene	18.79195	51.61667	20.90197
Phenanthrene	69.61778	219.7251	30.42354
Anthracene	153.7817	245.1306	81.02906
Fluoranthene	331.8467	228.3201	126.4932
Pyrene	230.634	840.0592	341.0519
Benzo (a) anthracene	506.6761	154.5222	363.4944
Chrysene	184.5762	215.092	127.4479
Benzo (b) flouranthene	623.3372	1073.55	73.91197
Benzo (k) flouranthene	292.1468	495.2156	91.24673
Benzo (a) pyrene	289.7963	97.06339	54.5168
Indeno (1,2,3-c,d) pyrene	73.77786	182.9528	34.48176
Benzo (g,h,i) perylene	30.39291	15.16077	0
TPHΣ	4106.503	190.5444	1361.24

Isolation and identification of bacteria

Four bacterial strains were isolated and diagnosed from oil contaminated soil samples by using serial dilution and culture on nutrient agar by aeroplate count technique. The enumeration was the best method to study the bacterial strains that able to degrade hydrocarbons [30]. Therefore, the bacterial colonies have been enumerated on the nutrient agar and it is easy to perform and very small number of organisms can be counted as CFU/ml [31]. The bacterial population isolated from crude oil contaminated soils had total aerobic counts ranging from ($1.5X10^4 – 2.9X10^4$) CFU/ml as show in Table 7.These results are close to the study of[19] that conducted to isolating bacteria from oil contaminated soil.

Table 7. Enumeration of bacterial colonies on nutrient agar plate by the aeroplate count.

Soil samples	Site	CFU/ml of dilution
1	A	$2.8 X 10^4$
2	B	$2.9 X 10^4$
3	C	$1.5 X 10^4$

Several subcultures were made to obtained pure culture for identification bacteria (Figure 1). Gram stain was showed that gram negative bacteria were most bacterial strains. This is proven by many studies that have observed that the percentage of gram negative bacteria is more than that of gram positive bacteria in oil contaminated soil [32, 33] The forms of bacteria were ranged from cocci, bacilli and variable pleomorphic (Figure 2). The colony characteristic was also identified such as colony color, size, form, elevation and margin as show in Table 8.

Table 8. Morphological characterization of bacterial strains.

Bacteria isolates	Gram stain	form	Colony color	Colony size	Colony form	Colony elevation	Colony margin
A1	+ve	Bacilli	White	Medium	Circular	Convex	Irregular
B1	-ve	Bacilli	Cream	Medium	Circular	Convex	Entire
B2	-ve	Bacilli	Off-white	Large	Circular	Convex	Entire
C1	-ve	Bacilli	Yellow	Large	Circular	Convex	Entire

Note: -ve: gram negative, +ve: gram positive

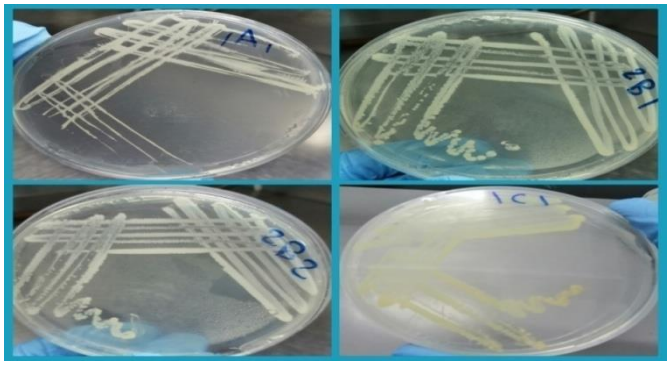


Figure 1. A pure culture of bacterial strains.

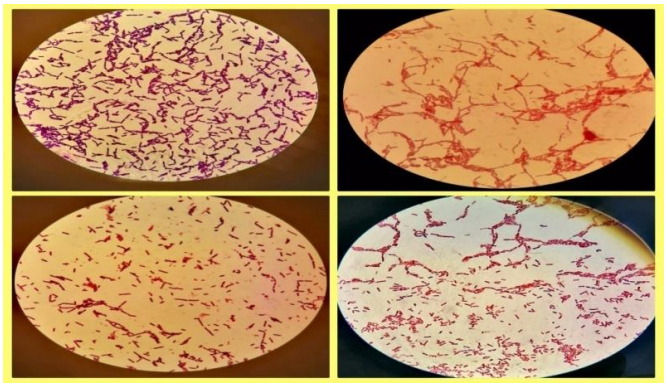


Figure 2. Gram stain of bacterial strains 1000x.

Table 9 illustrates the growth of bacterial isolation on Blood agar and MacConckey agar as well as the results of String test. All the bacterial strain has shown good growth on the Blood agar with different patterns of hemolysis. The gram positive bacteria didn't show any growth on the MacConkey agar while the gram negative bacteria were growing very well. Furthermore these bacteria were interacted with the KOH in the String test as compared with the gram positive bacteria, as they become viscous and String out whereas gram positive bacteria were not affected, this corresponds to a study of [34].

Table 9. Bacterial strains growth on Blood agar and MacConkey agar and interaction with string test.

Bacteria isolaties	Blood agar	MacConkey agar	String test
A1	+	-	-
B1	+	+	+
B2	+	+	+
C1	+	+	+

Note: (+) growth. (-) no growth. (+) reaction. (-) no reaction.

Biochemical and molecular identification of bacterial

Table10 represents results for biochemical and molecular identification. Based on the results of VITEK®2 Compact and 16S rDNA sequence homology studies.

Table 10. Biochemical and Molecular identification of bacterial strains.

Bacterial code	Vitek 2 system	16S rDNA sequence
A1	*Staphylococcus lentes*	*Bacillus subtilis*
B1	*Pseudomonas putida*	*Pseudomonas putida*
B2	*Sphingomonas paucimobilis*	*Novosphingobium subterraneum*
C1	*Sphingomonas paucimobilis*	*Sphingomonas paucimobilis*

Pairwise Sequence Alignment of the Sequencing data was obtained by using NCBI-BLAST (Nucleotide BLAST) as shown in Table11. The bacterial universal primer pair specific to 16S rDNA gene fragment was used to identify bacterial isolates(Figure 3A).Furthermore, some bacterial isolates were identified using primer pair specific to 16S rDNA gene fragment specific for the genus Pseudomonas (Figure 3B).

Table 11. Bacterial identification based on 16S rDNA sequencing data.

Bacterial strains	Maximum score	Total score	Query coverage	E. value	Identity %	Accession no.
Bacillus subtilis	717	717	35%	0.0	95.75%	Kr 999939.1
Pseudomonas putida	1982	1982	99%	0.0	98.07%	Kt 984874.1
Novosphingobium subterraneum	1951	1951	98%	0.0	98.3%	Kj573537.1
Sphingomonas paucimobilis	327	327	16%	6e-85	98.4%	Mk829514.1

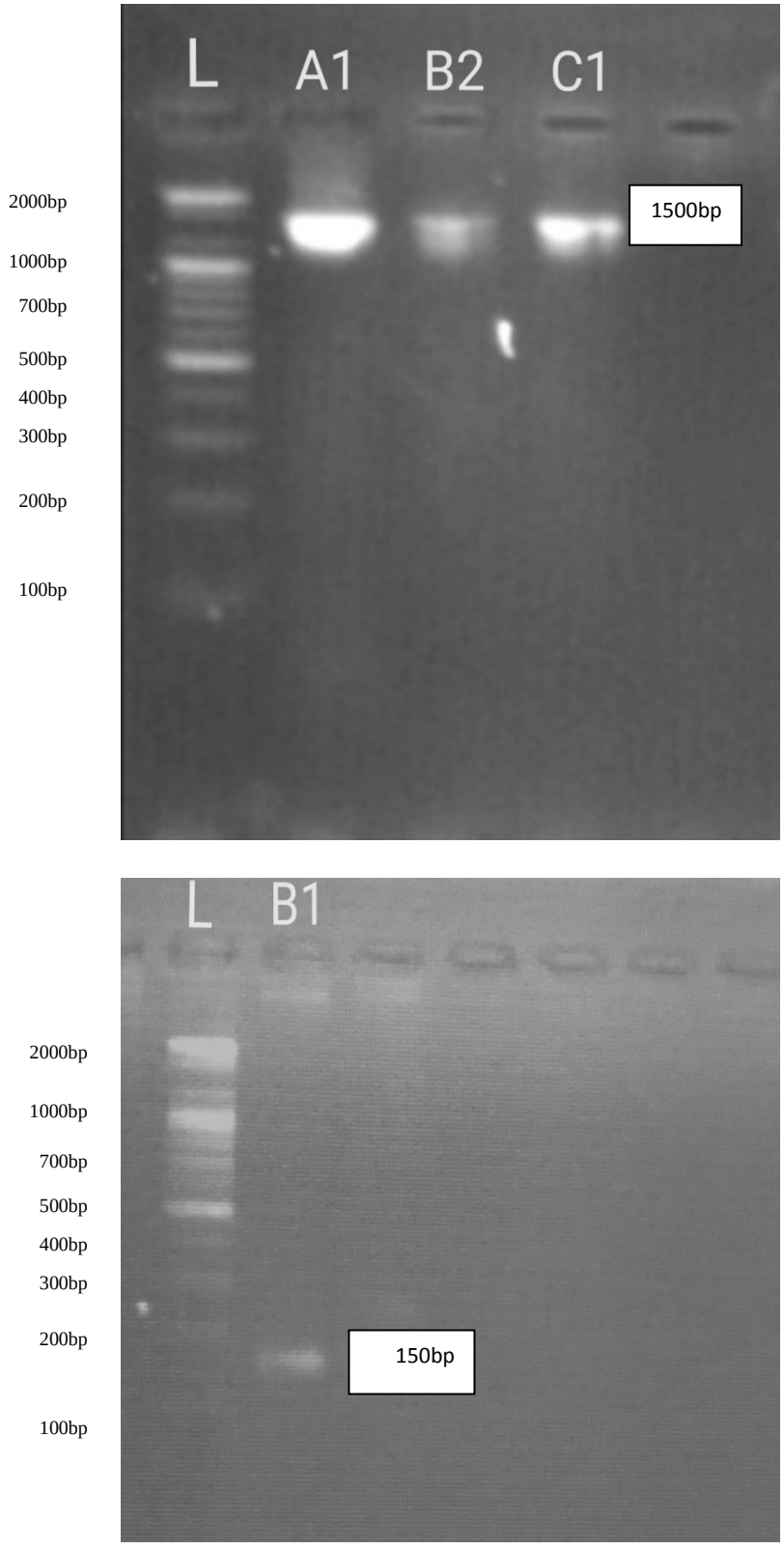
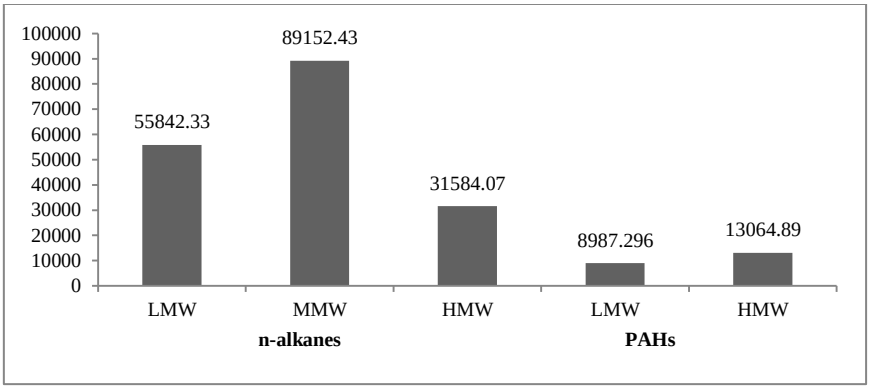


Figure 3. Ethedium bromide stained gel electrophoreses of the 16s rRNA gene of bacterial strains, lane(L) represents the molecular ladder (100bp).(A) lanes(A1, B2 and C1) represents positive PCR product size (1500 bp) of universal primer.(B) lanes(B1) represents positive PCR product size(150 bp) of Pseudomonas sp. primer.

Biodegradation of crude oil by bacterial strains

To examine the ability of bacterial strains to degrade hydrocarbons (aliphatic and aromatic fractions), GC analysis of the control (only crude oil without bacteria) at the concentration 0.5% showed that it was a mixture of different aliphatic fraction (n-alkanes) including low molecular weight (LMW), medium molecular weight (MMW) and high molecular weight (HMW) and different aromatic fraction (PAHs) including low molecular weight (LMW) and high molecular weight (HMW) as showed in Figure 4. Crude oil was exposed to degradation by bacterial isolates individually during the weekly incubation periods.



Figurer 4. The concentration of crude oil components in control sample.

Biodegradation of crude oil by Bacillus subtilis

According to Figure (5 A and B) *B.subtilis*was showed its ability to degrading n-alkanes hydrocarbons components through three incubation periods. *B.subtitlis* degradation were (10311.29, 4389.198 and 2840.431) µg gm^{-1} dry weight for LMW, whereas 31954.42, 24221.32 and 21119.55 µg gm^{-1} dry weight for MMW and 7851.607, 8059.836 and 5355.59 µg gm^{-1} dry weight for HMWat 7, 14 and 21 days respectively. As for PAHs hydrocarbons was gradually degraded from LMW (3057.059, 648.446 and 635.323) µg gm^{-1} dry weight to HMW (4942.129, 2578.591 and 2452.194) µg gm^{-1} dry weight at 7, 14 and 21days of incubation periods respectively. In accordance with the results of [35, 36] *B. subtilis* have been reported to have the potentials to utilize several compounds of n-alkanes and PAHs hydrocarbons as a sole source of carbon and energy. *Bacillus* species are more tolerant to high levels of oils due to their resistant endospores; they are known to possess a more competent and active oil degrading enzymes than other bio degraders [37].

(A)

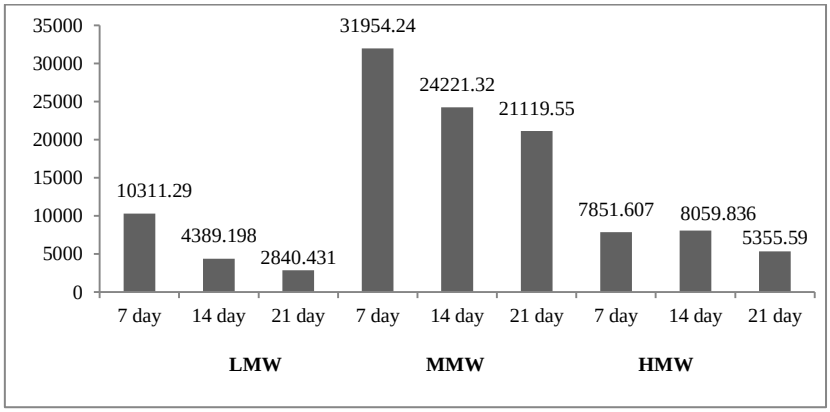


Figure 5. Biodegradation of crude oil by *B.subtilis*. (A) n-alkanes. (B) PAHs.

(B)

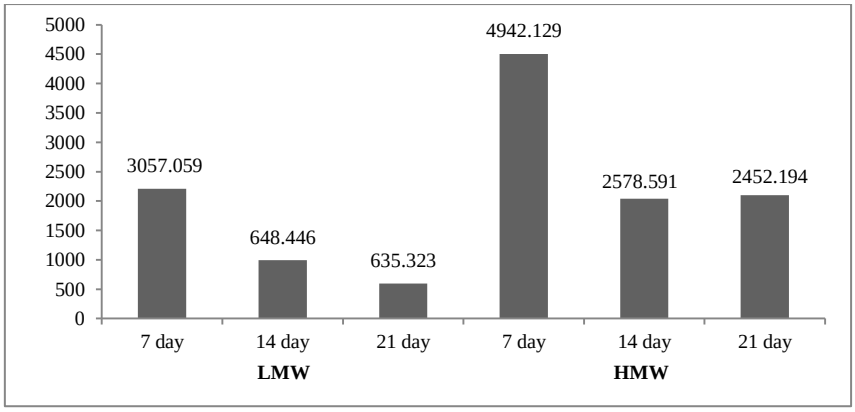


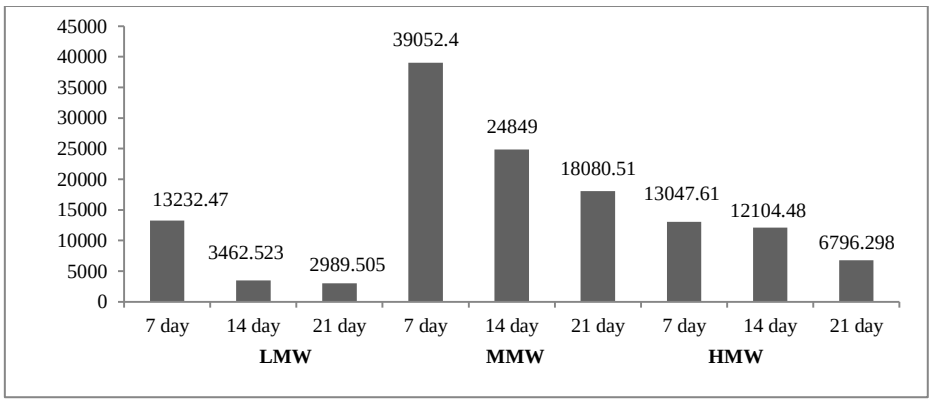
Figure 5. Continued.

Biodegradation of crude oil by Pseudomonas putida.

The results of bacterial GC analysis applying a pure bacterial culture of *P. putida* revealed that after 21 days, it is possible to degrade LMW and HMW of n-alkanes and PAHs hydrocarbons, The results are displayed in Figure6 A and B. It is apparent from the obtained results that the application of this bacterial culture is suitable for the degradation of LMW (13232.47, 3462.523 and 2989.505) µg gm^{-1} dry weight, MMW (39052.4, 24849 and 18080.51) µg gm^{-1} dry weight and HMW (13047, 12104 and 6796.298) µg gm^{-1} dry weight. Additionally degradation of PAHs were 3266.957, 1363.97 and 852.6932 µg gm^{-1} dry weight for LMW and 7359.838, 2949.902 and 1606.603 µg gm^{-1} dry weight for HMW at the three incubation periods.

P.putida has the ability to degrade and remove n-alkanes and other PAHs hydrocarbons, because these bacteria has normally involves the enzymatic activity to degradation almost crude oil compounds [38, 39].

(A)



(B)

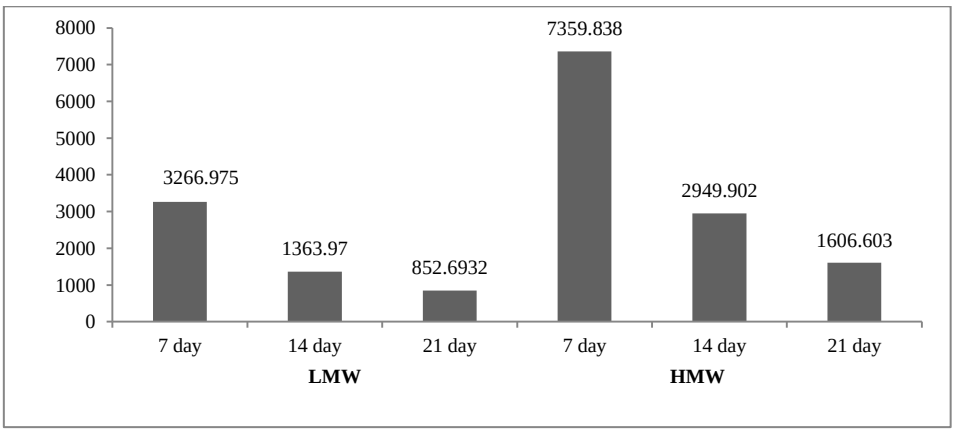


Figure 6. Biodegradation of crude oil by *P.putida*. (A) n-alkanes. (B) PAHs.

Biodegradation of crude oil by *Sphingomonas paucimobilis*

Figure7 A and B showed the degradation of n-alkanes by*S.paucimobilis*was6274.135, 4872.661 and 6343.411 µg gm^{-1} dry weight for LMW , 65386.59, 35527.96 and 21472.91 µg gm^{-1} dry weigh for MMW and 28800.02, 23135.06 and 8152.779 µg gm^{-1} dry weigh for HMW. In addation the degradation of PAHs were 2372.169, 870.3337 and 574.44 µg gm^{-1} dry weights for LMW and 7520.524, 2878.527 and 881.2927 µg gm^{-1} dry weigh for HMW. The ability of *S.paucimobilis* to degrade n-alkanes compounds and this in agreement with our results as reported by [40] (Figure7 A). *S.paucimobilis* has shown a high ability to degraded PAHs hydrocarbons from crude oil as showed in Figure7B. Based on these findings, *S.paucimobilis*is considered as an excellent agent in biodegradation soil polluted with both hydrocarbons types as reported by [41] who found that *S. paucimobilis* bacteria was typical in hydrocarbon degradation.

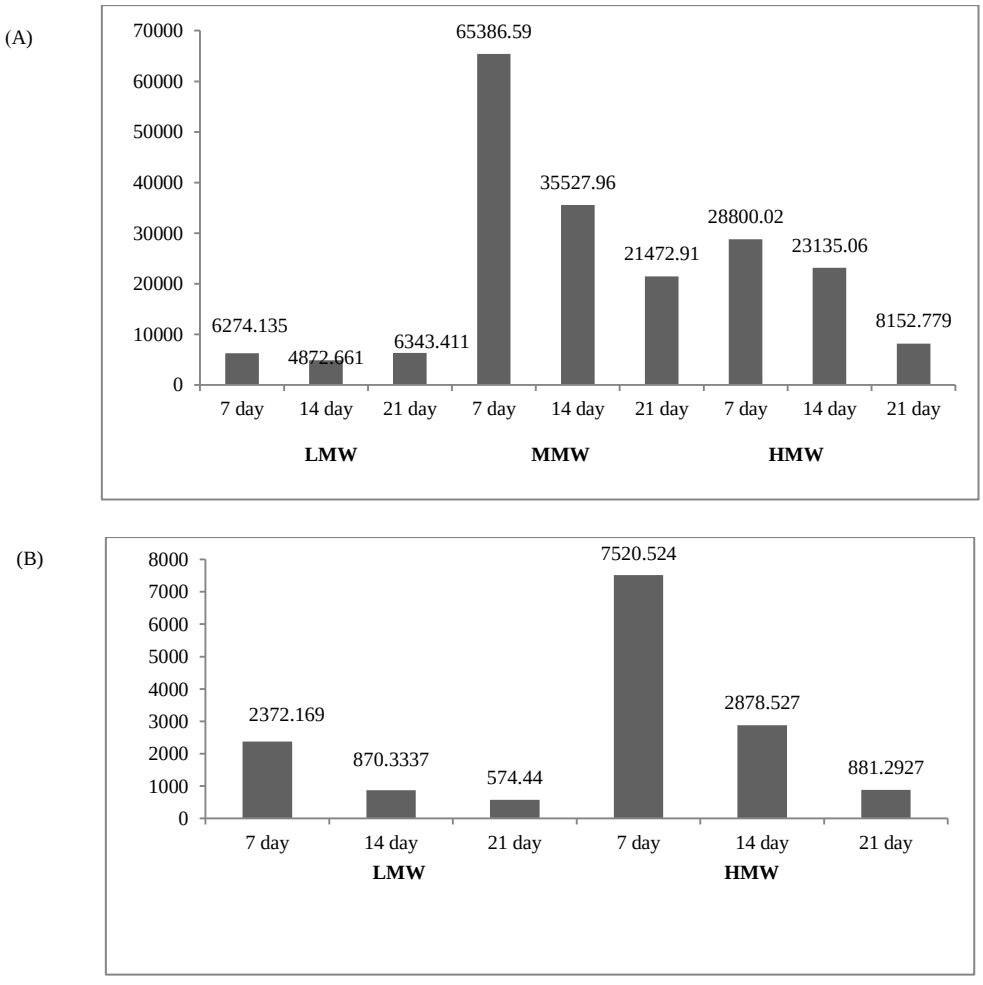


Figure 7. Biodegradation of crude oil by *S.paucimobilis*. (A) n-alkanes. (B) PAHs

Biodegradation of crude oil by Novosphingobium subterraneum

As shown in the current results the degradation of n-alkanes compounds by *N. subterraneum* through three incubation periods (7, 14 and 21 days) started from LMW (9240.062, 5736.827and 7051.718) µg gm^{-1} dry weigh to MMW (32128.37,28774.71 and 23407.95) µg gm^{-1} dry weigh and HMW in the end of periods were 10960.98, 7013.325 and 8064.589 µg gm^{-1} dry weigh. On the other hand, the degradation of PAHs compounds

also started from LMW (5255.347, 650.5566 and 883.2648) µg gm^{-1} dry weigh to HMW (7187.88, 2929.407 and 880.0631) µg gm^{-1} dry weight in the same periods by *N. subterraneum* in compared with control concentration. The decreasing in the concentration of crude oil compounds as show in Figure8A and B due to the ability of *N. subterraneum*to uses the crude oil as a carbon source. [42, 43] considering *N. subterraneum* one of the most important bacteria which used to treatment and removal oil from soil because they noted its high ability to degrade oil. The ability of strain *N. subterraneum* to spontaneously form biofilm on several surfaces could allow the adhesion of cells to soil, Moreover, its ability to form emulsions reduces the need to use detergents which are often required in biodegradation treatments and its capacity to encapsulate oil drops and to preferentially remove the crude oil components may avoid the dispersion of toxic hydrocarbons components in the environment [44].

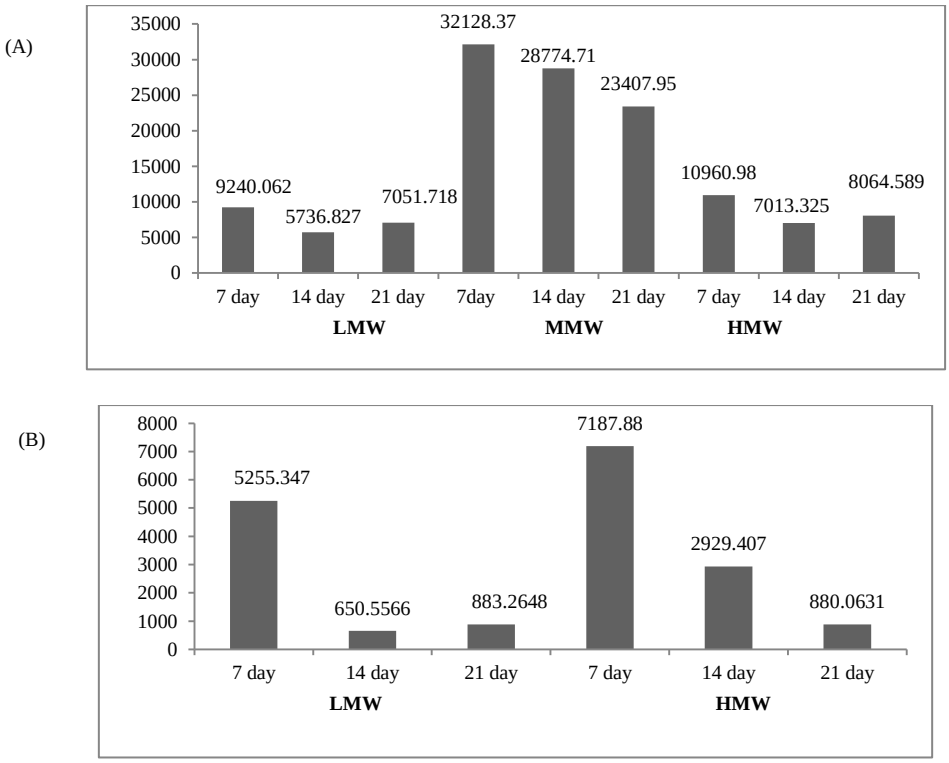


Figure 8. Biodegradation of crude oil by *N.subterraneum*. (A) n-alkanes. (B) PAHs

The Percentages of total concentration of crude oil degradation by bacterial strains

As seen the results in the Table 12 the higher percentage of the degradation rate of n-alkanes at the end of the incubation periods(21 days) by *P.putida* (84.21%) followed by *B.subtilis* (83.39%), *S.paucimobilis (*79.63%) and *N.subterranium* (78.18%), while the higher percentage of the degradation rate of PAHs at the end of the incubation periods (21 days) occured by *S.paucimobilis (*93.39%)followed by *N.subterranium* (92%), *P.putida* (88.84%) and *B.subtilis* (85.99%). In fact, the biodegradation of crude oil was required to complete mechanisms and this not available in one organism because the hydrocarbon mixture varies markedly in the volatility, solubility, tendency to biodegradable and the certain enzymes cannot be gained in a single organism [45].

Degradation of oil by these bacterial strains shows that they have specialized co-metabolic capacities, in oil polluted environments, specialized bacterial strains are abundant because of their adaptation ability to pollutants, but every organism has its own level of biodegradation [46].

Table 12. Percentage of total concentration of crude oil degradation by bacterial isolates.

Bacterial isolates	Incubation periods					
	% degradation of crude oil components					
	7days		14days		21days	
	Aliphatic	Aromatic	Aliphatic	Aromatic	Aliphatic	Aromatic
B.subtilis	71.61%	63.27%	79.23%	85.3%	83.39%	85.99%
P.putida	63%	84%.51	77.11%	80.43%	84.21%	88.84%
S.paucimobilis	43.1%	56.5%	64.01%	83%	79.63%	93.39%
N.subterranium	70.36%	43.57%	76.48%	83.76%	78.18%	92%

CONCLUSIONS

The current study showed that the bacterial strains isolated from oil contaminated soil identified by biochemical and molecular testes have a different ability to degrade the petroleum hydrocarbons. The higher percentage of the degradation rate of n-alkanes by *P.putida* (84.21%), while the higher percentage of the degradation rate of PAHs by *S.paucimobilis* (93.39%).

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Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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