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ORIGINAL PAPER

Biodegradation of low viscosity spindle oil causing environmental pollution

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Abstract

Three hydrocarbon degrading microorganisms were isolated from the soil samples collected from the storage sites of low viscosity spindle oil containers and identified on the basis of morphological and biochemical characteristics as *Aeromonas hydrophila*, *Bacillus subtilis* and *Staphylococcus aureus*. The study has revealed high ability of these microorganisms for oil biodegradation. The results have indicated that all isolates had the potential to breakdown the hydrocarbon. The most efficient bacteria among these examined was *Aeromonas hydrophila* which biodegraded almost all tested hydrocarbon giving a treatment percentage of 98% within 30 days which was considered as the perfect period for degradation. Also, a small scale was designed to treat the spindle oil with the using of oxidation process and all the tested organic materials were biodegraded in a treatment percentage of 100% within retention time of 20 days.

Keywords Bioremediation · Oil spills · *Aeromonas hydrophila*

Introduction

Hydrocarbons and oil products are regarded as one of the most crucial environmental pollutants being contained significant number of hydrocarbon structures associated with other compounds. However, its general structure is saturated with branched alkene chains and cyclo paraffin rings usually containing carbon, hydrogen and oxygen. Apparently, biodegradation of environmental pollutants, particularly those of raw oil, depends on the ability of microorganisms inhabited such ecosystems to digest most of the oil hydrocarbons via various metabolic routes and convert them into intermediate metabolic products and final products such as CO_2 and H_2O (Pérez Silva et al. 2006; Basuki 2017; Wua et al. 2018).

This process of biodegrading oil natural spills and removing hydrocarbons from the environment is crucial but the measured biodegradation differs significantly (Prince et al. 2016). It is worth mentioning that genetic engineering applications of microorganisms in biodegradation has given excellent and worthy findings to demonstrate the biodegradation of hazardous waste under laboratory conditions, where such results have shown that genetically biodegraded bacteria has higher ability in biodegradation. Currently, many ecosystems have been changed due to their impact on growing human activity and as a result, many people have become part of the environmental awareness of protecting these ecosystems and controlling any imposed risk.

During the past years, incidents and frequent risks of oil pollution has led carrying out considerable amount of researches, studies and workshops, since most oil products may leach to the environment systems by oil spills either from marine tankers or from oil refineries. It seems important that approximately 5 million tons of crude and refined oils are dissipated to the environment every year due processing activities (Fritsche and Hofrichter 2008).

Several microorganisms isolated from polluted sites such as bacteria, fungi and algae have shown significant ability in breaking down various hydrocarbons into carbons which may act as energy source (Bhattacharya et al. 2015; Ibrahim, 2016; Wang et al. 2019). However, hydrocarbon hydrolysis has shown to be as natural components of fossil fuels formed under incomplete combustion process of organic materials. Furthermore, such hydrocarbons release toxic substances

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that affect the environment in terms of inducing mutagenicity, distorting and cancer of various organisms (Wang et al. 1999).

Certain microorganisms are capable of utilizing the hydrocarbons as a main source of carbon for energy to demonstrate the biotic activities, since the biodegradation process is a complex and depends upon the nature of the oil derivative and the amount of hydrocarbon. In addition, these microorganisms can utilize such hydrocarbons depending on the chemical nature of these compounds of oil derivative. Also, bacterial biodegradation is considered as a one of the most important process in biodegrading oil derivatives and it seems as first primarily analyzer of oil spills and similar incidents due to bacteria societies already colonized these habitats, where there are many microorganisms having the ability to digest oil under lab or field conditions (Alsulami et al. 2014).

The current study has focused on isolating and identification of bacterial groups that have the ability to digest expired spindle oil which presented at significant quantities up to 7000 barrels That al-Swary state company of Industry and Minerals Ministry by isolating microorganisms from oil spilling sites within the company stores. As oil spills are hydrocarbon compounds contaminating the environment. The oil spill is a yellow liquid insoluble in water with neutral pH but it is flammable and explosive at higher temperature (more than 185 °C), where it produces carbon monoxide when burned. However, the spill oil is well known to have health risks in short term such as lung irritation, eye inflammation and sensitivity and derma problems, while in a long term, it may cause various types of cancer particularly respiratory system.

Regarding the process of biodegradation by bacteria is one of the first techniques used to remove oil pollution from the environment by biodegrading it for the purpose of obtaining energy. In fact, the biodegradation process seems to be cheaper than the remaining of the techniques for the removal of oil derivatives such as chemotherapy or incineration by furnaces. Also, the biological treatment process does not produce secondary materials that affect the balance of the ecosystem. The idea of this research is to develop the ability to invent scientific and technological solutions to meet environmental, economic and social challenges. So, the employing the outputs of this study in a small field system may give the necessary information to design a larger system for treating these pollutants locally especially the most applied treatments are locally to mix these oils with diesel fuel and burned directly in the absence of the resulted gas treatment system.

So, the current work focuses on treating low viscosity oil spills using efficient microorganisms in digesting organic compounds by applying small system based on the oxidation process (bioreactor) and the experimental work was designed to give the actual values to be adhered to in the case of designing a system treat in a factory.

Materials and methods

All samples were randomly collected from the oil spills of al-Swary state company of Industry and Minerals Ministry which contain approximately 7000 barrels of expired spindle oils at depths 1–5 cm of the topsoil using a sterile ladle. The samples were placed in sterile, tightly sealed plastic bottles. The samples were transferred to the laboratory and stored at 4 °C in preparation for subsequent laboratory analyzes of isolation and diagnosis of bacteria that are already endemic in these soil environments (Abbawi and Hassan 1990). One liter of de-mineral salt media was prepared to provide the appropriate conditions for the growth of bacteria with pH 7.2 ± 0.2 from the following components as follows:

NH_4Cl 0.5 g, NaCl 4 g, KH_2PO_4 0.5 g, $MgSO_4$ 0.5 g and N_2HPO_4 1 g.

These components were mixed thoroughly with 1 L distil water and a sterilized using a wet sterilizer (Autoclave) at a temperature of 121 °C for 15 min and under a pressure of 1.5 bar. The collected spindle oil samples were purified using filter paper (0.45 µm), placed in a 500 ml volumetric conical flask and received 100 ml of saline medium solution with 1 ml of spindle oil. 1 g of the collected soil was added and the conical flask was placed in a shaking incubator at 37 °C and 150 rpm for 48–72 h. The bacterial growth was carried out in this medium by feeding bacteria on the carbon resulting from the breakdown of the oil spill. After incubation period, 1 ml of the bacterial culture was taken and planted on the Petri dishes containing the nutrient agar medium prepared according to the manufacturer's instructions (Oxoid) to assess the numbers of the growing bacteria. Further transplanting was carried out using the method of planting as lines on dishes of nutrient agar and growing colonies were prepared for the subsequent biochemical tests of isolation and identification, where three isolates of bacteria were emerged through differential implant and the use of the method of biochemical tests (IMVIC tests) which were applied to identify the species and genus of growing bacteria according to the Bergey's Manual (Garrity et al. 2004). The isolated bacteria were kept in refrigerator by placing it on Slant tubes for further subsequent tests.

To obtain a standard bacterial trap (containing the saline medium and bacteria), each type of bacterium isolated with a rich medium (Tryptone Soy Broth) was grown at a temperature of 37 °C for 24 h using shaking incubator at a speed of 150 rpm. After that all bacteria were gathered in a glass tube using the cooling centrifuge at 4 °C at 5000 rpm. The bacteria settled in the tube bottom was washed by normal saline solution, after that each isolated bacteria was

suspended in saline solution (Mineral Salt Media) and the bacteria density was monitored using a Spectrophotometer with a absorbance of 0.5 at a wavelength of 540 nm. In case of preparing the bacterial stuck for each bacterium at a rate of 10% v / w, the results of the colony formation units were 1.62×10^7 cfu. Then 100 ml of this bacterial stuck (saline solution and bacteria) was mixed with 1 ml of purified spindle oil (after purification) in a 500 ml conical flask. Nitrogen and phosphorus were added in a ratio of 1: 0.5 and incubated in shaker incubator at 37 °C at a speed of 150 rpm. Then both bacterial growth monitored and oil decomposition for specific time periods (7 days, 15 days, 30 days) were monitored for the best time of decomposition testing. However, these processes were applied to each bacterium separately within the specified time periods in addition to making a mixture of bacterial cultures to study the efficiency of isolated bacterial groups when mixed in the treatment process with the control sample of saline solution and oil spill without pollination for the comparison.

The concentrations of the organic substance were calculated based on the peaks area obtained from the GC graph by applying the following equation according to (Silverstein et al. 2005):

$$C\,sample = C\,st \times A\,sample \,/\, A\,st$$

where:

C sample = Sample concentration.

C st = Primary sample concentration.

A sample = Peak area after treatment.

A st = Peak area before treatment.

Genomic DNA was isolated from growing bacteria using Geneaid kit (Korea) depending on manufacture instruction, followed by amplification of 16srRNA gene using universal primer F5- CCAGCAGCCGCGGTAATACG-3 R5-ATC GGCTACCTTGTTACGACTT-3. The amplified product were sequenced in Macrogene company and analyzed using NCBI blast (nlm.nih.gov/Blast.cgi).

Field system

For the purpose of transferring the data of the research experiment to the practical application, a small scale field system was designed to provide the information required for the application of the biological treatment process in a larger and wider scale. This system includes a plastic container with a capacity of 244 L with one opened end consisting a perforated tube installed in the bottom. The container is connected to an air pump to pump air during the treatment process with flow rate of 8 m/ min with galvanized iron buckle to hold the dispersed filter to the air, placed inside the container amid a saline solution of 200 L and then 2 kg of sterile soil was added (soil sterilized by wet sterilizer with temperature of 121 °C and a pressure of 1.5 bar was left for 24 h, and then sterilized again at the same conditions to ensure that the soil was free of bacterial boards that turn into the vegetative phase in a period of 24 h). Finally, 2 L of spindle oil were added to the container and the system was inoculated with the bacterial culture by 1 L of the enrichment medium, and the system was operated by pumping air for 20 days, where the biodegradation rate of examined hydrocarbons was measured (Fig. 1).

Results

Based on the ability of the organism to grow on hydrocarbons medium and its ability to analyze the examined material as a source of carbon, three types of bacteria were isolated which were *Aeromonas hydrophila*, *Bacillus subtilis* and *Staphylococcus aureus* using biochemical tests to determine the genus and species of bacteria isolated according to the Bergey's Manual (Table 1). However, bacterial isolates were checked using VITEK 2 with special kits which gave the same results. Also, each type of bacteria isolated was grown on nutrient agar by dish pouring technique to measure their numbers and determine the prevailing ones from the first dilution 10–1 of the examined samples by calculating the number of cells in 1 ml equaled to the number of colonies multiply by the dilution inverse. This is shown in Table 2, which displays the isolated bacterial species and their total numbers. The isolated bacterial groups showed different abilities to digest and break down the spindle oil.

The biological treatment ability of each bacterium was measured individually for the digestion and analysis of the hydrocarbon material. During the experiment of analyzing

Fig. 1 Field treatment system

Table 1 Biochemical test results of tested bacterial strains

Characteristic Properties	Bacteria		
	Bacillus subtilis	*Aeromonas hydrophila*	*Staphylococcus aureus*
Catalase	+	+	+
Citrate	+	–	+
Gas forming	–	+	–
Gelatin	+	+	+
Gram stain	+	–	+
H_2S	–	ND	–
Hemolysis	+	+	+
Indole	–	+	–
Motility	+	+	–
Methyl red	–	+	+
Oxidase	–	+	–
Urease	+	–	+
Glucose	+	ND	+
DNase	+	+	+
Lactose	+	+	+
Lipase	+	+	+
Ornithine decarboxylase	ND	–	ND
Esculin hydrolysis	ND	+	ND

ND Not done

Table 2 All isolated bacteria species examined and their total plate count

Microorganism	Treatment capability	Total plate count
Aeromonas hydrophila	+	880 cell/ml
Bacillus subtilis	+	690 cell/ml
Staphylococcus aureus	–	280 cell/ml

[+] Good ability to digest the oil spills and used as carbon source

[–] Poor ability to digest the oil spills and used as carbon source

the organic matter for each bacterium individually, the results showed that *Staphylococcus aureus* was the weakest in the analysis process. Nevertheless, it was included in a mixture experiment of bacteria as the actual behavior during oil biodegradation in natural contaminated environments does not depend on pure cultures (Janbandhu and Fulekar 2011; Cerqueira et al. 2011). Moreover, a mixed inoculation has been made for the three types of isolated bacteria to study their joint efficiency in breakdown the oil spills to be used as a carbon source via measuring the degradation results using GC mass for specified time periods (7, 15, 30 days) and determining the biodegradation percentage of treatment and the ability to analyze the organic matter according to the peaks areas extracted later in the readings

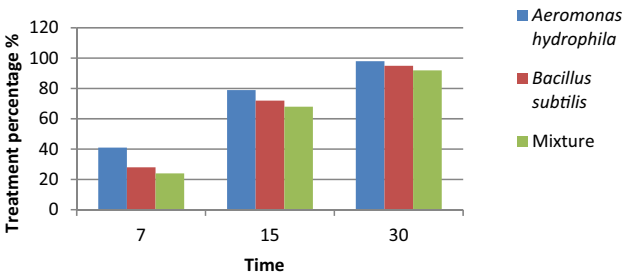


Fig. 2 Treatment percentage of the isolated bacterial groups within study specified time

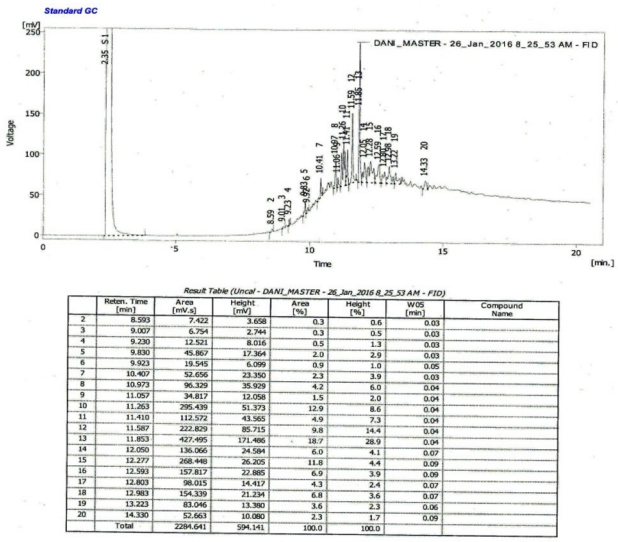
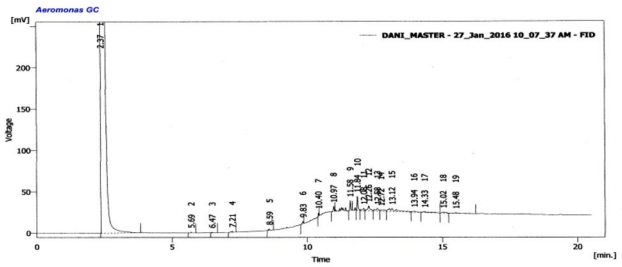


Fig. 3 GC-mass spectrum of purified spindle oil

of the gas chromatography, as shown in Fig. 2. The results have shown that the time period of 30 days was the best period of treatment and adopted in the experimental work experiment to prove this percentage without a change in the tests conducted up to 45 days.

Figure 3 shows the results of the chromatographic test of the examined oil spindle which was purified as a standard material for comparison with the changes occurred in the biodegradation processes later, where the results have been recorded at the minute of 11.853 of the retention time at the highest concentration being the tested material was pure and standard with area percentage reached 18.7%.

Figure 4 displays the results of biodegradation of the oil spills by *Aeromonas hydrophila,* which had the highest biodegradation rate among the isolated bacterial species recording 98% after 30 days from commencing the experiment, where the biodegradation rate has remained at the same efficiency even after 30 days and up to 45 days with the same standard retention time.



Result Table (Uncal - DANI_MASTER - 27_Jan_2016 10_07_37 AM - FID)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.370	10731.929	993.072	96.7	93.3	0.17	
2	5.690	2.020	1.005	0.0	0.1	0.03	
3	6.467	1.567	0.708	0.0	0.1	0.03	
4	7.210	3.591	1.127	0.0	0.1	0.03	
5	8.587	5.628	1.717	0.1	0.2	0.03	
6	9.827	6.764	2.923	0.1	0.3	0.03	
7	10.400	6.116	4.271	0.1	0.4	0.03	
8	10.967	15.627	6.523	0.1	0.6	0.04	
9	11.577	26.010	11.994	0.2	1.1	0.04	
10	11.840	45.009	17.593	0.4	1.7	0.04	
11	12.077	17.664	3.206	0.2	0.3	0.06	
12	12.260	42.155	6.226	0.4	0.6	0.06	
13	12.580	28.769	3.450	0.3	0.3	0.09	
14	12.720	23.168	1.935	0.2	0.2	0.22	
15	13.123	84.001	3.937	0.8	0.4	0.06	
16	13.940	14.693	1.442	0.1	0.1	0.16	
17	14.330	19.786	1.387	0.2	0.1	0.12	
18	15.020	10.326	1.277	0.1	0.1	0.08	

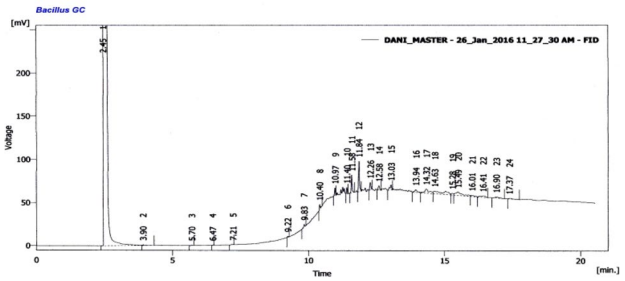
Fig. 4 Biodegradation process of oil spills by *Aeromonas hydrophila*

Genomic DNA was extracted from lysed *Aeromonas hydrophila* cells and subjected to 16 s rRNA gene amplification using PCR method. The PCR products were electrophoresed on agarose gel. The results indicated that the presence of the16s rRNA gene. For further confirmation, the sequencing result of PCR product was subjected to similarity search in GenBank database using Basic Local Alignment Search Tools (BLAST). High similarity (100% homology, Data not shown) was indicated between DNA sequence of tested *Aeromonas hydrophila* strain and the corresponding sequences of *Aeromonas hydrophila* strain Ha14 (Gen-Bank accession number MH477726.1).

Figure 5 shows the results of the biodegradation of the hydrocarbon material by *Bacillus subtilis*, which was relatively less than that of the *Aeromonas hydrophila* bacteria reaching upto 95% after 30 days from the experimental commencing date with the same retention time.

A vitally stuck was made consisting of the three isolated bacterial species at similar percentages to measure their joint efficiency in the biodegradation process and the biological analysis of the examined organic matter and to compare it with biodegradation of each bacterium. The results showed that there was a relative decrease in the treatment process, reaching 91% after 30 days from commencing experimental work depending on the area of the peaks and their percentages, as shown in Fig. 6.

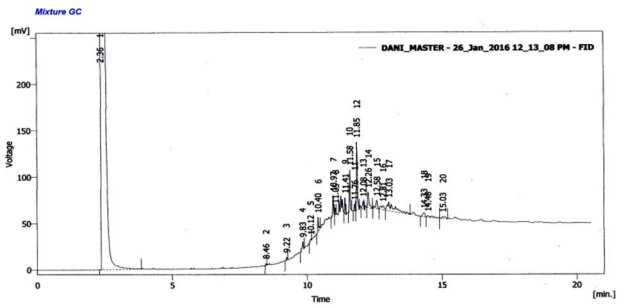
From the obtained laboratory results of this study and for the applying the data of the biodegradation process in the field, a small practical biodegradation system was designed to treat oil spills to provide sufficient information to apply the biodegradation process on a larger scale in certain factory or institution producing such organic matter as form of depleted waste to be disposed by environmentally friendly biodegradation processes that do not affect the ecosystem balance by not producing any secondary pollutants harmful to the environment, where the final outputs of this system are carbon dioxide and water. The biodegradation process system was provided with a device to pump air during this process. It is worth mentioning that the system was left to work for 72 h continuously to get rid of any possible concentrations of chlorine associated with drinking water to ensure that the toxic chlorine does not affect the organisms used in the experiment.



Result Table (Uncal - DANI_MASTER - 26_Jan_2016 11_27_30 AM - FID)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.453	9279.950	993.069	94.0	88.6	0.15	
2	3.903	5.534	0.941	0.1	0.1	0.05	
3	5.697	1.220	0.641	0.0	0.1	0.03	
4	6.467	0.498	0.313	0.0	0.0	0.03	
5	7.210	1.013	0.353	0.0	0.0	0.03	
6	9.223	1.950	1.215	0.0	0.1	0.03	
7	9.827	6.264	3.245	0.1	0.3	0.03	
8	10.403	12.424	5.883	0.1	0.5	0.03	
9	10.970	18.226	8.728	0.2	0.8	0.04	
10	11.403	13.846	7.044	0.1	0.6	0.04	
11	11.580	47.032	20.405	0.5	1.8	0.04	
12	11.843	87.153	35.391	0.9	3.2	0.04	
13	12.263	25.274	8.702	0.3	0.8	0.05	
14	12.580	18.566	5.177	0.2	0.5	0.06	
15	13.030	27.390	6.060	0.3	0.5	0.05	
16	13.940	22.314	3.145	0.2	0.3	0.09	
17	14.323	57.529	4.929	0.6	0.4	0.11	
18	14.633	95.659	3.194	1.0	0.3	0.24	

Fig. 5 Biodegradation process of oil spills by *Bacillus subtilis*



Result Table (Uncal - DANI_MASTER - 26_Jan_2016 12_13_08 PM - FID)

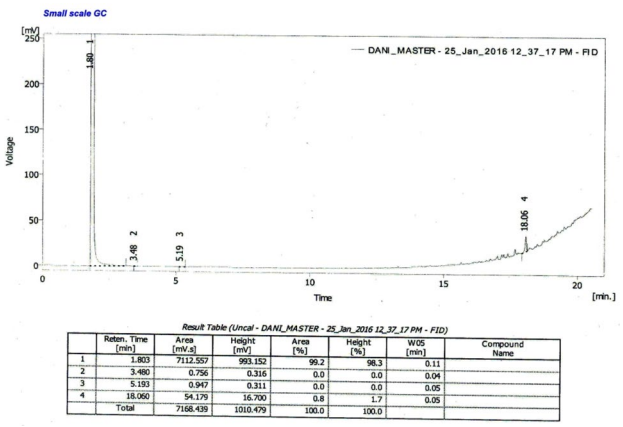
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.363	11769.160	992.824	93.1	79.1	0.19	
2	8.457	3.228	2.100	0.0	0.2	0.03	
3	9.223	5.810	3.551	0.0	0.3	0.03	
4	9.827	24.044	8.841	0.2	0.7	0.03	
5	10.123	5.964	2.482	0.0	0.2	0.04	
6	10.403	32.596	15.498	0.3	1.2	0.03	
7	10.970	51.420	22.100	0.4	1.8	0.04	
8	11.050	13.620	7.520	0.1	0.6	0.03	
9	11.407	33.668	17.215	0.3	1.4	0.03	
10	11.583	97.773	45.188	0.8	3.6	0.03	
11	11.760	15.341	7.582	0.1	0.6	0.03	
12	11.847	178.849	73.270	1.4	5.8	0.04	
13	12.080	24.672	8.851	0.2	0.7	0.05	
14	12.263	65.589	16.940	0.5	1.3	0.05	
15	12.583	42.334	9.111	0.3	0.7	0.06	
16	12.810	40.637	4.193	0.3	0.3	0.18	
17	13.033	164.090	9.638	1.3	0.8	0.06	
18	14.333	27.853	4.498	0.2	0.4	0.10	

Fig. 6 Biological analysis of oil spill by mixed isolated bacteria

The system was inoculated only with *Aeromonas hydrophila*, being had the highest percentage of treatment when grown in nutrient agar and inoculating the entire system components with it. The results showed the high efficiency of the system on the treating and digesting of the organic matter with a rate of 100%, as shown in Fig. 7 which explains the total disappearance and decomposition of the organic hydrocarbon used in the study during a period of 20 days from the start of the experiment under the surrounding natural field conditions such as temperature (the system water temperature reached 32 °C), where the system has been operated continuously for 20 days.

Discussion

The microorganisms that biodecrading petroleum derivatives have the ability to use these compounds as an energy source and are widely spread in various environments such as water, air, and soil (Magot 2005). In this study, three bacterial isolates were identified depending on their shape, characteristics, and biochemical properties using Bergey's Manual. These three bacterial isolates were capable of biodegrading the oil spill as a carbon source, which supported by the results of many laboratory studies (Hamzah et al. 2010; Ugur et al. 2012; Malik and Ahmed 2012) who have used the same saline medium despite some slight differences used in composition and concentrations, such as adding nutrients and biological supported enzymes responsible for the biodegradation process. Also, adding hydrocarbon to the medium as a single source of carbon is not sufficient, because organisms need other nutrients such as nitrogen and phosphorus which should be added in acceptable concentrations in the saline medium to complete the biological treatment process (Atlas 1984). The addition of the saline medium has caused an increase in the numbers of living organisms already occurred in the soil. This increase is due to the presence of inorganic nutrients, where it is possibly shown that the high contents of oil derivatives in the examined soil samples have caused a disturbance in the ratio of the distribution of inorganic nutrients necessary for the activity bacteria (Thomas et al. 1992). The assumed required nutrient ratio of C: N: P should be 100: 10: 1 to enable endemic microbes to grow and carry out their analytical activity. The reason of these fluctuated percentages in the examined soil samples is due to unintended oil spills resulted from improper use of these derivatives which has led to the increase of carbon percentage on both nitrogen and phosphorus percentages.



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	1.803	7112.557	993.152	99.2	98.3	0.11	
2	3.480	0.756	0.316	0.0	0.0	0.04	
3	5.193	0.947	0.311	0.0	0.0	0.05	
4	18.060	54.179	16.700	0.8	1.7	0.05	
	Total	7168.439	1010.479	100.0	100.0		

Fig. 7 Biodegradation of oil spills by *Aeromonas hydrophil* in the field treatment system for 20 days

The phenotypic changes of the oil layers in the glass vessels were the result of the bacterial species growth and this confirms the ability of these species to use the organic oil and convert it initially into small drops, as evidence that these isolated organisms have emulsified the organic oil biologically due to their specialized enzymes that break down chemical bonds and use carbon as an energy source with no any change in the standard vessel that does not contain bacterial groups (Naser 2000).

Petroleum oil is a mixture of both short and long-chain hydrocarbons and this is what all the manufacturing standard data sheet (MSDS) by produced companies manufacturers according to scientific references, and therefore, the shortness of these chains makes it easier for microorganisms to digest and break them to get carbon as an energy source.

The prevalence of growth of *Aeromonas hydrophila* and its good ability to digest organic compounds is due to its high tolerance to live in environments containing oil derivatives in large concentrations, where these bacteria can produce a wide range of enzymes to digest the organic compounds such as esterase, lipase, aminopeptidase, N -acetyl-beta-glucosidase, phosphoamidase, acid phosphatase, butyrate esterase and other enzymes and auxiliary compounds that contribute in breaking down the chemical bonds of organic compounds to get carbon as a source of energy, as well as capable of tolerating a wide range of temperatures (Waltman et al. 1982). Also, *Bacillus subtilis* bacteria has a high tolerance to live in environments containing high concentrations of oil derivatives and hydrocarbons due to the ability of these bacteria to produce spores resistant to various conditions in addition to producing many enzymes to analyze organic materials such as amylase, protease, pullulanase, chitinase, xylanase, lipase and other enzymes to obtain carbon as an energy source. These bacteria have high ability to produce specific enzymes by modulating the cell's genetic material (DNA). This explains the growth of these bacteria in such polluted habitats as these organisms contribute to cleaning the environment from oil stains and spills (Ghazali et al 2004). Multidisciplinary enzymes and their ability to resist

different growing conditions and adaptations found in both *Aeromonas hydrophila* and *Bacillus subtilis* are higher than those in *Staphylococcus aureus* and this explains the presence and relatively poor ability to digest organic matter compared to other examined bacterial species (Shekhar et al. 2015; Turkey et al. 2018).

We used a microbial consortium to evaluate the ability of the mixed bacterial isolates to increase the possibility of complete degradation of tested oil. In natural oil-contaminated environments, the mechanisms of oil biodegradation can be complex and influenced by many factors such as Physical, chemical, and biotic factors (Mukred et al. 2008; Cerqueira et al. 2011; Ibrahim 2016). In view of this, the indicated decrease in biodegrading percentage when used the bacterial consortium may be attributed to the reduced bacterial activity due to unknown factors that antagonized against bacterial growth. This agrees with (Friello et al. 2001) and (Al-Wasify and Hamed 2014) who reported that a wide variety of metabolic and physiological factors are required for the degradation of different compounds in diesel oil.

The high levels of hydrocarbon removal were found in the case of short alkene chains compared to long chains and this corresponds to the results of the current study regarding that the short chains of the examined compound are easier to biodegrade due to their hydrophobicity, which facilitates the access of the microbial cell to the organic compound. Also, the quality of soil components plays a significant role in the biodegradation process, where the soil used in the experiment has a low clay content, which allows the fluids loaded with nutrients and gases to flow between their molecules, thus accelerating the efficiency of the biodegradation process by utilizing oxygen as an oxidizing agent that contributes to break down the chemical bonds of the organic compound during the biodegradation process (Del 'Arco and Franca 2001). This explains the high efficiency of the biodegradation process in the designed system, where the air is pumped continuously to increase the oxygen levels needed for the oxidation process in a less time. The results of the current study were backed by the results of previous similar studies (Ghazali et al. 2004; Shekhar et al. 2015) carried out on the biodegradation process of crude oil derivatives in polluted soils.

The current study has highlighted the possibility of bacterial communities isolated from soils contaminated with hydrocarbons to the process of biodegradation, as it provides an effective process of decomposition of many hydrocarbons with a wide range of concentrations and retention periods. Therefore, the biodegradation of toxic hydrocarbons in soils, spills and oil slicks would be a better choice to use the organisms originally adapted in their natural environment, which are efficient in detoxification and stability of biodegradation processes with low economic cost and the preventing any environmental risk to preserve the ecosystem balance.

The study leads to the following conclusions:

1. *Aeromonas hydrophila* has great potential for analysis of organic hydrocarbons.
2. The biodegradation method of hydrocarbons is an environmentally sound method as it does not produce environmentally harmful secondary materials, where the end products are carbon dioxide and water.
3. The air pumping unit in the manufactured system contributes greatly to the decomposition of organic matter faster through the provided oxygen to the oxidation processes.
4. The current work recommends to examine the effect of different variables such as temperature, pH, concentration of organic matter and the size of soil particles on the biodegradation process using other microorganisms.

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Compliance with ethical standards

Conflict of interest Sufyan Mohammed Shartooh declares that he has no conflict of interest. Author Mohammed Fadhil Abood declares that he has no conflict of interest. Haidar Kadum Yahob declares that he has no conflict of interest.

Informed consent Informed consent has been obtained. The study has been approved by the Environment and Water directorate, Iraqi Ministry of Science and Technology [Ethical Approval/ 2015/ (T.B.M 25/740)].

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