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Isolation, identification and evaluation of oil hydrocarbon decomposing bacteria from contaminated areas of oil fields

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ABSTRACT

Microbial biodegradation is known as an effective and harmless method to overcome environmental pollution with oil hydrocarbon. Some bacterial species were isolated from the Sarvestan oilfields (Iran, Fars province), then identified and applied for oil hydrocarbon decomposition. A carbon-free minimum medium (CFMM) containing 1% crude oil was used to isolate bacteria through incubation at 30°C in the dark at 200 rpm for 7 days. Different methods were used to identify the hydrocarbon oil decomposing bacteria: gram staining, squalene hydrolysis, catalase, production of arginine dihydrolase, gelatin liquefaction, hydrogen sulfide production, levan production, methyl red, oxidase, nitrite reduction, oxidative/fermentative, starch hydrolysis and Tween-80 hydrolysis tests. Nine different oil decomposing bacterial species were isolated. All the species grew well at 28 and 35°C, while four isolates containing of *Bacillus* sp. SA13, *Pantoea* sp. SA1112, *Pseudomonas aeruginosa* sp. SA21, and *Bacillus* sp. SA23 were capable of growing in a temperature of up to around 42°C. The minimum salt tolerance for isolates, except for *Enterobacter* sp. SA711, was 8%; *Bacillus* sp. SA212 had the highest tolerance of 15% sodium chloride. *Acinetobacter* sp. SA172, *Enterobacter* sp. SA711, *Pseudomonas* sp. SA75, *Bacillus* sp. SA212 and *Bacillus* sp. SA23 had the most growth rate in the CFMM. The highest percentages of oil removal obtained were 89% for *Enterobacter* sp. SA711, 86% for *Acinetobacter* sp. SA172, and 68% for *Pseudomonas* sp. SA75. The three isolated bacterial strains from the contaminated soil of the Sarvestan area had a good ability to degrade oil hydrocarbon. Therefore, they could be used commercially for the bioremediation of this region.

1. Introduction

Oil products are widely used as fuel and energy sources in the world. It is believed that the total amount of oil leaked into the sea through human or natural activities can cover the surface of all the oceans of the planet with a thickness of 20 molecules. Oil spillage into natural ecosystems can lead to severe environmental pollution and harm the growth and health of plants, animals, aquatic animals and humans [1]. The accidental discharge and leakage of these materials can occur during the extraction, refining and distribution of petrochemical products; it is also associated with accidents such as burst and broken pipes, fire and blasting wells, broken tanks, sinking ships, and wars. Crude oil can cause fires, and also pollute groundwater and air. Therefore, it should be cleaned as soon as possible for a safe environment [2]. It usually takes from several days to months to decompose hydrocarbon oil that has spilled into water. In contrast, oil that leaks to shoreline areas are likely to be more concentrated and, therefore, needs more time to decompose [3]. There are some standard and common physical, chemical and biological methods for decontamination of petroleum products. Some of these methods are less likely to be implemented due to their high cost and low efficiency. Biodegradation methods that typically involve the conversion of contaminated materials to non-toxic substances using microbial processes are more effective and harmless. High enzymatic and biochemical

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reactions must be employed in order to biodegrade oil hydrocarbons [4]. Different bioremediation technologies have been developed in recent years using a wide range of microorganisms to remove the petrochemical hydrocarbons [5], heavy metals [6] and some other environmental pollutant materials. Bacteria are more important than other microorganisms due to their different degrading enzymes. The most important of these are *Bacillus*, *Pseudomonas*, *Enterobacteriaceae*, *Streptomyces* and *Mycobacterium*. One important feature of using bacteria to decompose oil hydrocarbon contaminants is to increase the hydrocarbon solubility by producing surface factors such as bio-surfactants [7]. Subathra et al. (2013) reported an oil hydrocarbon removal efficiency of 55% by using *Pseudomonas aeruginosa* in areas close to the Ennore creek in India [8]. Geetha et al. (2013) isolated 14 different bacteria from petroleum contaminated areas using enrichment methods [9]. According to their studies, the gram-negative bacterium RP12 was identified as the best bacterial isolate grown in most oil hydrocarbon, more particularly diesel, which could disperse diesel within 12 hours. There are also some bacteria with different capabilities that can decompose other petroleum products. For example, the *Bacillus sp.* bacteria isolated from underground water was capable of growing in wide pH and temperature ranges as well as in the presence of hydrocarbons such as crude oil, diesel and gasoline [10]. Studies investigating the Deepwater Horizon (DH) oil spill in the Gulf of Mexico showed that oil contamination had a considerable effect on the large quantity and community composition of the bacteria species (*Gammaproteobacteria* and *Alphaproteobacteria* as the main oil degrading species) in the Gulf beach sands [11]. Sunlight and other environmental factors such as temperature, soil composition and hydrostatic pressure have a significant impact on the type of bacterial flora in the oil contaminated areas [12-14]. Bacosa et al. (2015) reported that sunlight could reduce the cyanobacterium *synechococcus* and instead increase *Bartonella*, *Marinobacter*, *Alteromonas*, *Halomonas*, *Labrenzia* and *Sandarakinotalea* in the Northern Gulf of Mexico [15]. Usually a group of different types of bacteria are active in areas polluted with oil, but the importance and relative performance of each of them varies based on the type of existing hydrocarbons. Bacterial species such as *Colwellia*, *Cycloclasticus* and *Pseudoalteromonas* are usually seen in the presence of aromatic hydrocarbons while *Oceanospirillaceae* and *Pseudomonas* are reported in the contaminated areas containing *n*-alkanes and cycloalkanes [16]. Sarvestan is one of the oil-rich regions of Fars province in Iran. In recent years, the extraction of crude oil from the oil fields in this region and the feeding of adjacent refineries are being pursued more seriously. Therefore, a discussion on the environmental impacts and the elimination of pollution are important, and the appropriate scientific and applicable research is required to better understand the challenges. The purpose of this study was to isolate, identify and evaluate the performance of new microorganisms that can degrade the hydrocarbon contamination in the Sarvestan oilfields.

2. Materials and methods

2. 1. Collect soil samples

Soil samples were collected in April of 2017, after removing surface soil from oil contaminated areas in Sarvestan (29.2661° N, 53.2234° E) of Fars province (as a polluted area of hydrocarbon oil). Samples were stored in sterile containers at 4°C until the start of the experiments. The pH and electrical conductivity were measured for soil samples.

2. 2. Counting the total number of live bacteria

Each sample was diluted in succession with a 0.85% sodium chloride solution. The dilutions of 10^{-1} to 10^{-4} were spread on nutrient agar medium in three replicates, and the media were incubated at 30°C for 24 hours. The number of colonies appearing on the culture medium was counted. Considering the dilution level, the average number of colonies in the three replicates was reported as the total number of live bacteria in the soil samples.

2. 3. Isolation of oil hydrocarbon decomposing bacteria

A carbon-free minimum medium was used to isolate the hydrocarbon oil decomposing bacteria. The medium composition included (g/L): NH_4NO_3 3; Na_2HPO_4 2.2; KH_2PO_4 0.8; $MgSO_4.7H_2O$ 0.01; $FeCl_3.6H_2O$ 0.005; and $CaCl_2.2H_2O$ 0.005 [17]. The pH of the medium was adjusted to 7.5 using a standard sodium hydroxide solution, and then sterilized at 121°C for 15 min. To prepare a solid culture medium, 15 g/L of agar was added to the pre-autoclaving medium. Crude oil was added as a single source of carbon; the energy supply was separately sterilized and added to the CFMM medium by 1% before inoculation with the bacteria. 1 gram of the soil sample was added to 50 ml of the CFMM medium containing 1% crude oil, and the suspensions were shaken up to 200 rpm for two weeks at 30 °C. The serial concentrations were prepared from the obtained suspensions and spread on a solid CFMM medium containing 1% crude oil for the three replicates. The inoculated plates were kept at 30°C and inspected daily. The bacterial colonies were selected based on morphological characteristics and transferred to a new CFMM culture medium. The pure bacterial isolates were kept at 4 °C for further experiments.

2. 4. Investigating the oil hydrocarbon decomposing ability of bacterial isolates

Each of the isolated bacteria was incubated in the nutrient broth medium until OD_{600} = 1 at 30 °C and a 200 rpm agitation speed. 1 ml of the bacterial culture was then transferred to 1.5 ml tubes and centrifuged at 4°C and 4000

rpm for 5 minutes. Under sterile conditions, the supernatant was removed and the pellet of bacteria that equaled 8×10^8 cells m/l was dissolved in 500 µl of the CFMM medium. Then, the bacterial cells were transferred to 50 ml of CFMM medium containing 1% crude oil, as a source of carbon and energy, and incubated at 30°C in the dark at 200 rpm for 7 days. The samples from each culture medium were taken after 24, 48 and 72 hours and finally, 7 days. The bacterial biomass was measured three times for each sample using a spectrophotometer (Unico 2100, USA) at a wavelength of 620 nm [18]. A CFMM medium containing 1% crude oil, without any bacterial inoculation, was used as the control. The high growth ability isolates were selected as oil hydrocarbon degrading bacteria.

2. 5. Assessment of the ability to remove crude oil from the medium

The bacterial culture media that showed high growth ability after seven days were selected to evaluate the ability to remove the crude oil. The samples were dissolved in dichloromethane, and their optical density was compared with the control (pure dichloromethane) using a spectrophotometer (Unico 2100, USA) at 420 nm [19].

2. 6. Identification of bacteria

Various biochemical tests were used to identify the bacteria with the ability to degrade oil hydrocarbon: gram staining [20], squalene hydrolysis test [21], catalase test [22], production of arginine dihydrolase test [21], gelatin liquefaction test [21,23], hydrogen sulfide production test [24], levan production test [21], methyl red test [21], oxidase test [21], nitrite reduction test [21], oxidative/fermentative test [21], starch hydrolysis test [21] and Tween-80 hydrolysis test [25]. Based on the results obtained from the bacteriological tests, bacterial identification was performed to the species level. In addition to biochemical tests, the ability to grow isolates at different temperatures (28-42°C) and with a sodium chloride concentration of 1 to 15% in the culture media were also used to describe the isolates.

2. 7. Statistical analysis

The experiments were carried out in a completely randomized design with three replications. The results were reported within a standard error range. The analysis of variance in the GLM process of the SAS software was used for the statistical analysis of data and a significant level of 95% ($P < 0.05$) was considered. Duncan's multi-domain test was used to compare the averages. Microsoft Excel 2016 was used to draw the charts.

3. Results and discussion

3.1. Specifications of the soil samples

The 12 oil samples collected from the Sarvestan oil fields in Fars province had similar chemical properties. A variation range of 7.2 to 7.56 was recorded for the pH of the samples. These conditions were neutral and many microorganisms were capable of growing in this pH range. The electrical conductivity included a wider range, from 3.2 to 3.5 dS m^{-1}, which included relatively low to medium salinity. The chemical and physical properties of the soil had a direct impact on the microbial population of each site, and the change in these parameters changed the composition of the microorganism's population. Eskandari et al. (2013) reported a soil pH of 7.24 and an electrical conductivity of 3.4 for oil contaminated soils around the petroleum and gasoline tanks in the Isfahan refinery [26].

3. 2. Bacterial variety in soil samples

The various spectra of bacteria with different morphology were observed on the nutrient agar culture medium as a result of exposure to dilute suspensions of oil-contaminated soils. This bacterial variety (Figure 1) could be promising candidates to isolate the bacteria belonging to the different genera or bacterial species with the ability to decompose the hydrocarbon oil. The average number of 2.6×10^6 colony forming units per ml represents the favorable conditions for the growth of heterotrophic bacteria in this region. Parach et al. (2017) achieved a maximum of 9.50×10^9 cfu m/L for *Rhodococcus ruber* KE1 isolated from the Persian Gulf water and sediment from Khark Island, southern Iran; Ansari et al. (2018) reached to 2×10^6 colony forming units per g of oil decomposing bacteria in desert soil [27,28].

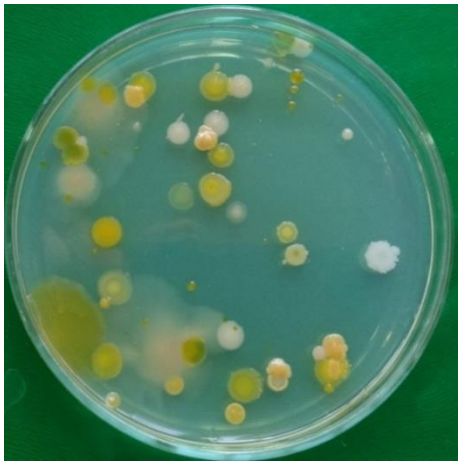

Fig. 1. Bacterial variety of the initial soil samples collected from oil polluted areas in Sarvestan and cultured on nutrient agar medium at 30°C for 24 hours

3. 3. Screening of hydrocarbon decomposing bacteria

The bacterial isolation was performed using a CFMM medium containing 1% of crude oil with the focus on the ability to use this compound as the only source of energy. Then, isolates with a different appearance were purified on the nutrient agar culture medium (Figure 2).

At the next stage, 64 isolates were selected and purified from the primary experiments. After preliminary biochemical tests, based on their apparent characteristics

and growth rate on the CFMM containing crude oil, the number of treatments were reduced and repeated isolates were deleted to allow the maintenance of complementary tests to be carried out. The first step in classifying and identifying the bacteria was performed based on morphological characteristics such as the shape, color and size of the colonies on the medium.

3. 4. Biochemical tests results

The results of the biochemical tests on the isolated bacterial species were used for identification of isolates and are presented in Table 1. Gram staining was used to determine the gram positive or negative isolates. The results of the catalase test, which indicate the presence or absence of the catalase enzyme in the bacterial cell, are presented in Table 1. Gas production, as a result of catalase enzyme activity, was observed in catalase-positive bacteria. The catalase enzyme was an important factor in protecting bacteria against toxic by-products of oxygen metabolism and played an important role in the removal of hydrogen peroxide, which is a lethal agent in bacteria. In the present work, all isolates that exhibited significant oil hydrocarbon decomposing ability were catalase-positive (Table 1). The oxidase test was mainly used to identify the species of the gram-negative bacteria. Therefore, the positive result of the oxidase test indicated the presence of cytochrome C. All the investigated isolates, except for *Enterobacter* sp. SA711 and *Acinetobacter* sp. SA172, were diagnosed as positive oxidase bacteria (Table 1). The result of the nitrite reduction test was positive for some species with high oil hydrocarbon oil decomposing ability including *Bacillus* sp. SA13, *Enterobacter* sp. SA711, *Pseudomonas aeruginosa* sp. SA21, *Acinetobacter* sp. SA172, and *Bacillus* sp. SA23 (Figure 3 and Table 1). The basis of the squalene hydrolysis test is the convertion of the squalene to the glucose. In this experiment, the presence of a black spot around the colonies indicated the hydrolysis of the squalene. *Bacillus* sp. SA13, *Bacillus* sp. SA212, *Pseudomonas aeruginosa* sp. SA21 and *Bacillus* sp. SA23 had a positive response to this test (Table 1). Also, *Pseudomonas* sp. SA75 and *Pseudomonas aeruginosa* sp. SA21 showed arginine dihydrolysis ability (Table 1). The gelatin liquefaction test was conducted to determine the ability of bacteria to produce proteolytic-like enzymes such as gelatinase. All isolates had a negative response to this test, and none of them were able to melt the gelatin (Table 1). Even though, only three isolates, namely *Pseudomonas aeruginosa* sp. SA21, *Acinetobacter* sp. SA172 and *Bacillus* sp. SA23, were able to produce hydrogen sulfide among the hydrocarbon oil degrading isolates (Table 1). In the Levan production test, the bacterial isolates were evaluated after three days based on the formation or absence of glazed and dome colonies. None of the studied hydrocarbon oil decomposing bacteria were positive for this test (Table 1). The methyl red test was conducted to investigate the ability of the bacterial isolates to produce and maintain the final acidic products derived from glucose fermentation as well as to overcome the buffering capacity of the system. The results showed that only the *Bacillus* species had such a feature (Table 1). The oxidative/fermentative test was performed to detect oxidants from the fermenters species, and the results are presented in Table 1. Starch hydrolysis was investigated by pouring Lugol's solution on the medium. The presence of a halo around the colonies indicated the absence of amylose and amylopectin. The iodine content of the Lugol's solution can absorb amylose to make a violet color (Figure 4). The Tween-80 hydrolysis test was performed to confirm the presence of a bacterial lipase enzyme and the ability of fats hydrolysis to fatty acids; only two isolates, *Pseudomonas aeruginosa* sp. SA21 and *Acinetobacter* sp. SA172, showed positive results (Table 1).

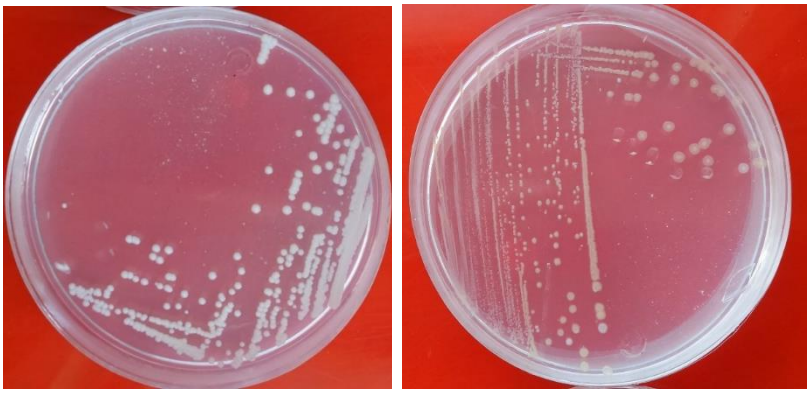

Fig. 2. The purified bacteria of the initial soil samples collected from oil polluted areas in Sarvestan with the ability to decompose oil hydrocarbons and cultured on nutrient agar medium at 30°C for 24 hours

Table 1. Biochemical tests results for the ability of purified bacterial isolates to decompose hydrocarbon oil from oil polluted areas in Sarvestan

Bacterial sp. / Biochemical test	*Bacillus* sp. SA13	*Bacillus* sp. SA212	*Pseudomonas* sp. SA75	*Pseudomonas* sp. SA515	*Enterobacter* sp. SA711	*Pantoea* sp. SA1112	*Pseudomonas aeruginosa* sp. SA21	*Acinetobacter* sp. SA172	*Bacillus* sp. SA23
Gram staining	+	+	-	-	-	-	-	-	+
Catalase	+	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	-	+	+	-	+
Nitrite reduction	+	-	-	-	+	-	+	+	+
Squalene hydrolysis	+	+	-	-	-	-	+	-	+
Arginine dihydrolase	-	-	+	-	-	-	+	-	-
Gelatin liquefaction	-	-	-	-	-	-	-	-	-
hydrogen sulfide production	-	-	-	-	-	-	+	+	+
Levan production	-	-	-	-	-	-	-	-	-
Methyl red	+	+	-	-	-	-	-	-	+
Oxidative/ fermentative	optional anaerobic	optional anaerobic	forced aerobics	forced aerobics	forced aerobics	forced aerobics	optional anaerobic	forced aerobics	optional anaerobic
Starch hydrolysis	+	+	-	+	+	+	+	-	+
Tween-80 hydrolysis	-	-	-	-	-	-	+	+	-

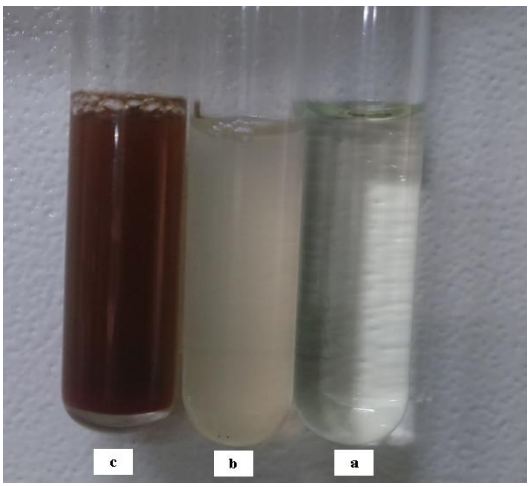

Fig. 3. Nitrite reduction test, from right to left: control (a), inability to reduce nitrite in *Pseudomonas* sp. SA75 (b), and formation of red color as a result of nitrite reduction after adding reagent to *Bacillus* sp. SA13 (c)

3.5. Survey the temperature and salt tolerance of the isolates

The results showing the ability of bacterial isolates to use different sugars as a carbon source are presented in Table 2. The different species of a genus have different properties in sugars, which can be used to separate bacterial isolates.

All the isolated bacteria with the ability to degrade the crude oil grew well at 28 and 35 °C. But with an increase in the temperature up to 42 °C, four isolates including *Bacillus* sp. SA13, *Pantoea* sp. SA1112, *Pseudomonas aeruginosa* sp. SA21, and *Bacillus* sp. SA23 were capable of growth. Also, the bacterial isolates growth was investigated on a nutrient agar containing 2-15% of sodium chloride.

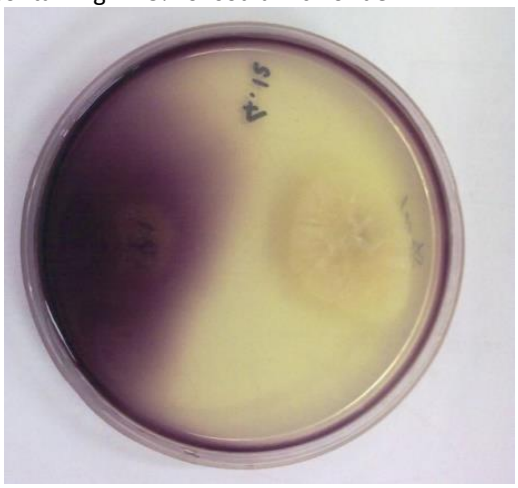

Fig. 4. Starch hydrolysis test. *Bacillus* sp. SA212 is capable to hydrolysis the starch and is seen in white (on the right side) *Pseudomonas* sp. SA75 did not have such ability and is seen in violet (on the left side)

The separated isolates showed a high relative resistance to the presence of salt in the culture medium. The minimum salt tolerance for isolates, except for *Enterobacter* sp. SA711, was 8% salt in the medium. *Bacillus* sp. SA212 had the highest tolerance so that it was able to grow in a culture medium containing 15% sodium chloride.

3.6. Investigating the ability of oil hydrocarbons decomposition

The ability to use crude oil as the sole source of carbon and energy in the minimum salt medium of CFMM was measured by measuring their growth in a liquid medium with bacterial isolates from the Sarvestan oil fields. As shown in Figure 5, the bacterial isolates had different growth patterns at various sampling times (24, 48 and 72 h after incubation). The *Acinetobacter* sp. SA172 showed the highest growth in the crude oil containing medium compared to other isolates after 24 h. However, the isolates of the *Enterobacter* sp. SA711 and *Bacillus* sp. SA23 also had a good growth rate during this period (Figure 5). The same growth pattern was observed after 48 hours, so that *Acinetobacter* sp. SA172 had the highest growth rate; the isolates of *Enterobacter* sp. SA711 and *Pseudomonas* sp. SA75 also had a favorable growth (Figure 5). After 72 h of incubation, it was shown that the differentiation of potent bacteria in the use of petroleum hydrocarbons was possible with other isolates. The *Acinetobacter* sp. SA172 had the same growth rate at this time; the *Enterobacter* sp. SA711 and *Pseudomonas* sp. SA75 isolates maintained the same level with the same growth. Then at the next level, with a significant difference, the *Bacillus* sp. SA23 and *Bacillus* sp. SA212 isolates were ranked as shown in Figure 5. After 7 days of incubation, the bacterial isolates with the ability to decompose crude oil had a significant difference in growth ($P< 0.05$); the isolates *Enterobacter* sp. SA711 and *Acinetobacter* sp. SA172, *Pseudomonas* sp. SA75, *Bacillus* sp. SA212 and *Bacillus* sp. SA23 had the highest growth rates, (optical density above 1 at 620 nm). Based on the obtained results, the *Pseudomonas aeruginosa* sp. SA21 had the least amount of growth in the CFMM containing crude oil, and the *Pseudomonas* sp. SA515 did not differ significantly after seven days ($P <0.05$). During the 7 day incubation time, *Enterobacter* sp. SA711 had a relatively stable growth trend; it also had the highest growth rate at the end of the incubation period in comparison to the other isolates. The ability to uniformly grow in the presence of oil hydrocarbons and the lack-of-effect on the production of metabolites on the survival and growth of this strain was very important. These capacities make it a suitable microbial strain for removing oil hydrocarbons from oil polluted lands.

Table 2. The ability of oil hydrocarbon decomposing bacteria isolates from oil polluted areas in Sarvestan to use various sugars

Bacterial sp. / Carbon source	*Bacillus* sp. SA13	*Bacillus* sp. SA212	*Pseudomonas* sp. SA75	*Pseudomonas* sp. SA515	*Enterobacter* sp. SA711	*Pantoea* sp. SA1112	*Pseudomonas aeruginosa* sp. SA21	*Acinetobacter* sp. SA172	*Bacillus* sp. SA23
Fructose	+	+	+	+	+	+	+	-	+
Galactose	+	+	+	+	+	+	+	-	+
Lactose	-	+	+	+	+	+	+	-	+
Maltose	-	-	+	+	-	+	-	-	+
Xylos	+	+	+	+	+	+	+	-	+

Toledo et al. (2005) studied the decomposing ability of isolated bacteria from crude oil in the multi-ring aromatic hydrocarbons including naphthalene, fluoranthene, phenanthrene and pyrene. They showed the ability of the *Enterobacter* species to remove them from an environment polluted with hydrocarbons [29]. Hua et al. (2010) also confirmed the ability of *Enterobacter cloacae* (GenBank accession number, GQ426323) to decompose alkanes and polycyclic aromatic hydrocarbons (PAHs) [30]. *Acinetobacter* sp. SA172 showed a high growth potential in the crude oil containing medium as the only source of energy. The initial growth rate of this strain was much higher in the first 48 hours than other isolates. After that, the growth rate decreased and nearly 7 days later, the stagnant phase was approached. Several studies have indicated the ability of various species of *Acinetobacter* to remove oil hydrocarbon contaminants. Nkem et al. (2016) reported the acceptable growth of *Acinetobacter baumannii* ATCC 19606 on diesel-oil as the sole carbon source with a 58.1% hydrocarbon degrading yield [31]. Shao et al. (2015) also identified the bacterial strain *Acinetobacter* sp. WSD with a phenanthrene-degrading ability; it was isolated in a coal-mining area of the Guozhuang karst water system in Shanxi province, northern China [32]. This strain of *Acinetobacter* degraded 90 % of fluorine, 90 % of phenanthrene and 50 % of pyrene after 6 days of incubation. The different abilities of the species of a microbial genus to eliminate biological pollutants was due

to the various physical, chemical and molecular mechanisms of these processes. *Pseudomonas stutzeri* CET 930 showed a 95, 78 and 82% overall biotransformation yield of phenanthrene, pyrene and benzanthracene, respectively [33]. Bacillus genus has also been reported as a hydrocarbon decomposing bacteria in some studies. Bisht et al. (2014) reported a decomposing efficiency of 83.4% anthracene and 75.1% naphthalene for *Bacillus* sp. SBER3 after six days of incubation [34]. Barin et al. (2014) reported the ability of *Bacillus subtilis* tb1 and *Pseudomonas aeruginosa* to produce biosurfactant and biodegrade kerosene hydrocarbons [35]. Parach et al. (2017) reported the hydrocarbon degradation ability of about 90% for *Rhodococcus ruber* KE1 after a week [27].

3. 7. Investigating the amount of oil removing from the bacterial culture

After 7 days of inoculation in the CFMM medium containing 1% crude oil, the amount of oil remaining in the culture medium was determined using a spectrophotometer at 420 nm [28]. The percentage of oil removal from the medium by different isolates of the bacterium is shown in Figure 6. The results showed a significant difference ($P < 0.05$) among isolated bacteria in terms of oil removal ability. The highest removal rate for *Enterobacter* sp. SA711 and Acinetobacter sp. SA172 was measured at 89% and 86%, respectively. The *Pseudomonas* sp. SA515 and *Pseudomonas aeruginosa* sp. SA21 isolates had an 18 and 26% crude oil reduction, which showed the lowest removal ability. In contrast to these two isolates, *Pseudomonas* sp SA75 had a high ability to remove crude oil, and after 7 days, it reached a 68% removal efficiency (Figure 6).

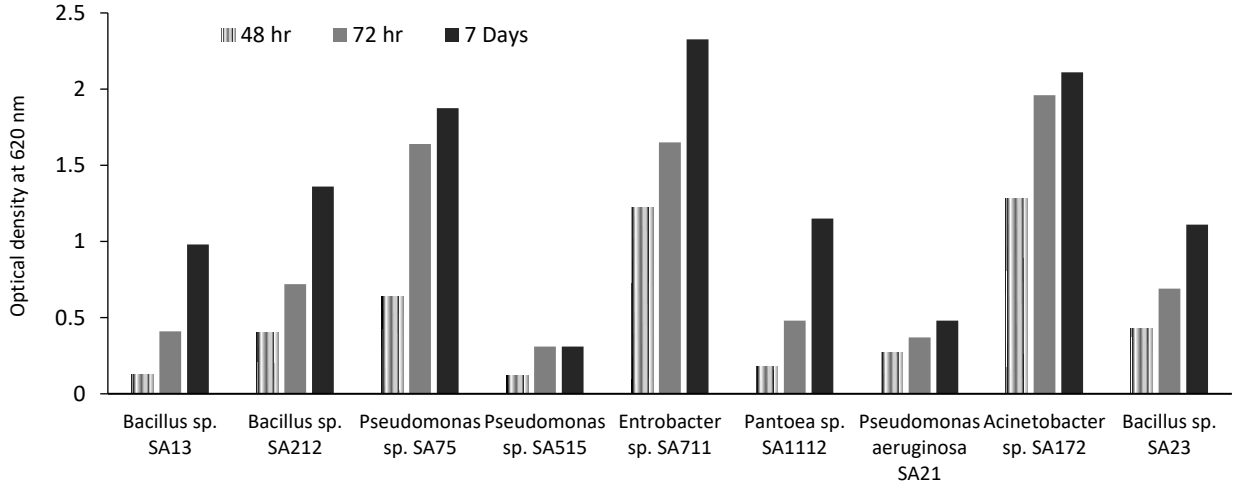

Fig. 5. Growth diagram of bacterial isolates from oil polluted areas in Sarvestan with the ability to decompose oil hydrocarbons over a period of 24 hours to 7 days

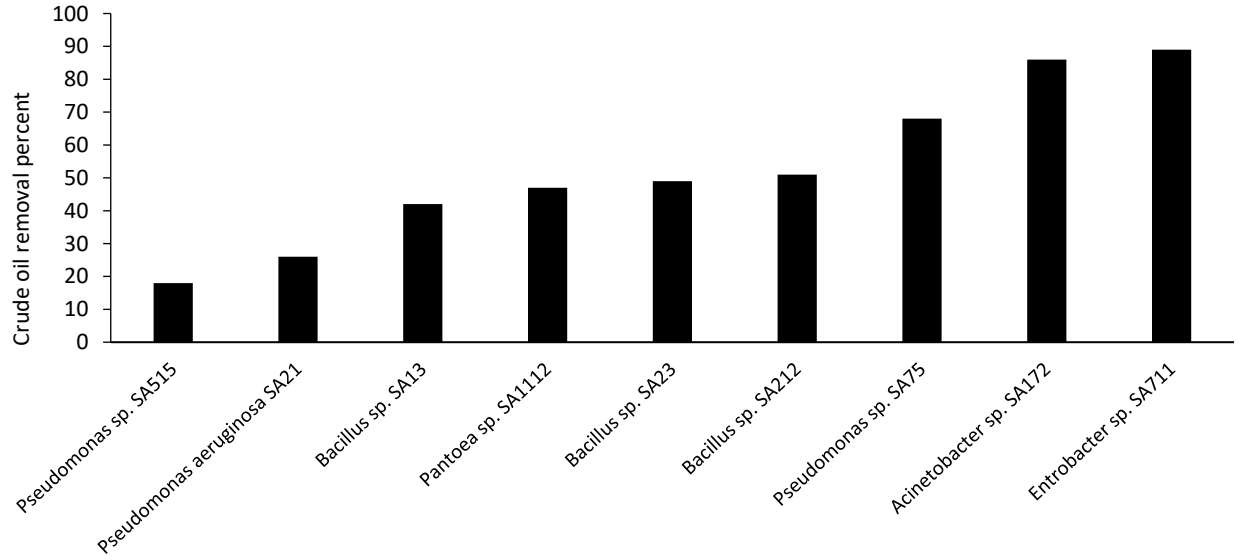

Fig. 6. Crude oil removing efficiency of bacterial isolates from oil polluted areas in Sarvestan with the ability to decompose oil hydrocarbons over a 7 days incubation period in a CFMM medium containing 1% crude oil

4. Conclusions

The present work carried out for the first time to our knowledge the isolation, identification and performance evaluation of oil hydrocarbon degrading bacteria from the contaminated soil of the Sarvestan oilfields. *Enterobacter* sp. SA711, *Acinetobacter* sp. SA172 and *Pseudomonas* sp. SA75 were introduced as three powerful bacterial species with a high ability to remove oil hydrocarbons pollutants from contaminated soils. Today, the elimination of natural ecosystems contaminants, especially pollution in oil-rich regions, is one of the serious challenges due to its harmful impacts on plants, animals, aquatic animals and humans. Bacterial bioremediation is one of the most effective and economical methods to remove oil pollutants from the environment and could be the dominant method in the future.

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