Plant Archives Vol. 19 No. 1, 2019 pp. 971-977 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ISOLATION AND IDENTIFICATION OF MICROBIAL SPECIES FOR HYDROCARBON DEGRADATION IN CONTAMINATED SOIL AND WATER

Asia Fadhile Almansoory[1]*, Ammar Talal[1], Nassir Abdullah Al-yousif[1] and Mohammad Hazaimeh[2]

[1*]Department of Ecology, Science Collage, Basrah University, Iraq, Basrah,
[2]Department of Biology, College of Science in Zulfi, Majmaah University, Majmaah-11952, Saudi Arabia

Abstract

Hydrocarbon contamination is one of the major environmental problems caused by different human activities related to the petrochemical industry. The accidental release of organic pollutants into the environment is a particular concern. In this study, 16 bacterial isolates were obtained from hydrocarbon-contaminated water and soil samples cultured for 48 hours in nutrient agar supplemented with 1% (vol/vol) crude oil. The growth requirements and crude-oil degradation ability of the isolates were determined. *Micrococcus* spp., *Pseudomonas* spp. and *Bacillus* spp. were obtained as common isolates from all of the water and soil samples. Some of the obtained isolates, specifically *Micrococcus* spp. and *Pseudomonas* spp., can utilise hydrocarbons as growth substrates in mineral salt medium (MSM) supplemented with type of crude oil, at 1% (vol/vol) concentration. Gas chromatography analysis revealed that *Micrococcus* spp. and *Pseudomonas* spp. provided crude oil degradation rates of 58% and 65%, respectively. These rates are higher than those of other isolates.

***Key word**:* biodegradation, crude oil, biochemical tests, *Pseudomonas* spp., *Micrococcus* spp.

Introduction

Recent studies have confirmed the close relationship between oil spills and damage to soil and aquatic environments (Kayode–Isola *et al.* 2008; Plaza *et al.* 2008). Oil spills in aquatic environments results from vehicle collisions or from the transport of refined petroleum products, such as petrol, kerosene and diesel (Subathra *et al.* 2013). Contamination by these products is common in Iraq, where unrecovered spilled petroleum products migrate to streams and rivers and ultimately accumulate in soil. Bioremediation is the best treatment for environmental oil pollution resulting from crude oil leakage (Maintains *et al.* 2006; Latha and Kalaivami 2012). Numerous bacterial species that have been characterised as good hydrocarbon degraders have been isolated from different ecosystems, including soil, activated sludge and marine habitats. These bacteria can tolerate high hydrocarbon concentrations (Ma *et al.* 2012). The biodegradation of crude oil may be limited by several factors (Latha and Kalavani 2012). Specifically, the low biodegradability and solubility of crude oil are the most important factors that limit the bioremediation of oil-polluted soils and define the crude oil as a persistent pollutant that can cause serious damage to human health and the ecosystem (Panda *et al.* 2013).

The microbial removal of oil from contaminated soil occurs through two important processes: microbial biodegradation and uptake. Microbial biodegradation involves the transformation of organic compounds, whereas microbial uptake involves the direct removal of pollutants through the adsorption of compounds onto the surfaces of bacterial membranes (McPherson 2007). Many bacterial species degrade hydrocarbons in solution through emulsification, which involves the production of active surface agents, such as biosurfactants, that increase cell adhesion to the substrate (Hassanshahiant, *et al.* 2012). Prior to their field application as technologies for environmental bioremediation, bacterial species must be isolated and characterised, and their ability to utilise

***Author for correspondence :** E-mail : asian.almansoory@yahoo.com

hydrocarbons must be assessed. Mechanical methods used for hydrocarbon degradation are expensive and time consuming. Meanwhile, bacterial systems for hydrocarbon degradation and utilisation have been proven to provide carbon and energy (Latha and Kalavani 2012).

The main aim of our study is to describe the ability of bacteria to degrade crude oil and its related products under laboratory conditions. We aim to isolate and identify microbes that can efficiently degrade hydrocarbons. We also attempt to identify approaches for accelerating the biodegradation rate of the isolates. The outcome of this work has potential applications in the remediation of hydrocarbon contamination and bioaugmentation.

Materials and Methods

Sampling areas

Soil and water samples for the isolation of crude-oil-degrading bacteria were collected from different contaminated sites in Iraq. Soil samples were collected from the surface layer (0–5 cm) of three contaminated sites in Basrah City, Iraq: an excavation site in Albrjsuia and two refineries in Rumaila and Shuaiba. Water samples were acquired from three hydrocarbon-contaminated sites in Shatt al-Arab River. All samples were collected during October 2016 and stored in sterile glass bottles.

Isolation and enumeration of bacteria

Random sampling was conducted to ensure the equal representation of each family of bacteria in a bacterial population from a geographical area. Sterilised spatulas were used to collect soil samples at random sites in the study area. The samples were placed in sterile bags, transferred to the laboratory within 30 min and stored at 4°C. Prior to sample processing, the laboratory bench was cleaned with cotton wool soaked in ethanol to prevent contamination. Bacteria were isolated and enumerated through the pour-plate method. Briefly, 1g of dried soil was added to 99mL of distilled water (DW). Then, 1mL of soil solution was taken from the solution and added to 9mL of DW. The dilution was then agitated vigorously at 150rpm for 1h to release adhering microorganisms. Different aqueous dilutions were prepared (10^{-1}–10^{-10}) from the original soil suspension. Next, 0.1 mL of each dilution was streaked onto plates containing agar medium. The plates were incubated for 24h at 37°C with gentle agitation. After incubation, bacteria were transferred from mixed culture plates onto new plates and incubated at 37°C for 24 h. Plates containing pure cultures were stored at 4°C until further examination. Water samples were stored at room temperature, processed and analysed within 24h as described above.

Preparation of culture medium

Media were prepared by mixing 28g of nutrient agar powder with 1,000mL of DW in a conical flask. The media were homogenised through agitation, sterilised through autoclaving at 121°C for 15min, dispensed into sterile petri dishes containing diluted soil samples and left to gel on the bench. Slants were prepared by pouring 8mL of media into sterile bottles and then allowed to gel while tilted. Mineral salt media (MSM) were prepared for tests on the ability of the isolates to degrade crude oil. MSM was prepared using DW (g/L). Each L of MSM contained l0g of NaCl, 2g of Na_2HPO_4, 0.17g of $MgSO_4.7H_2O$, 0.1g of $MnSO_4 \cdot H_2O$, 0.025g of $CuCl_2$, 0.025g of $(NH_4)6MO_7O_{24} \cdot 4H_2O$, 0.025g of $CO(NO_9)_2 \cdot 6H_2O$, 0.025 g of ZnCl and 0.01 g of NH_4NO_3. To prepare media, MSM was mixed with 1,000mL of DW. The mixture was then homogenised using a homogeniser. The pH of the media was adjusted to 7.2. Subsequently, the media were autoclaved at 121°C for 15 min (Arulazhagan *et al.* 2010; Gargouri *et al.* 2011). Next, 9mL of DW was pipetted into five different test tubes labelled from 1 to 5. Next, 1 g of soil sample was weighed and then transferred to test tube 1 and then to test tube 2. Different pipettes were used to avoid cross-contamination. Test tubes were then homogenised through agitation. These steps were repeated with all soil samples. Meanwhile, 0.1mL of the diluted sample was pipetted into the labelled petri dishes. Nutrient agar medium were plated at 45°C. The plates were then incubated at 37°C for 24h. The growth of microbial colonies on the plates was then observed. Colonies were picked by using a wire loop (flamed to red) and then transferred to freshly prepare nutrient agar plates.

Identification of isolates

The most potent bacterial crude oil degrader was identified on the basis of cellular morphological characteristics, including cell morphology, colony morphology and structural appearance. Biochemical tests were performed in accordance with the standard procedures outlined in Bergey's Manual of Systematic Bacteriology (Holt *et al.* 1998). Identification tests, such as Gram staining, indole, methyl red, Voges–Proskauer, H_2S, urea, catalase and oxidase tests were performed in accordance with the standard procedures provided in Bergey's Manual of Systematic Bacteriology (Holt *et al.* 1998).

Screening of isolates for the ability to degrade crude oil

The ability of bacterial isolates from hydrocarbon-contaminated samples to degrade crude oil was screened

using MSM containing 1% crude oil ($V_{crude\ oil}/V_{water}$) as the carbon source. Bacteria with tolerance for crude oil were subjected to additional screening.

Preparation of standard inocula

Pure cultures were obtained after 18–24h of growth in 50 mL of sterilised NB. Samples were centrifuged for 10 min at 4,000rpm and 4°C by using an Eppendorf T-type 5810 R centrifuge (USA). Thereafter, pellets were suspended in 10mL of normal saline (8.5g/L NaCl). The standard inoculum for each of bacterial isolate was prepared at the concentration of 10% (v/v) in a 250mL conical flask filled with 100 mL of MSM.

Degradation of crude oil

The degradation rates of all isolates were quantified. First, 10% of the standardised inoculum was inoculated into 100mL of MSM with 1% (v/v) crude oil (pH 7.0) and incubated for 7 days at 37°C and 150 rpm. Inoculated medium was used as the control. Then, the residual hydrocarbon content was extracted from the culture medium with 100mL of chloroform in a 500mL separator funnel. The solvent was removed through evaporation using a rotary evaporator at 50°C. The residual hydrocarbons were transferred to a 10mL vial and then evaporated for 2 days under an overhead fume hood. The extract was concentrated to 2 mL, and the total obtained hydrocarbon was weighed. The degradation rate for each sample was determined using the equation below:

$$\text{Degradation rate}\ (\%) = [(H_0 - H_h)/H_0] \times 100 \quad (1)$$

where H_0 denotes the hydrocarbon content at 0h, and H_h represents the hydrocarbon content of each sample after 5 days.

Results and Discussion

Isolation and identification of bacteria

We used soil and water samples from different hydrocarbon-contaminated sites. During the period between March 2016 and June 2016, we obtained 16 isolates of hydrocarbon-degrading bacteria from surface soil and water samples. We characterised bacterial isolates through morphological and biochemical techniques in accordance with the taxonomic scheme in Bergey's Manual of Determinative Bacteriology (Holt *et al.* 1994). The bacterial populations of all of the tested samples were predominated by Gram-positive bacteria. Some cells may not form colonies and some colonies may fuse under overcrowding. These behaviours may introduce errors to measurements. Thus, the samples must be diluted for the correct enumeration of colonies. In this work, we diluted solution samples to 10^{-5}. We used the spread-plate method to transfer the diluted samples to nutrient agar plates. By referring to Bergey's Manual of Determinative Bacteriology (Holt *et al.* 1994), we identified bacteria from water and soil as *Bacillus* spp., *Pseudomonas* spp. and *Micrococcus* spp., as listed in Table 1. Isolate A_1 colonies appeared yellow and diffuse with irregular edges and uneven surfaces.

Isolate C appeared as circular colonies with irregular edges and diameters of 4 mm. Colonies of isolate D were cream in colour and exhibited irregular borders with flat centres. Colonies of E_1 isolates were 5 mm in diameter and presented sticky and flat surfaces. E_2 colonies were medium-sized, circular and light orange in colour. E_3 colonies were circular, sticky and orange in colour and had consistently irregular borders. F_1 colonies were small and circular. F_2 colonies were small and transparent with regular edges. Images of the bacterial colonies isolated from water samples are shown in table 1. Isolates from soil a appeared as yellow and diffuse colonies with irregular edges and uneven surfaces, whereas those from B_1 and B_2 appeared as transparent, medium-sized, flat, irregular and green colonies. B_3 isolates appeared as irregular white colonies with uneven edges. B_4 colonies were irregular and circular with flat, translucent, creamy and transparent edges. C_1 colonies were translucent, medium-sized, circular and green with flat edges. Isolate H_1 colonies were translucent, small and circular with regular and defined edges. I_2 colonies were highly elevated, sticky, small and circular with flat, regular edges. The images of the colonies are shown in table 1.

Biochemical characterisation

To identify and characterise the bacterial isolates, we conducted Gram staining and biochemical tests, such as oxidation/methyl red, lactose, indole and citrate tests. As shown in table 1, the number of Gram-negative bacterial isolates (A_1, C, D, E_2 and F_2) was higher than that of Gram-positive bacterial isolates (E_1, E_3 and F_1). Isolate E_1 was positive for the H_2S test, whereas all other isolates were negative. Isolates A_1, C, D and E_2 provided negative results for the urea test, whereas F_2 and E_1 provided positive results. Given these results, F_2 and E_1 can produce ammonia. C, E_1, E_3, F_1 and F_2 provided negative results for the methyl red test, whereas E_2, D and A_1 provided positive results. Thus, isolates E_2, D and A_1 can produce organic acids that decrease environmental pH and change the colour of the test medium to red. Isolates A_1, C, D, E_1, E_2 and F_2 were positive for the glucose test, whereas A_1, C and E_2 were negative. F_2 was negative for the oxidase test, whereas E_1, D, E_3 and F_1 were positive and exhibited a dark purple colour. A_1, C, D, E_1, E_2 and F_2 were positive for the indole test. Only isolate F_1 was positive for the citrate

Table 1: Bacterial colonies isolated from hydrocarbon-contaminated water and soil samples

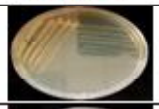
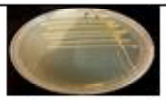
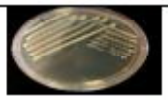
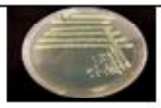
Bacteria isolated from water				Bacteria isolated from soil			
Code	Name of bacteria	Streak bacteria	Gram stain	Code	Name of bacteria	Streak bacteria	Gram stain
A_1	*Bacillus sp.*			**A**	*Bacillus sp.*		
C	*Bacillus sp.*			**B_1**	*Bacillus sp.*		
D	*Cocci sp.*			**B_2**	*Bacillus sp.*		
E_1	*Bacillus sp.*			**B_3**	*Bacillus sp.*		
E_2	*Bacillus sp.*			**B_4**	*Bacillus sp*		
E_3	*Bacillus sp*			**C_1**	*Pseudomonas sp*		
F_1	*Micrococcus sp.*			**H**	*Bacillus sp.*		
F_2	*Bacillus sp.*			**I_2**	*Bacillus sp.*		

Table 2: Biochemical characterisation of isolated bacteria

Bacteria isolated from water									
Code	Name of bacteria	H2S	Urea test	Methyl red test	Lactose test	Glucose test	Oxidase test	Indole test	Citrate test
A_1	*Bacillus* sp.	-	-	+	-	+	-	+	-
C	*Bacillus* sp.	-	-	-	-	+	-	+	-
D	*Cocci* sp.	-	-	+	-	+	+	+	-
E_1	*Bacillus* sp.	+	+	-	-	+	+	+	-
E_2	*Bacillus* sp.	-	-	+	-	+	-	+	-
E_3	*Bacillus* sp	-	-	-	-	-	+	-	-
F_1	*Micrococcus* sp.	-	-	-	-	-	+	-	+
F_2	*Bacillus* sp.	-	+	-	-	+	-	+	-

test presented in table 2.

The results for the biochemical tests are as shown in table 3. Isolates A, B_1 and H were positive for the H_2S test, and B_2, B_3, C_1, I_2 and B_4 were negative. The H_2S test is used to determine the ability of bacteria to produce H_2S, which is produced in the form of a black precipitate. All isolates were negative for the urea test, except for isolate B_2. B_2 can produce ammonia gas, which increased the pH of the test medium and caused its colour to change to red. B_1, B_2, B_3, B_4, H and I_2 were positive for the methyl red test. These isolates produced organic acids that decreased the pH and changed the colour of the test medium to red. Isolates A, B_1, B_2, B_3, B_4, C_1 and H were all negative for the lactose test. Isolates I_2, C_1, B_2, B_3 and B_4 were positive for the glucose test. Isolates A, B_1 and I_2 were negative for the oxidase test. Isolates B_2, B_3, B_4, C_1 and H were positive for the oxidase test. Isolates A, B_1, B_3, B_4 and H were negative for the indole test, whereas B_2 and I_2 were positive. All isolates, except for A, B_1 and B_4, were positive for the citrate test.

Screening isolates for the ability to degrade crude oil

Screening experiments were performed for 48 h with

Table 3: Biochemical characterisation of isolated bacteria.

Bacteria isolated from water									
Code	Name of bacteria	H2S	Urea test	Methyl red test	Lactose test	Glucose test	Oxidase test	Indole test	Citrate test
A	*Bacillus* sp.	+	-	-	-	-	-	-	-
B_1	*Bacillus* sp.	+	-	+	-	-	-	-	-
B_2	*Bacillus* sp.	-	+	+	-	+	+	+	+
B_3	*Bacillus* sp.	-	-	+	-	+	+	-	+
B_4	*Bacillus* sp.	-	-	+	-	+	+	-	-
C_1	*Pseudomonas* sp	-	-	-	-	+	+	-	+
H	*Bacillus* sp.	+	-	+	-	-	+	-	+
I_2	*Bacillus* sp.	-	-	+	+	+	-	+	+

100µL of crude oil. Some bacterial isolates degrade and convert hydrocarbons into carbon for use a growth substrate, as shown in (Fig. 1). After 48 h of incubation, hydrolytic zones appeared on nutrient agar plates supplemented with crude oil. The appearance of these zones indicates the ability of bacteria to degrade crude oil. The results show that the ability to biodegrade crude oil varied among bacterial groups and species and is dependent on the enzymatic activity of each species. Bacteria that can degrade crude oil were isolated from environments that are heavily contaminated with crude oil. These bacterial species can break down hydrocarbons and increase the activities of enzymes related to hydrocarbon decomposition. Notably, the hydrocarbon biodegradation rate began to increase to extremely high levels during the 7-day incubation period. Meanwhile, some bacterial isolates, such as B_4, lacked the ability to degrade hydrocarbons.

Among all tested isolates, isolates C_1 and F_1 yielded the highest biodegradation rates of 65% and 58%, respectively, over the 7-day incubation period (Fig. 2 and 3). These isolates were obtained from water samples. The characteristics of these bacteria are consistent with those reported by Das and Mukherjee (2007), who stated that *Pseudomonas* is a common hydrocarbon degrader. Given the ability of *Pseudomonas* spp. to degrade petroleum hydrocarbons, *Pseudomonas* strains should be isolated from areas receiving petroleum waste discharges. In addition, *Pseudomonas aeruginosa* is a component of a bacterial consortium isolated from sandy and loamy soils and showing the ability to degrade hydrocarbons from light fuel oil (Hawle-Ambrosch *et al.* 2007). Clinical isolates of *P. aeruginosa* can grow with hydrocarbon as the sole carbon source and show good oil degrading ability (Szoboszlay *et al.* 2003). Latha and Kalaivani (2012) reported that *Bacillus, Lactobacter*, *Arthrobacter*, *Pseudomonas* and *Micrococcus* predominated in soil polluted with crude oil given their ability to utilise hydrocarbons and to produce spores, which may shield them from the toxic effects of hydrocarbons.

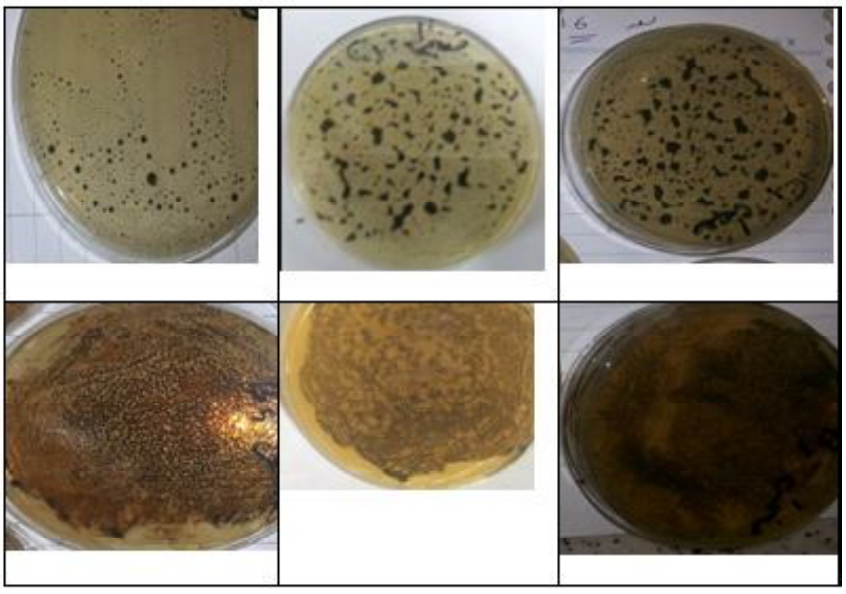


Fig. 1: Screening for crude-oil degradation ability in nutrient agar over a 48 h incubation period

Pseudomonas spp. is the primary isolates with the ability to degrade oil in aerobic conditions. In addition to enzymes related to oil degradation, whole bacterial cells have an important role in environmental adaptation to high oil concentrations. Specifically, bacterial cells provide the appropriate cytoplasmic surroundings for enzymatic activity or active transport via specialised membranes (Ma *et al.* 2006). *Pseudomonas* spp. and *Micrococcus* spp. could be potentially used in the bioremediation of oil-polluted areas. These hydrocarbon-degrading bacteria can also tolerate high petroleum concentrations; this characteristic is a vital criterion for the successful bioremediation of highly polluted environments. Nikhil *et al.* (2013) observed that some isolates that do not grow in crude oil because they have entered the stationary phase of growth. *Pseudomonas* spp. SA044 is an excellent hydrocarbon degrader given its potential to degrade phenanthrene,

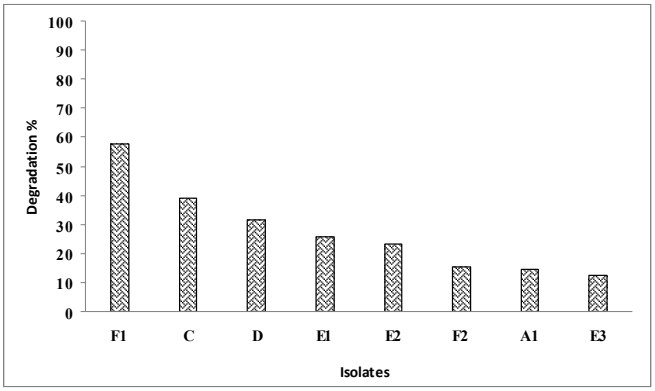


Fig. 2: Rates of crude oil degradation by isolates over 7 days of shaking incubation at 150 rpm and 37°C

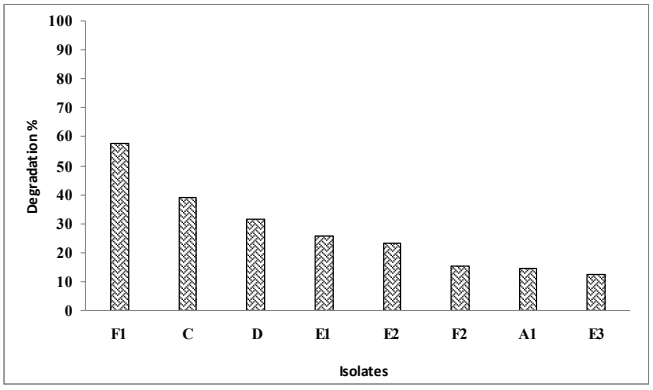


Fig. 3: Percentage of the study of degrading the isolates of crude oil after incubation for 7 days with temperature of 37°C and at a speed of 150 rpm

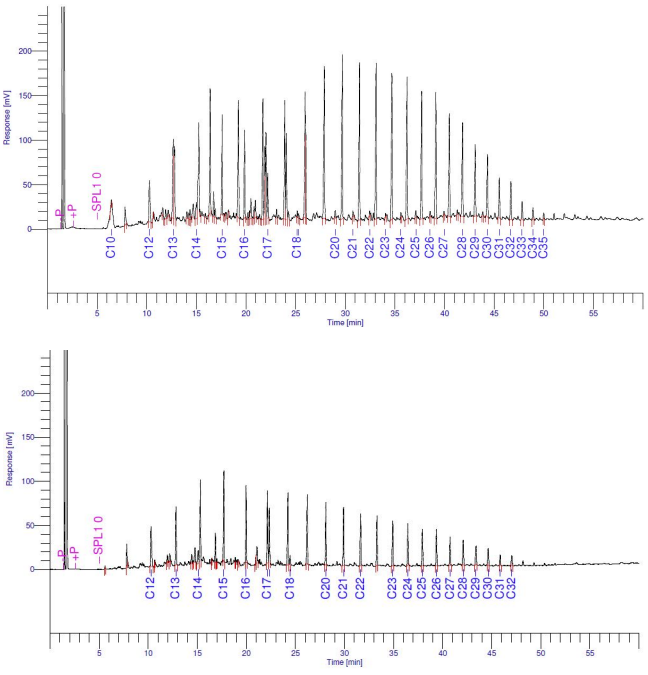


Fig. 4: Capillary gas chromatography analysis of (a): Control MSM + 1% crude oil without bacteria; (b): *P. aeruginosa was* grown in MSM + 1% crude oil after 7 days incubation

naphthalene, xylene, anthracene and biphenyl (Mujahid *et al.* 2015). Current research can focus on the cost-effective, large-scale applications of native bacteria for industrial crude oil degradation (Vinothini *et al.* 2015). Industrial crude oil contamination is an alarming problem because of its detrimental effects on the health of different organisms and humans. The oil biodegradation capacity of *Pseudomonas* sp. clearly indicates that this bacterial species uses hydrocarbon as a carbon source (Fig. 4). The detection of hydrocarbon degradation or residual toxicity after biodegradation highlights the need to test for changes in pollutant levels in bioremediation sites. The occurrence of contaminants in mixtures, like crude oil, is an important problem because the removal or degradation of one component can be inhibited by other compounds or by-products in the mixture, and different conditions may be required to treat different compounds within the same mixture (Plaza *et al.* 2008).

Conclusion

This study showed that three species of bacteria, *Pseudomonas* sp. and *Micrococcus* sp., isolated from contaminated soil and water were able to degrade crude oil. They showed optimum degrade crude oil by *Pseudomonas* sp. as 65% and 58% *Micrococcus* sp. at highest percentage compared to other isolates. Both bacterial genera Gram positive and negative were isolated from water and soil and assumed to have a common pattern of tolerance and degradation of crude oil. Bacterial isolates capable of degrading complex hydrocarbons present in the environment have a potential to be used as an effective tool for removing ecotoxic compounds.

Acknowledgment

The authors would like to thank the University of Basrah, Department of Ecology. This research project under University of Basrah, Science College, Department of Ecology.

References

Arulazhagan. P., N. Vasudevan and I.T. Yeom (2010). Biodegradation of polycyclic aromatic hydrocarbon by a halotolerant bacterial consortium isolated from marine environment. Int. *J. Environ. Sci. and Tech.,* **7**: 639-652

Das, K. and A.K. Mukherjee (2007). Crude petroleum-oil biodegradation efficiency *of Bacillus subtilis* and *Pseudomonas aeruginosa* strains isolated from a petroleum-oil contaminated soil from North-East India. *Bio. Tec.,* **98**: 1339-45

Gargouri, B., F. Karray, N. Mhiri, F. Aloui and S. Sayadi (2011). Application of a continuously stirred tank bioreactor (CSTR) for bioremediation of hydrocarbon-rich industrial

wastewater effluents. *J. Haz. Mat.,* **189**: 427–434

Hawle-Ambrosch, E., W. Riepe, M. Dornmayr-Pfaffenhuemer, C. Radax, A. Holzinger and H. Stan-Lotter (2007). Biodegradation of fuel oil hydrocarbons by a mixed bacterial consortium in sandy and loamy soils. *Biotech J.,* **2**: 1564-1568.

Hassanshahian, M., G. Emtiazi and S. Cappello (2012). Isolation and characterization of crude-oil-degrading bacteria from the Persian Gulf and the Caspian Sea. *Mar. Poll. Bull.,* **64**: 7–12.

Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Stanley and S.T. William (1994). Bergey's Manual of Determinative Bacteriology. Baltimore: William and Wilkins.

Holt, S.G., N.R. Kriey, P.H.A. Sneath, J.T. Stanley and S.T. William (1998). Bergy's Manual of Determinative for Bacteriology. Williams and Wilkins, New York.

Kayode-Isola, T.M., K.I.T. Eniola, A.B. Olayemi and O.O. Igunnugbemi (2008). Response of Resident Bacteria of a Crude Oil-Polluted River to Diesel Oil. *Am-Euras J. Agron,* **1(1)**: 06-09

Latha, R. and R. Kalaivani (2012). Bacterial Degradation of Crude Oil by Gravimetric Analysis. *Adv. Appl. Sci. Res.,* **3(5)**: 2789-2795

Ma, B,, Y. He, H. Chen, J. Xu and Z.Rengel (2010). Dissipation of polycyclic aromatic hydrocarbons (PAHs) in the rhizosphere: Synthesis through meta-analysis. *Environ. Poll.,* **158**: 855–861

McPherson, A. (2007). Monitoring Phytoremediation of Petroleum Hydrocarbon Contaminated Soils in a Closed and Controlled Environment. Thesis Submitted to the College of Graduate Studies and Research.*The Degree of Masters of Science,* P206.

Meintanis. C., K.I. Chalkou, K.A. Kormas and A.D. Karagouni (2006). Biodegradation of crude oil by thermophilic bacteria isolated from a volcano Island. Biodegradation DOI 10.1007/s10532-005-6495-6

Mujahid, T.Y., P.S.H. Abdul Wahab. S.A. Subhan, M.N. Baloch and Z.A. Pirzada (2015). Isolation and Characterization of Hydrocarbon Degrading Bacteria from Petrol Contaminated. *Soil. J. Basic & Appl.* Sci., **11**: 223-231

Nikhil T, Deepa V, Rohan G, Satish B (2013) Isolation characterization and identification of diesel engine oil degrading bacteria from garage soil and comparison of their bioremediation potential. Int Res J Environ Sci 2(2): 48-52

Panda, S.K., R.N. Kar and C.R. Panda (2013). Isolation and identification of petroleum hydrocarbon degrading microorganisms from oil contaminated environment. *Int. J. Environ. Sci.,* **3**: s5

Plaza1, G.A., K. £ukasik, J. Wypych, G. Nalêcz-Jawecki, C. Berry and R.L. Brigmon (2008). Biodegradation of Crude Oil and Distillation Products by Biosurfactant-Producing Bacteria. *Polish J. of Environ. Stud* ., **17(1)** : 87-94

Subathra, M.K., G. Immanuel and A.H. Suresh (2013). Isolation and identification of hydrocarbon degrading bacteria from Ennore creek. *Bioinformation;* **9(3)**:150-157 http://dx.doi.org/10.6026/97320630009150

Szoboszlay, S., B. Atzel and B. Kriszt (2003). Comparative biodegradation examination of *Pseudomonas aeruginosa* (ATCC 27853) and other oil degraders on hydrocarbon contaminated soil. *Com. Agr. Appl. Biol. Sci.,* **68**: 207-10

Vinothini, C., S. Sudhakar and R. Ravikumar (2015). Biodegradation of petroleum and crude oil by *Pseudomonas putida* and *Bacillus cereus. Int. J. Curr. Microbiol. App. Sci.,* **4(1)**: 318-329.