Egyptian Journal of Soil Science
http://ejss.journals.ekb.eg/

Characterization of Hydrocarbon-Utilizing Indigenous Bacterial Species Isolated from Crude Oil- Contaminated Soil in Sudan

Salma Yousif Elsheikh* and **Ismail M. A. M. Shahhat**

Northern Border University, Faculty of Sciences, Department of Biological Sciences, Arar, Saudi Arabia

POLLUTANTS in the soil can have serious environmental and health consequences in the surrounding. Soil pollution caused by mining, transportation, and petroleum, particularly oil, is a major problem in the twenty-first century because of the harmful repercussions. Oil contamination, primarily caused by petroleum oil, poses a significant global issue causing bacteria to thrive in oil-contaminated environments. A study isolated fourteen culturable strains of oil-utilising bacteria from pumping stations in Khartoum, Sudan, using enrichment culture techniques. These isolates were isolated from the soil contaminated with crude oil for initial identification. Morphological and biochemical analysis of pure cultures of bacterial isolates revealed that the isolates belong to 9 well known oil–degrading bacterial genera viz. *Bacillus*, *Citrobacter*, *Enterobacter*, *Klebsiella*, *Kocuria*, *Micrococcus*, and *Staphylococcus* from 3 orders and 3 classes within three phyla (Proteobacteria, Firmicutes, and Actinobacteria). The growth rate of isolates in MSM containing crude oil was assessed using an Apel spectrophotometer model PD-303 at OD620. Five bacterial strains identified as *Kocuria sp. (Isolate No 14; PS2), Bacillus lentus (Isolate No 13; PS1), Citrobacter diversus (Isolate No 12; PS2), Bacillus mycoides (Isolate No 11; PS1), and Bacillus sp (Isolate No 10; PS1)* capable of utilising hydrocarbons were selected to construct a consortium for further study. The findings of this study showed that isolates from seven genera found in the contaminated sites had exceptional potential for use in biotechnological processes like hydrocarbon-polluted site bioremediation. These isolates will also help to maintain a sustainable environment. As a result, this study indicated that these bacteria could have promising applications in hydrocarbon bioremediation.

Keywords: Spectrophotometer, crude oil, Isolate, bacteria growth rate, Enrichment technique.

1. Introduction

In addition to complex chemical molecules like aliphatic and aromatic hydrocarbons, petroleum is a viscous liquid that also contains mineral components. Crude oil has C_{10}-C_{28} hydrocarbons, while gasoline contains C_6-C_{10} hydrocarbons (Wu et al., 2013; Kuppusamy et al., 2020). Growing global industrialization and urbanization have led to a major increase in the consumption of crude oil and its byproducts in recent decades. During the extraction, shipping, processing, and use stages, petroleum leaks into the soil are all but inevitable and are usually brought on by inadequate management, insufficient combustion, old pipeline cracks, and unforeseen accidents. Although oil is an essential energy source, over drilling can lead to oil spills that contaminate soil, harming ecosystems and public health (Xu et al., 2018; Tahir et al., 2024). Petroleum pollution in soil can have a serious negative impact on the environment and human health in the surrounding area because of how harmful petroleum and its byproducts are. In the twenty-first century, soil contamination from mining, transportation, and petroleum especially oil is a serious problem because of its detrimental effects (Mambwe et al., 2021; Lv et al., 2022). As cities continue to expand and civilization advances, the environmental harm caused by petroleum hydrocarbons has increased to the point that it requires quick rehabilitation. A diverse range of environmental microbes can break down petroleum hydrocarbons, producing energy and carbon. Many times, using microorganisms with specific characteristics is part of the biological treatments used to restore oil-damaged areas (Aziz et al., 2024).

contaminated areas endanger the environment and human health, limiting urban development and economic progress. Petroleum components in oilcontaminated locations degrade slowly, which damages neighbouring species. Petroleum aromatics are difficult to remove from soil because of their complicated molecular structure. Compounds having a high carbon count, insoluble in water, and toxicity are difficult to remove. Urgent research is required for cost-effective and efficient remediation solutions to enable sustainable soil usage for food production and healthy living environments (Lv et al., 2022). One of the biggest challenges that oil refineries confront is the correct management and disposal of oily sludge as a hazardous waste. The Khartoum Refinery is

*Corresponding author e-mail: yousifsalma9@gmail.com
Received: 24/10/2024; Accepted: 16/12/2024
DOI: 10.21608/EJSS.2024.330875.1900

no exception. Extreme caution must be used to avoid these oily wastes from entering and damaging the environment. Bioremediation, which employs certain microbes, is an effective technique for cleaning up contaminated environments. The bacterial strains successfully decomposed petroleum oil, showing the possibility of bioremediation in polluted locations (Varjani and Upasani, 2016; Varjani, 2017; Ranjani et al., 2024; Tahir et al., 2024).

Bioremediation is a successful strategy for cleaning up contaminated regions by utilizing the inherent capacity to degrade toxic organic compounds, thereby regenerating the ecosystem (Ishaya et al., 2023). Numerous studies have shown that petroleum hydrocarbons may be broken down and used as the main source of carbon and energy by a variety of microorganisms (Panda et al., 2013, Dwivedi et al., 2019; Kumari et al., 2020;; Augustine, 2023; Ahirwar and Dehariya, 2013; Muthukumar et al., 2022; Wang et al., 2011). From diverse oil-contaminated natural settings, more than 200 species of hydrocarbon-degrading microbes have been found (Nwaogu et al., 2008; Musa, 2019; Wang et al., 2020; Aqeel et al., 2021; Issa et al., 2023; Aladwan et al., 2024; Tahir et al., 2024).

Many hydrocarbon-degrading bacteria have been isolated from soil (Hamzah et al., 2010; Sun, et al., 2018; Obafemi, et al., 2018; Ra, et al., 2019; Soltanighias, et al., 2019; Chikere, et al., 2020; El-Shennawy, 2022; Atai et al., 2023) including crude oil contaminated sites (Nadarajah et al., 2002; Okoh, 2003), refined petroleum products polluted sites (Kebria et al., 2009; and Eze & Eze, 2010) and sludge (Wang et al., 2011; Panda et al., 2013; Ahirwar and Dehariya, 2013; Lima et al., 2019). The exploration of unique bacterial genera and novel isolates effective in hydrocarbon degradation reveals several promising candidates. Among these, the *Rhodococcus qingshengii* GOMB7 strain stands out for its ability to degrade long-chain alkanes under microaerobic conditions, a novel capability within its genus. This strain, isolated from the Gulf of Mexico, demonstrates potential for energy-efficient bioremediation in low-oxygen environments (Juárez et al., 2023). *Fictibacillus phosphorivorans* RP3 is a novel strain identified for its ability to degrade kerosene and diesel, achieving over 90% degradation efficiency. This strain also produces biosurfactants, which enhance its hydrocarbon degradation capabilities by increasing the bioavailability of hydrophobic compounds (Pandey et al., 2021). While these strains and genera show promise, the effectiveness of bioremediation can be influenced by environmental conditions and the specific hydrocarbons present. The adaptability of indigenous strains, such as Bacillus sp. SEA18 and Serratia sp. Tan611, underscores the importance of local microbial communities in bioremediation efforts (Swetha et al., 2020; Semai et al., 2021). Abd El-Hafez (2014) suggested that *Bacillus megaterium* sp. can be useful for the remediation of hydrocarbons and heavy metal contaminated soils.

This research aims to isolate and identify several hydrocarbon-utilizing bacteria from Sudan's crude oil polluted soils.

2. Materials and Methods

2.1. Soil sample collection

Random samples from crude-oil contaminated soil at depths of 0-20 cm were obtained from the following locations:

(i) Soil contaminated with crude oil from petrol filling station1 in Khartoum south (15.44404,32.83877).

(ii) Soil contaminated with crude oil from petrol filling station 2 in Khartoum north (16.654048,33.442155).

The soil samples were carefully mixed, and all stones and debris were eliminated. After that, they were taken to the lab, put through a 2 mm sieve, and stored at 4°C in a refrigerator for analysis.

2.2. The composition of Medium

Mineral salt medium (MSM) was utilized as the enrichment medium, 1% (v/v) Heglig Nile Blend (HNB) crude oil as sole carbon source for isolation of hydrocarbon-utilising bacteria. The investigation was conducted using the MSM Zajic and Supplisson, (1972) combination. Khartoum Oil Refinery at Algaili provided the HNB crude oil. MSM was used to cultivate the isolated bacteria. The pH of the medium was adjusted to 7.4 using 6 N hydrochloric acid or 1 N sodium hydroxide.

2.3. Isolation and enrichment of pure cultures of crude oil-utilising/ bacteria

Bacterial strains that use hydrocarbons were identified and purified using the following enrichment method: 99 mL of Zajic and Supplisson's (1972) MSM, which included 1% crude oil as the only carbon source, was used to suspend 1 g of contaminated soil in a 250 mL conical flask. For seven days, the flasks were shaken occasionally while being incubated at 35ºC ±2ºC with periodic shaking for seven days. Serial dilutions were carried out for all samples. Zajic and Supplisson's (1972) MSM agar, which contained 1% crude oil as the only carbon source, was

covered with one milliliter of each dilution. Plates were incubated at 35 degrees Celsius for three to seven days. Colonies of oil-using bacteria were chosen at random and purified by repeatedly streaking them over nutrient agar plates until pure colonies were formed. For testing purposes, the purified isolates were subcultured every 30 days onto nutrient agar slants, which were stored in a refrigerator at 4°C.

2.4. Characterization and identification of isolated bacteria

Using the methods described by Cheesbrough (1991), pure bacterial isolates were examined visually, microscopically, and biochemically. Standard taxonomic references such Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974), The Prokaryotes (Dworkin et al., 2006), and Bergey's Manual of Systematic Bacteriology (De Vos et al., 2009) were used to identify the isolated bacterial strains.

2.5.Morphological and microscopic characteristics of the bacterial isolates

The pure isolates were grown on nutrient agar medium. Shape, color, surface elevation, margin, transparency, pigmentation, opacity, and appearance of colonies examined using the same method described by Cheesbrough (1991). Two tests were performed on each bacterial isolate: endospore staining and Gram staining, which differentiates between Gram-positive and Gram-negative bacteria. Fresh, pure bacterial isolates were used in each assay. All isolates were subjected to Gram staining using the protocol outlined by Cappuccino and Sherman (1996).

2.6. Biochemical characteristics of the bacterial isolates

Oxidase, hydrogen sulfide generation, urease, indole, methyl red, nitrate reduction and Voges-Proskauer reactions, citrate utilization test, oxidative fermentation, catalase, and starch hydrolysis were among the biochemical tests conducted (Cheesbrough 1991).

2.7. Determination of bacterial growth by measuring turbidity of the culture broth

According to Rahman et al. (2002), the optical density of the liquid medium was measured at 620 nm using an Apel spectrophotometer model PD-303 in order to track the bacterial growth of each isolate. A conical flask with 100 mL of sterile MSM broth and 1 mL of crude oil as the sole carbon source was filled with a loopful of each bacterial isolate's overnight culture (18 hours). For three weeks, the flasks were kept in an incubator set at 35°C to track the growth of bacteria. To identify turbidity brought on by bacterial growth, the optical density was measured at 620 nm at the conclusion of the incubation period.

Bacterial growth was classified as:-

(i) excellent (OD620=0.81-2.0, indicated by ++++) or high (OD620=0.61-0.80, designated by +++), suggesting strong utilization ability.

(iii) Bacterial growth is moderate (OD620 = 0.41-0.60), as shown by the ++ symbol, suggesting moderate utilising ability.

(iv) Bacteria with limited growth (OD620 = < 0.40) are marked by +, suggesting low utilisation ability.

(v) no growth (-).

3. Results

3.1. Oil-utilising bacteria isolation

The hydrocarbon-utilising bacteria were isolated using MS (Mineral Salt) medium supplemented with Heglig Nile Blend (HNB) crude oil (1%). We observed bacterial growth on the medium after four days of incubation at 35^0C. Using the streak plate approach, colonies were then divided according to their morphological characteristics, all experimental steps was summarized in Figure 1. Fourteen isolates, referred to originally as Isolate-1, Isolate-2, Isolate-3, Isolate-4 Isolate-5, Isolate-6, Isolate-7, Isolate-8, Isolate-9, Isolate-10, Isolate-11, Isolate-12, Isolate-13, and Isolate-14, were shown to be viable candidates for use in bioremediation (Table 1). Given that the isolates were cultivated on media containing just burned Heglig Nile Blend (HNB) crude oil as a carbon source for their survival, development, and reproduction, it may be assumed that these isolates can absorb these substances as food and nourishment and can subsequently degrade them.

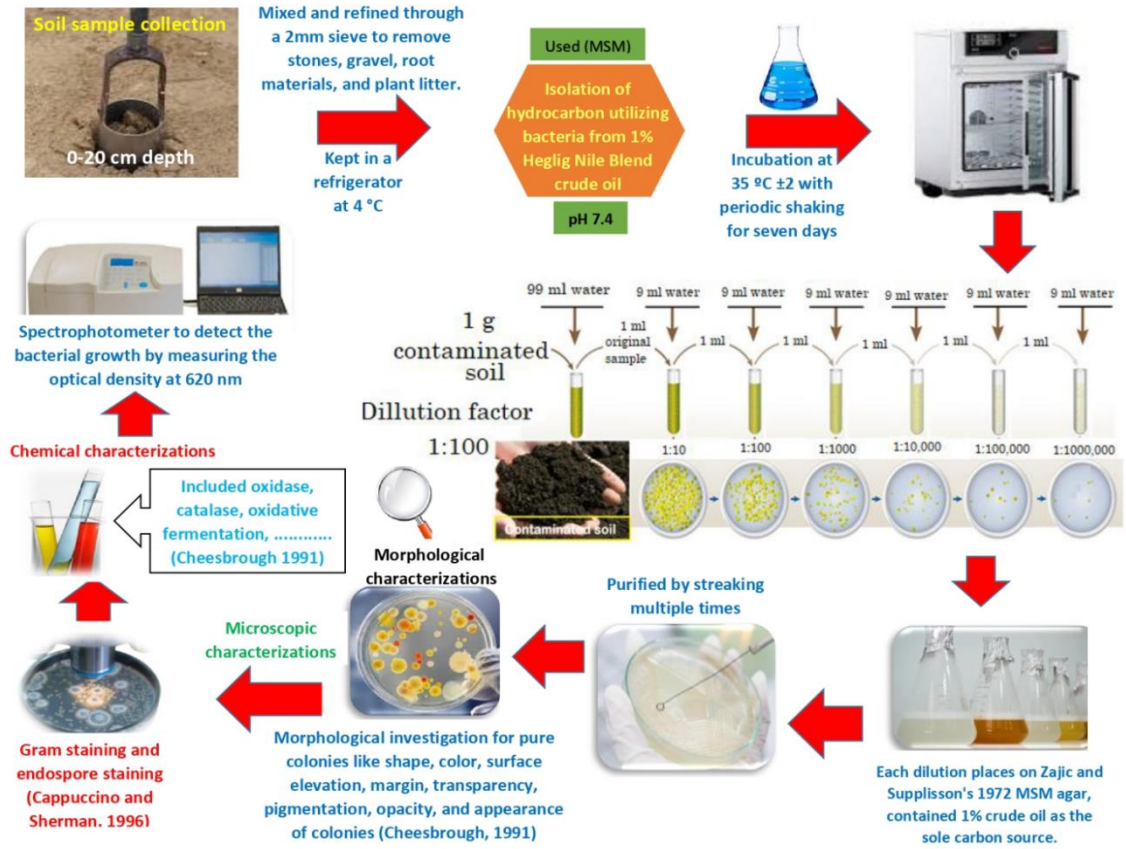

Fig. 1 Experiment processes of isolation, purification and identification of crude oil-utilizing bacteria fron oil contaminated soil.

Table 1: Growth performance of the fourteen identified strains measured at an optical density of 620 nm (OD620) in mineral salt medium (MSM) containing 1% crude oil.

Represented No.	Bacterial strains	Source of isolate	OD_{620}	Bacteria Growth
1	*Bacillus brevis*	PS2	0.206	+
2	*Bacillus firmus*	PS1	0.378	+
3	*Micrococcus roseus*	PS2	0.448	++
4	*Bacillus megaterium*	PS1	0.503	++
5	*Staphylococcus* sp.	PS1	0.539	++
6	*Bacillus amyloliquefaciens*	PS1	0.567	++
7	*Klebsiella oxytoca*	PS1	0.567	++
8	*Enterobacter asburiae*	PS2	0.580	++
9	*Enterobacter* sp.	PS1	0.588	++
10	*Bacillus sp.*	PS1	0.654	+++
11	*Bacillus mycoides*	PS1	0.670	+++
12	*Citrobacter diversus*	PS2	0.680	+++
13	*Bacillus lentus*	PS1	0.684	+++
14	*Kocuria* sp.	PS2	0.878	+++

PS1: the first petrol station; PS2: the second petrol station The data shows the averages that were taken after seven days of incubation.

3.2 Morphological characterization

Table 2 shows colony morphological characteristics such as shape, colour, surface elevation, margin, transparency, pigmentation, opacity, and appearance as described in Cheesbrough (1991). The colour and colony form of the bacteria allowed for a partial identification. Twenty-four hours later, the streaking results were seen.

Fourteen distinct colonies have been identified based on their morphology. The first group of isolates (1, 4, 6, 8, 9, 10, 12, and 13) comprised cream-colored colonies, while the second group (2, 5, 7, and 11) consisted of small white colonies. The third isolate, number 3, was a pink-colored colony, and isolate number 14 was an orange-colored colony.

Table 2: The morphological and cultural traits of the bacterial strains that were separated from various environments.

Represented No.	Source of isolate	Form	Colour	Surface	Elevation	Margin	Opaque	Pigmen	Appearance
2	PS1	Circular	White	Smooth	Raised	Undulate	Opaque	Non	Shine
13	PS1	Circular	Cream	Smooth	Raised	Serrate	Opaque	Non	Non
5	PS1	Circular	White	Granular	Raised	Entire	Opaque	Non	Non
11	PS1	Rhizoid	White	Granular	Umbonate	Lobate	Opaque	Non	Non
6	PS1	Circular	Cream	Smooth	Raised	Entire	Transparent	Non	Shine
9	PS1	Circular	Cream	Smooth	Raised	Serrate	Transparent	Non	Shine
7	PS1	Circular	White	Smooth	Convex	Entire	Opaque	Non	Non
4	PS1	Circular	Cream	Smooth	Flat	Entire	Transparent	Non	Shine
10	PS1	Circular	Cream	Smooth	Raised	Entire	Transparent	Non	Shine
8	PS2	Circular	Cream	Smooth	Flat	Entire	Transparent	Non	Shine
12	PS2	Circular	Cream	Smooth	Convex	Entire	Opaque	Pigmen	Non
3	PS2	Circular	Pink	Smooth	Raised	Entire	Opaque	Pigmen	Shine
14	PS2	Circular	Orange	Smooth	Raised	Entire	Opaque	Pigmen	Shine
1	PS2	Circular	Cream	Smooth	Raised	Entire	Opaque	Non	Non

PS1: petrol station1; PS2: petrol station2.

Table 3 displays endospore staining according to Cappuccino and Sherman's (1996) instructions as well as Gram staining to distinguish between Gram-positive and Gram-negative bacteria. The isolates numbers (1,2,4,6,10,11 and 13) are gramme positive, rod-shaped bacteria and endospore positive according to endospore staining except isolate number 10 is endospore negative. The isolates numbers (7,8,9 and 12) are gramme negative, short-rod-shaped bacteria. and endospore negative according to endospore staining. The isolates numbers (3,5, and 14) are Gram positive, cocci shaped bacteria according to Gram staining and endospore negative according to endospore staining. They are motile, according to a motility test.

Table 3: Microscopic and staining characteristics of bacterial strains isolated from the two hydrocarbon polluted habitats.

Isolatetete No	Source of isolate	Cell shape	Gram stain	Endospore stain	Genus
2	GPS 2.4	Rod	Positive	+	*Bacillus*
13	GPS 2.9	Rod	Positive	+	*Bacillus*
5	GPS 2.10	Cocci	Positive	–	*Staphylococcus*
11	GPS 2.13	Rod	Positive	+	*Bacillus*
6	GPS 2.14	Rod	Positive	+	*Bacillus*
9	GPS 2.15	Short rod	Negative	–	*Enterobacter*
7	GPS 2.16	Short rod	Negative	–	*Klebsiella*
4	GPS 2.17	Rod	Positive	+	*Bacillus*
10	GPS 2.19	Rod	Positive	–	*Bacillus*
8	SHS 3.3	Short rod	Negative	–	*Enterobacter*
12	SHS 3.5	Short rod	Negative	–	*Citrobacter*
3	SHS 3.21	Cocci	Positive	–	*Micrococcus*
14	SHS 3.25	Cocci	Positive	–	*Kocuria*
1	SHS 3.27	Rod	Positive	+	*Bacillus*

PS1: petrol station1; PS2: petrol station2

3.3 Biochemical characterization

Table 4 shows the biochemical tests such as oxidase, oxidation fermentation, catalase, hydrogen sulphide production, nitrate reduction, urease, methyl red, indole and Voges-Proskauer reactions, citrate utilization test, and starch hydrolysis were carried out as described by Cheesbrough (1991). The fifteen bacterial isolates' biochemical tests findings were performed as described by Cheesbrough (1991).

Table 4: Biochemical characteristics of bacterial strains isolated from the three hydrocarbon polluted habitats.

Isolate No	Strain No	Glucose	Lactose or\and sucrose	Gas	H_2S Production	Oxidase	MR	VP	Indole	Oxidation Fermentation	Starch	Nitrate	Urease	Citrate	Catalase
2	PS1	K	A	+	+	–	+	–	–	+	+	+	–	+	+
13	PS1	A	A	+	–	–	+	–	–	+	+	+	+	+	+
5	PS1	K	A	+++	–	–	+	–	–	+	+	+	+	+	+
11	PS1	K	A	+	–	+	+	–	–	+	+	+	–	+	+
6	PS1	K	A	+	+	–	+	–	+	+++	+	+	+	+	+
9	PS1	A	A	+	–	–	+	–	–	+	–	+	+	+	+
7	PS1	K	A	+++	–	–	–	+	+	+	–	+	+	+	+
4	PS1	K	A	++	–	–	+	–	–	+	+	+	–	+	+
10	PS1	K	A	–	–	–	+	–	–	–	+	+	+	+	+
8	PS2	A	A	++	–	–	+	–	–	+	+	+	+	+	+
12	PS2	A	A	+	–	–	+	–	+	+	+	+	+	+	+
3	PS2	K	K	–	–	–	+	–	–	–	+	+	+	+	+
14	PS2	A	A	–	–	–	+	–	–	+	+	–	–	+	+
1	PS2	K	A	–	–	–	+	–	–	+	+	–	+weak	+	+

PS1: petrol station1; PS2: petrol station2; K: alkali (–); A: acid (+).

3.4. Growth profile of isolated bacterial strains

In MSM containing 1 percent crude oil, the isolates' growth rate was assessed using the Apel spectrophotometer model PD-303, as described by Rahman et al. (2002). The strains isolated from contaminated soils (petrol filling station1 and petrol filling station 2) are shown in Fig. 2 with their growth rates. Out of all the isolates, the bacteria isolated from PS2 soil showed the greatest capacity to proliferate in the medium that included crude oil. The highest growth rate was observed in PSN14, identified as Kocuria sp. (shown in Fig. 2), while PSN12, identified as Citrobacter diversus, displayed the second highest growth rate. PSN8, identified as Enterobacter asburiae, exhibited a moderate growth rate, and PSN3, identified as Micrococcus roseus, showed the second moderate growth rate. Additionally, PSS13 (*Bacillus lentus*), PSS11 (*Bacillus mycoides*), and PSS10 (Bacillus sp.), isolated from petroleum station one samples, also demonstrated significant growth rates. Moderate growth rates were observed in PSS9, identified as Enterobacter sp., PSS7, identified as Klebsiella oxytoca, PSS6, identified as Bacillus amyloliquefaciens, PSS5, identified as Staphylococcus sp., and PSS4, identified as Bacillus megaterium. In contrast, other strains, such as PSS2 and PSN1, identified as *Bacillus firmus* and *Bacillus brevis* respectively, exhibited the lowest growth rates in oil-containing media (Fig. 2).

Several of the bacterial taxa that were isolated and identified in this study have also been found as effective hydrocarbon degraders and biosurfactant producers in marine environments. These consist of *Kocuria* sp., *Bacillus lentus*, *Citrobacter diversus*, *Bacillus mycoides*, *Bacillus* sp. *Enterobacter sp.*, *Enterobacter asburiae*, *Klebsiella oxytoca*, *Bacillus amyloliquefaciens*, *Staphylococcus* sp., *Bacillus megaterium*, *Micrococcus roseus*, *Bacillus firmus*, and *Bacillus brevis* [Al-Mailem et al., 2017; Erdoğmuş et al., 2013; Zenati et al., 2018; Gutierrez et al., 2020; Neifar et al., 2019; Abbas, et al., 2022; and El-Shennawy, 2022).

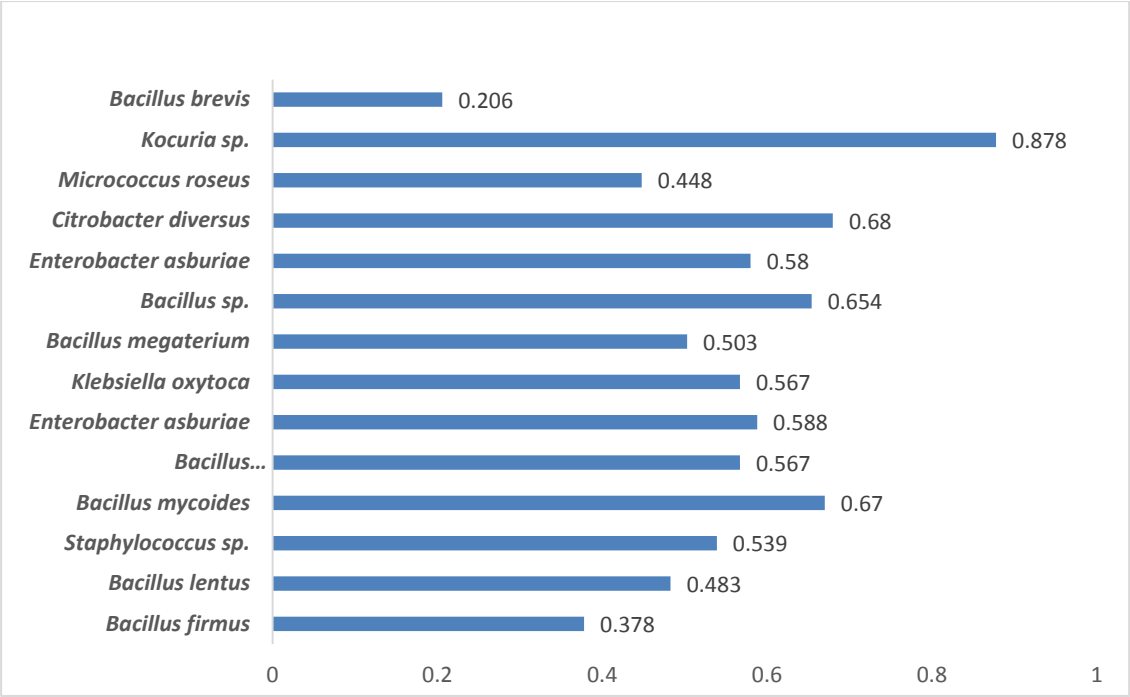

Fig 2. Oil degrading ability as measured by optical density at 620 nm due to turbidity.

4. Discussion

Crude oil degrading bacteria in natural habitats

Hydrocarbon degrading bacteria occur in natural habitats. According to Atlas (1995) the proportion of their population in hydrocarbon–contaminated environments is about 10% of the total bacterial population. Similarly, Rhykerd et al. (1999) reported that up to 10% of culturable organisms in unpolluted soil have been found to grow on and degrade hydrocarbons. Hydrocarbon degraders are widely spread in polluted soil where their numbers increase rapidly reaching 90% in extreme cases (Atlas and Bartha, 1992; Boboye et al., 2010). Since the survival of the inoculated acclimatized oil degrading bacteria is a key deciding factor on which the success of bioremediation depends, all bacteria in the present study were isolated from petroleum contaminated soils from gas petrol stations in Khartoum. The use of indigenous acclimatized bacteria as source of inoculum is in conformity with the procedure of Jasmine and Mukherji (2014). It is widely recognized that introduction of mixed indigenous consortia of hydrocarbon degraders to hydrocarbon polluted sites would be advantageous to effectively degrade all the hydrocarbons in crude oil (Atlas, 1995; Atlas and Bartha, 1992; Jasmine and Mukherji, 2014). The bacteria in this investigation were isolated from soil samples that had petroleum hydrocarbon contamination. To assess the degrading efficacy of the isolated bacteria, we employed burned Heglig Nile Blend (HNB) crude oil as their only carbon source in our experiment. These isolates have the capacity to break down Heglig Nile Blend (HNB) crude oil, but how well they do so depend on the biological, chemical, and physical factors.

The isolates were characterized morphologically and biochemically using the taxonomic scheme of Bergey's Manual of Determinative Bacteriology (Tables 2, 3 and 4) to identify them. The findings of morphological tests show that every isolated bacterium was gramme negative, motile, and cocci shaped. The isolates can detoxify hydrogen peroxide and use citrate as a carbon source, according to biochemical studies. A sugar utilisation test also verified that they could use a variety of carbohydrates as a source of nutrients, including lactose, sucrose, and glucose. Each isolate ferment lactose. They can hydrolyze starch and urea as well. The bacteria listed in Table 1 were also identified as hydrocarbon-utilising microorganisms by several workers (Tahir et al., 2024; Musa, 2019; Wang et al., 2020; Aqeel et al., 2021; Issa et al., 2023; Aladwan et al., 2024; Hassanshahian et al., 2014).

A total of fourteen bacterial isolates were shown to grow on SMS supplemented with crude oil as sole source of carbon. Ten isolates were precisely identified to named species while four were identified to the genus level. Morphological and biochemical analysis of pure cultures of bacterial isolates revealed that the isolates belong to nine well known oil-utilising bacterial genera viz. *Bacillus, Enterobacter, Klebsiella, Kocuria, Micrococcus,* and *Staphylococcus* from three orders and three classes within three phyla (*Proteobacteria, Firmicutes,* and *Actinobacteria*). Most of them were frequently reported to possess high potentials to biodegrade total petroleum

hydrocarbons (Erdoğmuş et al., 2013). The hydrocarbon-utilising abilities of bacteria and their development in MSM was evaluated by measuring the light transmittance in liquid medium spectrophotometrically based on the increase of bacteria in the liquid broth. Spectrophotometric reading results (OD620 nm) of the reproduction abilities of the fourteen bacteria isolated during the present study are given in Figure 1. Figure 1 shows oil-utilising ability as measured by optical density at 620 nm due to turbidity. As can be seen in the Figure, the best results were obtained from isolates: Kocuria sp. (Isolate No 14; PS2), Bacillus lentus (Isolate No 13; PS1), Citrobacter diversus (Isolate No 12; PS2), Bacillus mycoides (Isolate No 11; PS1), and Bacillus sp (Isolate No 10; PS1). In concordance with the current study (Afifi et al., 2015), indigenous bacteria (*Pseudomonas stutzeri* and *Klebsiella pneumoniae* strain) were extracted from petroleum effluent generated in the Abadan refinery, Iran, utilizing a mineral-based medium. Indigenous bacteria were evaluated based on their capacity for the diminution of total petroleum hydrocarbons.

Analogous findings were documented by (Hamzah et al., 2010) who extracted four species of bacteria, *Acinetobacter lwoffii*, *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, and *P. putida*, from soil tainted with hydrocarbons. The isolates were examined for their capability to biodegrade petroleum hydrocarbons by cultivating in Minimal Salts Medium (MSM) augmented with two varieties of crude oil, either Sumandak or South Azngsi at a 1% (v/v) concentration. Several of the bacterial taxa that were isolated and identified in this study have also been found as effective hydrocarbon degraders and biosurfactant producers in marine environments. These consist of *Kocuria* sp., *Bacillus lentus*, *Citrobacter diversus*, *Bacillus mycoides*, *Bacillus* sp. *Enterobacter* sp., Enterobacter asburiae, *Klebsiella oxytoca*, *Bacillus amyloliquefaciens*, *Staphylococcus* sp., *Bacillus megaterium*, *Micrococcus roseus*, *Bacillus firmus*, and *Bacillus brevis* [Al-Mailem et al., 2017; Erdoğmuş et al., 2013; Zenati et al., 2018; Gutierrez et al., 2020; Neifar et al., 2019; Abbas, et al., 2022; El-Shennawy, et al., 2022; Mussa, et al., 2024, Shalaby, et al., 2024].

5. Conclusion

In this investigation, the enrichment methodology was employed to isolate fourteen hydrocarbon-degrading bacterial strains from fuel stations utilizing crude oil as the sole carbon substrate. The five bacterial strains exhibiting the most optimal growth in crude oil are suggested for evaluation in subsequent studies prior to application in the field. *Bacillus lentus*, *Bacillus mycoides*, and *Bacillus* sp. were extracted from crude oil-contaminated soil of a PS1: Petrol Station 1, while *Kocuria* sp. and Citrobacter diversus were procured from crude oil-contaminated soil of a PS2: Petrol Station 2. It is proposed that bacterial strains can be applied in situ landfarming remediation of oil-polluted locations to diminish pollutants' concentrations to acceptable levels. The 16 s rRNA technique, biosurfactants, and biodegraded hydrocarbons of the isolates will be performed in future studies to identify the differences between these isolates and those published in the literature and other isolates obtained during this study.

Competing interests:

The authors declare that they have no conflict of interest in the publication.

Authors' contributions:

SE conceptualization of the study, Defined the topic of this Study. SE and IS wrote the original draft, edit and finalize approval of manuscripts.All authors read and agree for submission of manuscript to the journal.

Acknowledgment:

The authors extend their appreciation to the Deanship of Scientific Research at Northern Border University, Arar, KSA for funding this research work through the project number "NBU-FFR-2024-2585-01".

References

Abbas, A. S., Refae, R. I., Amin, G. A. E. F., & Elsayed, T. R. Characterization and potentials of microbial community in soil and water contaminated with crude oil in Egypt. *Egyptian Journal of Chemistry*, *2022*, *65*, 355-370.

Abd El-Hafez, A. A. Heavy Metal Resistant Bacteria Isolated from Hydrocarbons Contaminated Soil. *Egypt J. Soil Sci*, 2014, *54*, 1-6.

Afifi, A.; Motamedi, H.; Alizadeh, B.; Leilavi, H. Isolation and identification of oil degrading bacteria from oil sludge in Abadan oil refinery. Environmental and Experimental Biology, 2015, 13, 13-18.

Ahirwar, S.; Dehariya, K. Isolation and characterization of hydrocarbon degrading Biodegradation of oil tank bottom sludge using microbial consortia. Biodegradation, 2013, 18, 269-281.

Aladwan, M. M.; Dababneh, B. F.; Farah, H. S.; Abusalah, M. A. Identification of Oil Degrading Bacteria from Oil-Contaminated Soil in the Northeastern Part of Jordan. Journal of Ecological Engineering, 2024, 25(5), 306-320.

Al-Mailem, D. M., Al-Deieg, M., Eliyas, M., & Radwan, S. S. Biostimulation of indigenous microorganisms for bioremediation of oily hypersaline microcosms from the Arabian Gulf Kuwaiti coasts. *Journal of Environmental Management*, 2017, *193*, 576-583.

Aqeel, A.; Hussain, Z.; Aqeel, Q. U. A.; Zafar, J.; Ehsan, N.; Tariq, M. Enrichment and characterization of hydrocarbon degrading bacteria from various oil-contaminated sites in Pakistan. Geomicrobiology Journal, 2021, 38(7), 577-587.

Atai, E., Jumbo, R. B., Cowley, T., Azuazu, I., Coulon, F., & Pawlett, M. Efficacy of bioadmendments in reducing the influence of salinity on the bioremediation of oil-contaminated soil. *Science of the Total Environment*, 2023, *892*, 164720.

Atlas, R.M., and Bartha, R., Hydrocarbon biodegradation and oil–spill bioremediation. In: Marshall KC (ed) Advances in Microbial Ecology, 1992, vol. 12, Plenum Press, New York, pp. 287–338.

Atlas, R. M. Bioremediation of petroleum pollutants. *International Biodeterioration & Biodegradation*, 1995, *35*, 317-327.

Augustine, J. A. Degradative potential of bacteria isolated from crude oil contaminated soil. BioRxiv, 2023, 2023-10.

Aziz, Z. S.; Jazaa, S. H.; Dageem, H. N.; Banoon, S. R.; Balboul, B. A.; Abdelzaher, M. A. Bacterial biodegradation of oil-contaminated soil for pollutant abatement contributing to achieve sustainable development goals: A comprehensive review. Results in Engineering. 2024, 102083.

Boboye, B., Olukunle, O.F. and Adetuyi, F.C. Degradative activity of bacteria isolated from hydrocarbon–polluted site in Ilaje, Ondo State, Nigeria. *African Journal of Microbiology Research*, 2010, 4, 2484–2491.

Buchanan, R.E. and Gibbons, N.E. (eds.), Bergey's Manual of Determinative Bacteriology. Eighth Edition. Williams and Wilkins Company, Baltimore. 1974, 1268 pp.

Cappuccino, J.G. and Sherman, N. Microbiology: A Laboratory Manual. Fourth Edition. The Benjamin / Cummings Publishing Company, Inc. 1996, 477 pp.

Cheesbrough, M. Microbiology: Medical Laboratory Manual for Tropical Countries. Volume II. ELBS with Tropical Health Technology/Butterworth-Heinemann, 1991, 479 pp.

Chikere, C. B., Obieze, C. C., & Chikere, B. O. Biodegradation of artisanally refined diesel and the influence of organic wastes on oil-polluted soil remediation. *Scientific African*, 2020, *8*, e00385.

De Vos, P., Garrity, G.M., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K. H., Whitman, W.B. (Eds), Bergey's Manual of Systematic Bacteriology Second Edition, 2009, Vol 3. Springer, Dordrecht. 1422 pp.

Dwivedi, A., Chitranshi, S., Gupta, A., Kumar, A., Bhat, J. L. Assessment of the petroleum oil degradation capacity of indigenous bacterial species isolated from petroleum oil-contaminated soil. International Journal of Environmental Research, 2019, 13, 735-746.

Dworkin, M., Falkow, S., Rosenberg, E., Stackebrandt, E., Schleifer, K. H. (Eds.). The prokaryotes: a handbook on the biology of bacteria Vol. 1. 2006. New York: Springer.

El-Shennawy, S. Isolation And Characterization Of Crude Oil-Degrading Bacteria From Petroleum Oil Contaminated Egyptian Soils. Al-Azhar Journal Of Pharmaceutical Sciences, 2022, 65, 107-128.

Erdoğmuş, S. F., Mutlu, B., Korcan, S. E., Güven, K., & Konuk, M. Aromatic hydrocarbon degradation by halophilic archaea isolated from Çamaltı Saltern, Turkey. Water, Air, & Soil Pollution, 2013, 224, 1-9. https://doi.org/10.1007/s11270-013-1449-9.

Eze, V. C., & Eze, B. N. Isolation and characterization of microorganisms involved in the degradation of refined petroleum products polluted sites in Elele, Rivers State, Nigeria. *Int. J. Curr. Res*, 2010, *8*, 91-95.

Gutierrez, T., Morris, G., Ellis, D., Mulloy, B., & Aitken, M. D. Production and characterisation of a marine Halomonas surface-active exopolymer. *Applied microbiology and biotechnology*, 2020, *104*, 1063-1076. https://doi.org/10.1007/s00253-019-10270-x.

Hamzah A; Rabu A; Farzarul, R; Azmy, HR; Yussoff, NA. Isolation and characterization of bacteria degrading Sumandak and South Angsi oils. Sains Malaysiana, 2010, 39 (2): 161–168.

Hassanshahian, M.; Zeynalipour, M.S.; Musa, F.H. Isolation and characterization of crude oil degrading bacteria from the Persian Gulf (Khorramshahr Provenance). Marine Pollution Bulletin, 2014, 82, (1-2), pp. 39-44.

Ishaya, S.; Usman, S.; Nweke, O. D.; Adams, N. H.; Umar, R.; Ilyasu, N. S.; Yakasai, H. M. Degradation of used engine oil by alcaligenes sp. strain isolated from oil contaminated site: isolation, identification, and optimization of the growth parameters. Case Studies in Chemical and Environmental Engineering, 2023, 8, 100516.

Issa, H. K.; Dobara, M. I. A.; El-Sayed, A. K.; El-Bana, M. I. Bioprospecting of Lipase Producing Bacteria Isolated Form Oil Contaminated Sites of Port Said, Egypt. In The First International Conference & Expo on Green Science, 2023, September, (pp. 269-291). Cham: Springer Nature Switzerland.

Jasmine J, Mukherji S Evaluation of bioaugmentation and biostimulation effects on the treatment of refinery oily sludge using 2^n full factorial design *Environmental Science Processes Impacts*, 2014, 16, 1889-1896.

Juárez, K., Reza, L., Bretón-Deval, L., Morales-Guzmán, D., Trejo-Hernández, M. R., García-Guevara, F., & Lara, P. Microaerobic degradation of crude oil and long chain alkanes by a new Rhodococcus strain from Gulf of Mexico. World Journal of Microbiology and Biotechnology, 2023, 39, 264.

Kebria, D. Y., Khodadadi, A., Ganjidoust, H., Badkoubi, A., & Amoozegar, M. A. Isolation and characterization of a novel native Bacillus strain capable of degrading diesel fuel. *International Journal of Environmental Science & Technology*, 2009, *6*, 435-442.

Kumari, B.; Kriti, Singh, G.; Sinam, G.; Singh, D. P. Microbial remediation of crude oil-contaminated sites. Environmental Concerns and Sustainable Development: Volume 1: Air, Water and Energy Resources, 2020, 333-351.

Kuppusamy S, Maddela NR, Megharaj M, et al. Methodologies for analysis and identification of total pet-roleum hydrocarbons. In: Kuppusamy, S.; Maddela, N. R.; Megharaj, M.; Venkateswarlu, K. , editors. Total petroleum hydrocarbons: environmental fate, toxicity, and remediation. Cham: Springer International Publishing; 2020, 1-27.

Lima, S. D., Oliveira, A. F., Golin, R., Lopes, V. C. P., Caixeta, D. S., Lima, Z. M., & Morais, E. B. Isolation and characterization of hydrocarbon-degrading bacteria from gas station leaking-contaminated groundwater in the Southern Amazon, Brazil. *Brazilian Journal of Biology*, 2019, *80*, 354-361.

Lv, Y., Bao, J., & Zhu, L. A comprehensive review of recent and perspective technologies and challenges for the remediation of oil-contaminated sites. Energy Reports. 2022, 8, 7976-7988.

Mambwe, M.; Kalebaila, K. K.; Johnson, T. Remediation technologies for oil contaminated soil. Global Journal of Environmental Science and Management. 2021, 7(3), 419-438.

Musa, S. I. Isolation and identification of diesel oil-degrading bacteria in used engine oil contaminated soil. Journal of Applied Sciences and Environmental Management, 2019, 23(3), 431-435.

Mussa, E. S. E. S., Hewait, H. M., & El-sharnouby, S. F. Diesel Biodegradation Capacities of Bacillus subtilis OR632422, Micrococcus luteus OR632421 Isolated From petroleum-Contaminated Soil. *Egyptian Journal of Soil Science*, 2024, *64*, 1537-1547.

Muthukumar, B.; Parthipan, P.; AlSalhi, M. S.; Prabhu, N. S.; Rao, T. N.; Devanesan, S.; Rajasekar, A. Characterization of bacterial community in oil-contaminated soil and its biodegradation efficiency of high molecular weight (> C40) hydrocarbon. Chemosphere, 2022, 289, 133168.

Nadarajah, N., Singh, A., & Ward, O. P. De-emulsification of petroleum oil emulsion by a mixed bacterial culture. *Process biochemistry*, 2002, *37*, 1135-1141.

Neifar, M., Chouchane, H., Najjari, A., El Hidri, D., Mahjoubi, M., Ghedira, K., Naili F., Soufi L., Raddadi N., Sghaier H., Ouzari H., Masmoudi A. S. & Cherif, A. (2019). Genome analysis provides insights into crude oil degradation and biosurfactant production by extremely halotolerant Halomonas desertis G11 isolated from Chott El-Djerid salt-lake in Tunisian desert. *Genomics*, *111*(6), 1802-1814.https://doi.org/10.1016/j.ygeno.2018.12.003.

Nwaogu, LA; Onyeze, GO; Nwabueze, RN. Degradation of diesel oil in polluted soil using Bacillus subtilis. African Journal of Biotechnology, 2008, 7(12): 1939–1943.

Obafemi, Y. D., Taiwo, O. S., Omodara, O. J., Dahunsi, O. S., & Oranusi, S. Biodegradation of crude petroleum by bacterial consortia from oil-contaminated soils in Ota, Ogun State, South-Western, Nigeria. *Environmental technology & innovation*, 2018, *12*, 230-242.

Okoh, A. I. Biodegradation of Bonny light crude oil in soil microcosm by some bacterial strains isolated from crude oil flow stations saver pits in Nigeria. *African Journal of Biotechnology*, 2003, *2*, 104-108.

Panda, S.; Kar, R.; Panda, C. Isolation and identification of petroleum hydrocarbon degrading microorganisms from oil contaminated environment. International Journal of Environmental Science and Technology, 2013, 3(5), 1314-1321.

Pandey, R., Sharma, P., Rathee, S., Singh, H. P., Batish, D. R., Krishnamurthy, B., & Kohli, R. K. Isolation and characterization of a novel hydrocarbonoclastic and biosurfactant producing bacterial strain: Fictibacillus phosphorivorans RP3. 3 Biotech, 2021, 11, 105.

Ra, T., Zhao, Y., & Zheng, M. Comparative study on the petroleum crude oil degradation potential of microbes from petroleum-contaminated soil and non-contaminated soil. *International Journal of Environmental Science and Technology*, 2019, *16*, 7127-7136.

Rahman KSM; Rahman JT; Lakshmanaperumalsamy PI; Banat M. Towards efficient crude oil degradation by a mixed bacterial consortium. Bioresource Technology, 2002, 85: 257–261.

Ranjani, V. A.; Rani, G. T.; Jithendar, B.; Serafina, K.; Sri, K. J. Study on isolation and characterization of bacteria from soil samples of fuel station. World, 2024, 3(3).

Rhykerd RL, Crews B, Mclnnes KJ, Weaver RW Impact of bulking agents forced aeration, and tillage on remediation of oil–contaminated soil. Bioresource Technology, 1999, 67, 279–285.

Semai, A., Plewniak, F., Charrié-Duhaut, A., Sayeh, A., Gil, L., Vandecasteele, C., Lopez-Roques, C., Leize-Wagner, E., Bensalah F. & Bertin, P. N. Characterisation of hydrocarbon degradation, biosurfactant production, and biofilm formation in Serratia sp. Tan611: a new strain isolated from industrially contaminated environment in Algeria. Antonie Van Leeuwenhoek, 2021, 114, 411-424.

Shalaby, M., Ibrahim, S., Omara, A. E. D., & Abdelrazek, M. A. Biodegradation of Dimethoate 40% by Bacillus subtilis OQ347968 Isolated from Polluted soils. *Egyptian Journal of Soil Science*, 2024, *64*, 801-814.

Soltanighias, T., Singh, A. E., Satpute, S. K., Banpurkar, A. G., Koolivand, A., & Rahi, P. Assessment of biosurfactant-producing bacteria from oil contaminated soils and their hydrocarbon degradation potential. *Environmental Sustainability*, 2019, *2*, 285-296.

Sun, J. Q., Xu, L., Liu, X. Y., Zhao, G. F., Cai, H., Nie, Y., & Wu, X. L. Functional genetic diversity and culturability of petroleum-degrading bacteria isolated from oil-contaminated soils. *Frontiers in Microbiology*, 2018, *9*, 1332.

Swetha, S., Elakiya, V. P., Ammonica, B. K., Valli, N. C., & Prakash, P. Degradation of crude oil using the indigenous isolate Bacillus sp SEA18. Indian Journal of Biochemistry & Biophysics, 2020, 57, 317-326.

Tahir, U.; Zameer, M.; Frrukh, S. Y.; Ghauri, B. A.; Javed, M. A.; Ali, Q., Ali F., & Ali, D. Isolation, and Characterization of Petroleum Oil-Degrading Bacteria from Petroleum Contaminated Soil. Geomicrobiology Journal, 2024, 41,691-698.

Varjani, S. J. Microbial degradation of petroleum hydrocarbons. Bioresource Technology, 2017, 223, 277-286.

Varjani, S.J.; Upasani, V.N. Biodegradation of petroleum hydrocarbons by oleophilic strain of Pseudomonas aeruginosa NCIM 5514. Bioresource Technology, 2016, 222, 195-201.

Wang, R.; Wu, B.; Zheng, J.; Chen, H.; Rao, P.; Yan, L.; Chai, F. Biodegradation of total petroleum hydrocarbons in soil: Isolation and characterization of bacterial strains from oil contaminated soil. Applied Sciences, 2020, 10(12), 4173.

Wang, X. B.; Chi, C. Q.; Nie, Y.; Tang, Y. Q.; Tan, Y.; Wu, G.; Wu, X. L. Degradation of petroleum hydrocarbons (C6-C40) and crude oil by a novel Dietzia strain. Bioresource Technology, 2011, 102(17), 7755-7761.

Wu, M; Chen, L; Tian Y; Ding Y; Dick, WA. Degradation of polycyclic aromatic hydrocarbons by microbial consortia enriched from three soils using two different culture media. Environmental Pollution, 2013, 178: 152–158.

Xu, X.; Liu, W.; Tian, S.; Wang, W., Qi-Q.; Jiang, P.; Gao, X.; Li, F.; Li, H.; Yu, H. Petroleum hydrocarbon-degrading bacteria for the remediation of oil pollution under aerobic conditions: A perspective analysis. Frontiers in Microbiology, 2018, 9, 2885-2895.

Zajic, E., Supplisson B. Emulsification and degradation "Bunker C" fuel oil by microorganisms. Biotechnology and Bioengineering, 1972,14: 331–343.

Zenati, B., Chebbi, A., Badis, A., Eddouaouda, K., Boutoumi, H., El Hattab, M., Hentati, D., Chelbi, M, Sayadi S., Chamkha M. & Franzetti, A. A non-toxic microbial surfactant from Marinobacter hydrocarbonoclasticus SdK644 for crude oil solubilization enhancement. *Ecotoxicology and environmental safety*, 2018, *154*, 100-107. https://doi.org/10.1016/j.ecoenv.2018.02.032.