Multi-Knowledge Electronic Comprehensive Journal For Education And Science Publications (MECSJ)

ISSUE (20), June (2019)

*ISSN :***2616-9185**

www.mecsj.com

Study the ability of some marine oil-degrading bacteria to grow in different concentrations of salt solution and production of bio-emulsifiers

Hala Ezzat Abou El-Hassayeb[1]

[1]Department of Marine Microbiology, National Institute of Oceanography and Fisheries, Alexandria, Egypt

Email: dr.lola2010@yahoo.com

Abstract:

Back ground: Studies in the field of treatment of oil pollution and its derivatives have shown that microorganisms have a significant role in the treatment of environmental pollution and thus contribute to environmental balance as they have the ability to benefit from hydrocarbons as a good source of carbon.

Objectives: This study reviews the possibility of isolating the bacteria from crude oil from four locations in Egypt; East of Suez, North-east of Alexandria, North of Alexandria and Port Said

Methodology: Twenty-two bacterial isolates were isolated, all of which were cultivated in liquid nutrient media with addition of crude oil, were isolated as a single source of carbon and energy. The isolates were then cultured in five different concentrations of 1% crude oil, 5%, 10%, 25%, 50%

Results: Some isolates have shown the ability to grow unevenly up to 50% concentration، isolates were cultivated in four different concentrations of salt (1.2%, 2.4%, 3.6%, 5%) to determine the appropriate concentration for the growth of these marine bacteria. It was observed that the best growth rates during concentrations of 2.4%, 3.6% which is relative to the salt ratio in the Mediterranean Sea water. The solubility of isolates was estimated for the production of bio-emulsifiers on Hexane and Toulene compounds. The results showed that isolates capable of producing a bio-emulsion. The bacterial isolates were identified using 16S rRNA. Three types were identified: *Marinobacter hydrocarbonoclasticu, Vibrio alginolyticus* and *Eubacterium combesii*

Key words: marine oil-degrading bacteria, different concentrations of salt solution, production of bio-emulsifiers

1. Background

Oil fields are not homogenous in the world, but they are limited in regions such as the Arabian Gulf. Egypt is an international economic hub for the oil and natural gas trade. Egypt currently has the potential to be a central hub for exporting energy from its own sources and producing it to the main markets in Europe. (El Nady & Harb, 2010).

The Arab production of crude oil is more than 3 billion tons per year, and half of this material by sea. As a result, the global shipping of the oil is resulting in pollution of the marine environment for several reasons, including the numerous incidents that led to the sinking of carriers such as the Exxon Valdez accident in Alaska, has greatly affected the marine environment and drilling and exploration for oil and its sources is behind Severe marine pollution (Harayama et al., 1999).

It has been found that the method of biological treatment of pollution depends on the use of species of microorganisms capable of extracting or dismantling many of the pollutants present in water. As bacteria have the ability to disassemble many pollutants and compounds to less weight, complex and dangerous particles to facilitate the process of melting in water, which transforms them from dangerous substances harmful to less hazardous materials and less harmful as the bacteria rely on these compounds as a single source of carbon and energy (Radhakrishnan et al., 2011)

Oil pollution is one of the most dangerous contaminants of the seas and oceans due to its rapid spread according to the speed and direction of wind and sea currents. It reaches a distance of more than 300 kilometers from the source of pollution. Oil forms a thin layer above the surface of the water with a diameter more than 30 cm, and gives one ton of oil area of 12 km, and the oil forms a layer above the surface of the water with thickness ranges from parts of the micrometer to 2 cm (Smith et al., 2012)

With the increase in population growth and the spread of basic industrial processes, humankind witnessed the emergence of a civilization that began to have the greatest collective impact on the surrounding areas. The beginnings of environmental awareness were expected to occur in more advanced cultures, especially in denser urban centers. Studies show that 200,000 tons of oil are enough to turn the Baltic Sea from a biological point of view into an arid desert with no living organisms, as well as marine pollution sources. The process of cleaning tankers, which cause 10 million tons of oil to spill into the sea, oil is particularly high in the coastal areas, and the closed seas complain of the pollution problem. There is a warning by scientists to turn the Mediterranean Sea into a dead sea if pollution rates remain so, especially by France, Italy and Spain. So the French, Italian and Spanish costs were more affected by pollution of the Mediterranean Sea (Stephens et al., 2013).

Bio-emulsifiers are known as vital products that are effective at the surface tension level (Biosurfactants), they are divided into two phases: water-resistant (insoluble) and water-soluble, and can interfere with polar and non-polar materials. This phenomenon is known as bio-emulsification (Kiyohara et al., 1982), which are used by microorganisms when they grow in water-threatening environments, reduces the surface tension between these two phases, resulting in the formation of small concentrations of water-soluble molecules within the water-insoluble phase. The surface area available for microbial interference increases the bioavailability of these molecules and increases their consumption (Scott, 2000).

Biomass emulsions are produced by many microorganisms, especially bacteria, in aerobic conditions in large quantities and in different species, most of which are extracellular fatty complexes that either remain attached to the surface of the cell or are introduced into the growth medium (Macdonald et al., 1981).

Since the early 1990s, bioremediation has been associated with the environmental pollution of hydrocarbons as oil pollution is a major problem at present (Leahy et al., 1990).

The bio-emulsification mechanism is one of the most effective means of bio-remediation for oil pollution. It is an alternative to the chemical and physical methods used to remove hazardous pollutants from the environment, as well as being economically inexpensive and safe, and not a new burden on the polluted environment (Edelvio et al., 2009; Paul, 2007).

Microbes that tolerate high salinity levels can tolerate salinity up to 50%. Salt-tolerant bacteria and fungi need salinity 2.5-4%) to grow (and cannot grow well without salinity ( ), however Salinity is a chemical agent that inhibits the growth of microorganisms. Rarely exist microbes that can tolerate difficult environmental conditions alongside their ability to consume hydrocarbons at the same time. Sodium chloride plays an important role in reducing metabolism and inhibiting fermentation (Zaki And al-Kashkari, 1998).

For salt-tolerant organisms, the chlorine ion is used to maintain ionic balance during energy production in conjunction with sodium ion. For the marine and ocean environment, some biological processes in cells such as oxidation of organic matter and production of monocellular proteins, sugars and indole are encouraged by high concentration of salinity. Some enzymatic systems also require a weak increase in osmotic pressure. Sodium ion plays an important role in the transport of nutrients from the environment to the cell through the cell membrane (Exile, 2007).

Ajisebutu (1988) studied the effect of different concentrations of sodium chloride ranged between 0.5 - 1.5% (weight / volume) on the decomposition of crude oil by the *Aeromonas* and reported that the contrast of decomposition was associated with increased sodium chloride concentration.

Baraniecki et al. (2002) used low concentrations of sodium chloride in the study of biodegradation of diesel oil. Diaz et al. 2002 analyzed hydrocarbons in water under high salinity conditions (0-180 g / L), while Mukherji et al. (2004) studied biodegradation of diesel by farmer Bacteria isolated from the sediments of the Arabian Sea tolerate salinity with a concentration of more than 3.5% and adapt to them.

In general, toxicity is dependent on oil compounds and their concentrations. Refined oil is more toxic for the plants than crude oil. Acute toxicity occurs through compounds with low molecular weight, alkanes and aromatic compounds, whereas chronic toxicity is caused by PAH, the toxicity occurs at high temperature as large quantities of toxic compounds lose their toxicity through changes in weather conditions. When cooling of oil occurs in cold climates, the toxic chain is reduced, water solubility increases, hydrocarbons increase, and microbes can dissolve pollutants when their concentration is less than the rate of toxicity and their growth and vitality are limited, when the pollution is high and the toxicity is high, bacterial growth is limited and has little vitality. When a microbial killer does not occur and no complete biomass occurs here, we need engineering methods and high concentrations of dilution prior to biodegradation (Si-Zhong et al., 2009).

The aim of this study is to isolate and define bacterial strains from the study area. Which have a natural ability to crack and analyze hydrocarbons, and the study of some factors on the isolates such as: different concentrations of crude oil and the possibility of composition emulsion and different concentrations of saline.

2. Materials and methods

2.1. Study locations: from crude oil from four locations in Egypt; East of Suez, North-east of Alexandria, North of Alexandria and Port Said

2.2. Sampling:

Twelve samples from four different sites within the study area were collected every four samples from a different site. These samples include sea water at a depth of about 1 meter in sterile 100 ml dark color bottles. They were kept cold until they reached the laboratory and laboratory experiments were carried out, and were labeled as follows:

First location: (SA1 to SA3)

Second location: (SB1 to SB3)

Third location: (SC1 to SC3)

Fourth location: (SD1 to SD3)

2.3. Isolation of bacteria from crude oil from the study area:

Basal Media was prepared and 9 ml was placed in (25 ml) glass bottles. 0.1% (v / v) of crude oil was added as the sole source of carbon to the center. The bottles were then sterilized with their contents at the complex at 121 ° C for 15 minutes (Kozai et al., 1988).

2.3.1. Components of basal medium prepared (g / l):

K2HPO4	0.5 g/l	
NH4Cl	1.5 g/l	1 mL of collected sampl
Na2SO4	2.0 g/l	
KNO3	2.0 g/l	
MgSO4.7H2O	0.2 g/l	
Na Cl	1 g/l	

es from sea water were added to the previously prepared glass bottles in the basal media and crude oil, one bottle for each sample of 12 samples collected and studied for consideration. The number was 24 bottles. Crude oil was placed in the incubator at a temperature of 30-32 ° C and controlled bacterial growth for 7 days.

2.3.2. Purification of isolated bacterial strains

After the incubation period was completed (7 days) 0.1 mg (sterile) was taken from the bottles, all of which gave bacterial growth and cultivation by distributing it to Bushnell haas media, where this medium was added and 1% (v / v) of crude oil was added as the sole source of carbon and Na Cl 3.5% g / l. Bushnell haas medium is optional and is used to determine which of these isolates is able to grow and consume crude oil and placed the dishes at a temperature of 30 - 32 ° C in the incubator for 18 - 72 hours (Latha & Kalaivani, 2012)..

The bacteria, which gave growth to the center ofi8, were then cultured on nutrient agar. There was no growth on nutritious agar medium. Salt was added to the nutritious nutrient medium of 3.5% and we obtained results and growth. The dishes are then incubated at 30 ° C inside the incubator for 18-24 hours to obtain pure bacterial growth،

After the incubation period, another subculture were carried out for the purpose of purification of bacterial strains growing on the center of nutritious agar and quoted according to their different forms.

2.3.3. preservation of bacterial isolates:

The isolates were kept cool at a temperature lower than 4 ° C.

2.3.4. The ability of isolated bacteria to grow on different concentrations of crude oil:

In order to determine the ability of bacterial isolates to grow on different concentrations of crude oil, the selected bacteria were first cultured on the nutrient agar and placed in the incubator at 32 ° C for 18-24 hours. The Bushnell haas medium was prepared and placed 10 ml in glass bottles 25% and 10%, 25%, 50%, the prepared media were subjected to sterilization then incubated at a temperature of 32 ° C for 7 days and the growth was monitored for turbidity of the media by naked eye.

2.3.5. The selection of the best isolates has the ability to decompose different concentrations of crude oil:

Isolated and selected bacteria based on their ability to grow in different concentrations of crude oil were used as the sole source of carbon and energy. After evaluating the results, the best seven bacterial isolates were selected and used for further identification.

2.3.5. Effect of different concentrations of salinity on selected bacterial isolates:

To determine the ability of bacterial isolates selected to grow in different concentrations of saline، The Basal media were prepared and 10 mL of it was placed in glass bottles and different concentrations of saline were added: 1.2%, 2.4%, 3.6% and 5%. The different concentrations of the prepared media were then sterilized at 121 ° C for 15 minutes. Prior to that, the selected bacteria were placed on the nutrient agar media and incubated at 30 ° C for 18-24 hours، bacterial growths were added to sterile glass bottles and incubated at 30 - 32 ° C for 6 days and monitored for growth media turbidity by naked eye (Harayama et al., 1999).

2.3.6. Testing the ability of local isolates to the production of bio-emulsifiers

The selected bacteria were cultured in Nutrient broth and incubated at 30 °C for 24 hours, the cells were centrifuged at 6,000 rpm for 10 minutes, the precipitate was removed and the supernatant was taken. Two milliliters of bacterial filtration were added in sterile glass for each isolation type to 2 mL of different hydrocarbon compounds (toluene, hexane). The contents were mixed in glass at room temperature and at maximum speed of magnetic stirrer for two-minutes, then the glass left to settle at room temperature for 24 hours،

A negative control with hydrocarbon compounds was added without the addition of bacterial filtration and a positive control containing 4 m distilled water and Tween 80 was used. The optimum emulsification effect was then calculated to obtain the highest amount of bio-emulsifiers according to the following equation:

$$E24 = \frac{\text{Height of the formed emulsion}}{\text{Total height of the solution}} \times 100$$

2.3.7. Identification of selected bacterial isolates

The selected isolates were identified using16S rRNA.

3. Results

3.1. Isolation of bacteria that analyze crude oil

The results showed that the isolated bacteria from the studied area, which are four locations, from which four samples were taken, so that the number of samples 12 samples have activity and ability to grow in a medium containing crude oil shown in Table (1).

The results showed that all isolates grew without exception; and the best and fastest growth in the fourth site, the table shows that the first site gave all samples a good growth; the second location gave one excellent growth sample, one good growth sample and other weak growth sample; while the third site showed one excellent growth sample, a weak growth sample and a good growth sample; and the fourth site gave all isolates an excellent growth

Table (1) shows the growth of samples collected on basal media with a concentration of 0.1% (v / v) of crude oil.

Sample	**1st day**	**2nd day**	**3rd day**	**4th day**	**5th day**	**Sixth day**	**Seventh day**
S1a	-	+	+	+	+	++	++
S1b	+	+	+	+	+	++	++
S1c	-	+	+	+	+	++	++
S2a	+	+	+	+	++	+++	+++
S2b	+	+	+	+	+	++	++
S2c	+	+	+	+	+	+	+
S3a	+	+	+	+	++	+++	+++
S3b	+	+	+	+	+	++	++
S3d	-	+	+	+	+	+	+
S4a	+	+	+	+	+	+++	+++
S4b	+	+	+	+	+	++	+++
S4c	+	+	+	+	+	++	+++
Control –ve	-	-	-	-	-	-	-
Control +ve	-	-	-	-	-	-	-

3.2. Growth of the bacteria obtained on Bushnell haas media added to 1% (v / v) of crude oil + (Na Cl 3.5% g / l)*:*

The results showed in Table (2) that eight isolates gave excellent growth; one isolate gave good growth and two weak growth isolates

Table (2) Shows Bacterial growths on the Bushnell haas media + 1% (v / v) Crude oil + Na Cl 3.5% g / l

Sample	24 hours	48 hours	72 hours
S1a	+	++	+++
S1b	+	++	+++
S1c	+	++	+++
S2a	+	++	+++
S2b	+	++	+++
S2c	+	++	+++
S3a	+	+	+++
S3b	+	+	+++
S3c	+	+	++
S4a	+	++	+++
S4b	-	+	+
S4c	-	+	+

It is observed that all bacterial isolates from all sites have grown on the Bushnell haas media + 1% crude oil, and the emergence of dense growth in most sites.

3.3.preservation of bacterial isolates growing on Bushnell haas media and purified after isolation on nutrient agar:

The results showed that there was a heavy growth of bacterial isolates after purification on the nutritious agar media in all sites except for 2 samples of the second sites, and 22 isolates were obtained.

3.4.The ability of isolated bacteria to grow on different concentrations of crude oil*:*

After selecting 22 isolates, which gave growth on Bushnell haas media and their ability to grow, was measured in different concentrations of 1% crude oil, 5% N 10%, 25%, 50%

In Table (3) shows about (32%) of the isolates gave excellent growth on 1% concentration of crude oil، the table shows the results of growth rate on the concentration of 5% of crude oil that about (32%) of the isolates gave excellent growth. The growth result on the 10% concentration was about 27% of the isolates gave excellent growth. As the results of growth on the concentration of 25% of crude oil were observed that almost (9%) of isolates have excellent growth. The results of the growth on the concentration of 50% were about 9% of the isolates gave excellent growth; nearly (5%) of the isolates gave good growth and about 23% gave weak growth have the same concentration.

Table (3) Shows Growth of isolates on basal medium in different concentrations of crude oil

N	Sample	Different concentrations of oil				
		1%	5%	10%	25%	50%
1	S1Aa	+	+	+	-	-
2	S1Ab	+	+	+	+	+
3	S1Ba	-	+	+	+	+
4	S1Bb	+	-	-	-	-
5	S1Ca	+	-	-	-	-
6	S1Cb	+++	+++	+++	++	+
7	S2Aa	+++	+++	+++	+++	+++
8	S2Ab	+++	+++	+++	-	-
9	S2Ba	+	-	-	-	-
10	S2Bb	+	-	-	-	-
11	S2Ca	+++	+++	+++	++	+
12	S2Cb	+	-	-	-	-
13	S2Cc	+	+	+	+	-
14	S3Aa	+	-	-	-	-
15	S3Ab	+	-	-	-	-
16	S3Ba	++	++	+	+	++
17	S3Bb	+	-	-	-	-
18	S3C	+++	+++	+++	+++	+++
19	S4Aa	+++	+++	+++	+	+
20	S4Ab	+	-	-	-	-
21	S4Ba	+	-	-	-	-
22	S4Bb	+	-	-	-	-

From table (3) it is observed that (S2Ca, S3C) showed the best bacterial isolates that gave growth on concentration (1%) of crude oil.

(S2Aa, S2Ab) showed the best bacterial isolates that gave growth to 5% concentration of crude oil.

(S3Ba, S3C) showed the best bacterial isolates that gave growth to 10% concentration of crude oil.

(S2Ca, S4Aa) showed the best bacterial isolates that gave growth to 25% concentration of crude oil.

(S1Cb, S3C) showed the best bacterial isolates that gave growth to 50% concentration of crude oil.

The isolated 22 bacteria were adapted to grow at different concentrations of crude oil and use crude oil as the sole source of carbon and energy. After evaluating the results, seven types of isolated bacteria were selected, which were used for further study.

3.5.Effect of different concentrations of salinity on selected bacterial isolates:

After identifying the ability of these selected isolates to grow in different concentrations of crude oil and their ability to decompose different hydrocarbons, the effect of different concentrations of salinity on these isolates and determination of any salt concentration more suitable for their activity.

The results in Table (4) show that about 71% of the isolates gave an excellent growth rate of 1.2%; nearly 29% of them gave good growth ; the activity of 2.4% of the salinity alone gave about 86% of the isolates an excellent growth; Almost 14% of which are good growth; at the concentration of 3.6% the isolates gave weak growth; The activity of 50% concentration gave about 57% of the isolates a good growth ; The rest bacterial isolates showed weak growth on the same concentration.

Table (4) shows the growth of isolates on the basal medium in different concentrations of saline

N	Sample	Different salt concentrations			
		1.2%	2.4%	3.6%	5%
1	S1cb	+++	+++	+++	++
2	S2Aa	+++	++	+	+
3	S2Ab	++	++	+	+
4	S2Ca	+++	+++	+++	++
5	S3Ba	+++	+++	+++	++
6	S3C	+++	+++	+++	++
7	S4Aa	+++	+++	+++	+
8	Control	-	-	-	-

Table 4 revealed that activity of two bacterial isolates (S1Cb, S2Aa) on the concentration of 1.2% of salt. (S4Aa, S3C) showed the activity of two bacterial isolates on 2.4% concentration of salt. (S3Ba, S2Aa) activity of two bacterial isolates on concentration (3.6%) of salt. (S1Cb, S2Ca) activity of two bacterial isolates on the concentration of (5%) of salt.

3.6.Bio-emulsification

The results in Table (5) show that S3C and S4Aa have the ability to produce active emulsifiers on Toluene with medium activity. S2Aa, S2Ab, S2Ca and S3Ba have also been shown to have the potential to good biomass emulsifiers. S1Cb was found to have the ability to produce highly active emulsifiers.

Table (5) shows Bio-emulsification in Toluene

Isolates	**E24%**
S1cb	71
S2Aa	54
S2Ab	49
S2Ca	53
S3Ba	48
S3C	43
S4Aa	42
-ve control	0
Positive control	65

The results in Table (6) show that S2Aa and S2Ab has the ability to produce good biochemical emulsions on Hexane. S1Cb, S2Ca, and S4Aa have also been shown to have the ability to produce good biomass emulsions. S3C and S3Ba have been found to be able to produce highly active biomass emulsifiers.

Table (6) Biofuels in Hexane

Isolates	**E24%**
S1cb	48
S2Aa	29
S2Ab	42
S2Ca	54
S3Ba	61
S3C	55
S4Aa	53
-ve control	0
Positive control	65

Three bacterial isolates (S2Aa, S2Ca, S1Cb) were produced for the bio-emulsifier on Toluene

Three bacterial isolates (S4Aa, S2Ca, S2Aa) were produced for bio-emulsifier on Hexane.

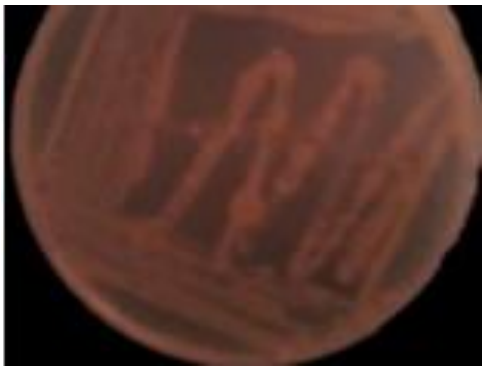
3.7.Identification of isolated and selected bacterial strains:

Some of the bacterial isolates selected by the 16R rRNA method were identified and the following table (7) illustrates the results of the identification of bacteria.

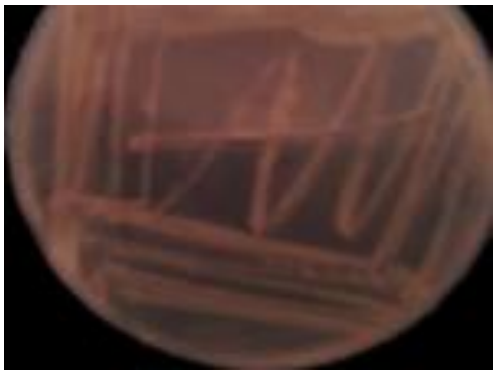
Table (7) Identification of bacterial isolates by 16S rRNA

N.	**Bacterial isolates**	**Identified bacteria**
1		*Marinobacter hydrocarbonoclasticu*
2		*Vibrio alginolyticus*
3		*Eubacterium combesii*
S2Aa		
S3Ba		
S4Aa		

S2Aa



S3Ba



S4Aa

Image (1) shows growth of selected bacterial isolates on nutrient agar media

4. Discussion

In this study, bacteria were isolated from four locations in Egypt; East of Suez, North-east of Alexandria, North of Alexandria and Port Said, the power generation stations and desalination. The number of samples to isolate the bacteria was 12 samples from each three samples from a different site to obtaining the best isolated bacteria that can help the decomposition of crude oil.

The isolates isolated from the samples and added 1% (v / v) of crude oil gave positive results. The isolates gave excellent and good growth by 42% and 42% while 16% gave weak growth. This may be due to the adaptation of bacteria to the environment contaminated with crude oil as microbial growth reflects the association with biodegradation (Hazen et al., 2003), in addition to the low concentration of crude oil in the growth media and this is consistent with Rahman et al. (2004).

The isolated bacteria after implantation on the Bushnell haas media and purified to 22 isolates. This is consistent with many studies in which marine bacteria were isolated from crude oil-contaminated areas that were capable of biodegradation of crude oil. Al-Saleh et al. (2008) isolated 272 types of crude oil biodegrading bacteria were isolated from several sites along the Kuwaiti coast. Another study isolates 24 species of marine crude oil biodegrading bacteria from several sites in the Gulf of Mexico (Kostka et al., 2011).

Estimation of the ability of growth of the twelve isolated bacterial strains on a selective medium was done using Bushnell haas media with addation of 1% (v / v) of crude oil as a sole source of carbon and energy. The results showed that 100% of the isolates had grown on Bushnell haas media, which is the selective media for hydrocarbon degradation (Okoh et al., 2001; Saieb and Elgaazwani 2008). Based on the growth in the selective medium, the isolates were purified and 22 isolates obtained.

When adding different concentrations of crude oil to the basal media as a sole source of carbon and energy and determine its effect on the growth of bacteria, it was found that the growth of bacteria decreased with the increase of the concentration of crude

oil and this is consistent with (Antai, 1990). In 50% concentration showed only 8 types of bacteria growth; both (Jia et al, 2005 and Johnsen et al, 2005) stated that the biodegradation rate is linked positively and directly the concentration of the substance that is the target for the enzyme, and also the very high levels organic chemicals can be disruptive or toxic for microbial concentrations. Willey et al. (2008) has stated that under certain conditions, the emergence of high concentrations can occur where there is no degradation of crude oil.

Several previous studies have indicated the use of different concentrations of crude oil. In Ijah and Antai study (2003), there was significant biological decomposition of crude oil by bacteria at concentrations of 10-20% compared to concentrations of 30-40%. Similarly, the study of Rahman et al. (2003) showed that the percentage of degradation by bacteria decreased from 78% to 52% with each increase in crude oil from concentrations of 1-10% and the reason for the low decomposition rate may be due to the toxicity of crude oil to microorganisms and due to the high concentrations of Oil 5 - 10% which in turn may have a negative impact on the activity of biodegradation of microorganisms. As agreed by the study of Rahman et al. (2002); Ijah and Antai 2003 as they suggested that high concentrations of crude oil can be frustrating at the early stages of microorganism growth.

Regarding to the ability of isolated bacteria to produce biomass emulsions, the results showed that these isolates could produce bio-emulsifiers, hexane and Toluene consumption on the basal medium at a concentration of 1% (v / v) each as a single source of carbon and energy. These results were in line with the findings of Ron and Rosenberg (2002) that microbes are one of the best methods of analyzing the saturated hydrocarbons contained in the synthesis of crude oil, which is based on the concept of bio-treatment of oil pollution. Biodegradability depends on the type of controlled hydrocarbons and the activity of society Microbial in the production of emulsifiers. A study also showed that biodegradation occurs more rapidly when the pollutant is soluble in water, but more hydrocarbons are insoluble in water, and that

the bioequivalence of hydrocarbons can increase for microscopic microorganisms with production of emulsifiers, where these compounds increase the water dispersion of these compounds (Al-Dahash and, Mahmoud, 2013)

Seven isolates were selected from 22 isolates that were examined for the selection and analysis of the most efficient bacteria to grow in crude oil. Three isolates (S2Aa, S3Ba and S4Aa) were the most efficient isolated species as they were able to grow in the selective media and in crude oil up to a concentration of 50% and capable of biodegradation. After their identification, this bacterium was Marinobacter.

References

1. Ajisebutu S.O. (1988). Effect of sodium chloride on biodegradation of crude oils by two species of Aeromonas.Appl. Microbiol .Biotechnol. 28:203-208
2. Al-Dahash L. M. , Mahmoud H. M. (2013). Harboring oil-degrading bacteria: A potential mechanism of adaptation and survival in corals inhabiting oil-contaminated reefs. Marine Pollution Bulletin 72: 364–374.
3. AL-Saleh E. , Drobiva H and Obuekwe C. (2008). Predominant culturable crude oil degrading bacteria in the coast of Kuwait. International Biodeterioration and Biodegradation . 63:400-406.
4. Antai S. P. (1990). Biodegradation of Bonny light crude oil by Bacillus sp. and Pseudomonas sp. Waste Management. 10: 61–64.
5. Baraniecki C.A. , Aislable J and Foght J.M.(2002).Characterization of Sphingomonas sp. Ant 17,an aromatic hadrocarbon degradation bacterium isolated from Antarctic soil .Microbial Ecology. 43:44-54
6. Diaz M .P ,.Boyd ,K. G .Grigson ,S .J. W. ,and Grant - Burgess ,J.(2002). Biodegradation of crude oil across awide range of salinities by an extremely halotolerant bacterial consortium MPD-M immobilized onto polypropylene fibers. Inc. Biotechnol.Bioeng.79:145-153.
7. Edelvio de. , Adriana B.G. , Rita de.U.S. , Maria de .C.M. , F. and Nei P.J.(2009). Biodegradation of stored jet fuel by a Nocardia sp. Isolated from contaminated soil, Braz arch Boil technol. 52: 1516 – 1520.
8. Harayama S. , Kishira H. , Kasai Y and Shutsubo K.(1999) . Petroleum Biodegradation in Marine Envioronments . Molec Microbiol . Rev 1:6370
9. Harayama, S., Kishira, H., Kasai, Y., & Shutsubo, K. (1999). Petroleum biodegradation in marine environments. *Journal of molecular microbiology and biotechnology*, *1*(1), 63-70.
10. Hazen T.C. , Tien A.J. , Worsztynowicz A. , Altman D.J. , Ulfig K and

Manko T.(2003). Biopiles for remediation of petroleum–contaminated soils :Apolish case study.Lawrence Berkeley national laboratory Institute for ecology of Industrial Areas.p:115.

11. Ijah U. J. J. , and Antai S. P. (2003a). Removal of Nigerian light crudeoil in soil over a 12-month period. Int. Biodeter. Biodegrad.51: 93-99.
12. Ijah U. J. J. and Antai S. P. (2003b). The potential use of chicken-drop microorganisms for oil spill remediation. Enviro., 23: 89–95.
13. Jia L. Q. , Quz Z. Q. and Quyang Z. y. (2005). Exological behacior of linear ylbenzene sulfonate (LAS) in soil –plant systems . Pedosphere. 15:216-224.
14. Johnsen A. R., Wick L. Y. and Harms H.(2005). Principles of microbial PAH degradation in soil . Encironmental pollution . 133: 71-84.
15. Kostka J. E. , Prakash O. , Overholt W A. , Green S J. , Freyer G. , Canion A, Delgardio J. , Norton N. , Hazen T. C and Huettel M.(2011). Hydrocarbon-Degrading Bacteria and the Bacterial Community Response in Gulf of Mexico Beach Sands Impacted by the Deepwater Horizon Oil Spill. Appl Environ Micrbiol. 77:7962–7974.
16. Kozai, T., Kubota, C., & Watanabe, I. (1988, May). Effects of basal medium composition on the growth of carnation plantlets in auto-and mixo-trophic tissue culture. In *Symposium on High Technology in Protected Cultivation 230* (pp. 159-166).
17. Latha, R., & Kalaivani, R. (2012). Bacterial degradation of crude oil by gravimetric analysis. *Advances in Applied Science Research*, *3*(5), 2789-2795.
18. Leahy J.C and Colwell R.P. (1990). Microbial degradation of hydrocarbons in the environment, Microbial Rev. 54: 305 – 315.
19. Macdonald C.R., Cooper D.G and Zajic J.E. (1981). Surface-active lipids

from Nocardia erythropolis grown on hydrocarbons, Appl Environ Micrbiol. 41: 117 – 123.

20. Mukherji ,S., Jagadevan, S. ,Mohapatra, G. and Vijay ,A .(2004) . Biodegradation of diesel oil by Arabian sea sediment culture isolated from the vicinity of an oil field . Bioresource Technology. 95:281-286
21. Okoh A. , Ajisebutu S. , Babalola G. , and Trejo-Hernandez M. R.(2001). Potential of Bukholderia cepacia RQ1 in the biodegradation of heavy crude oil. International microbiology. 4:83-87. 26
22. Paul E.A.(2007). Soil Microbiology, Ecology and Biochemistry, 3rd ed ., Academic press, USA. 250-261p.
23. Radhakrishnan, N., Kavitha, V., Madhavacharyulu, E., Gnanamani, A., & Mandal, A. B. (2011). Isolation, production and characterization of bioemulsifiers of marine bacteria of coastal Tamil Nadu.
24. Rahman K. S. M. , Rahman J .T. , Kourkoutas Y. , Petsas I. , Marchant R. , and Banat I. M. (2003). Enhanced bioremediation of n-alkane in petroleum sludge using bacterial consortium amended with rhamnolipid and micronutrients. Bioreso.Technol. 90:159–168.
25. Rahman K. S. M. , Rahman J .T. , Kourkoutas Y. , Petsas I. , Marchant R. , and Banat I. M. (2004). Enhanced bioremediation of n-alkane in petroleum sludge using bacterial consortium amended with rhamnolipid and micronutrients. Bioreso.Technol. 90:159–168
26. Rahman K. S. M. , Rahman J. T. , Lakshmanaperumalsamy P. and Banat I. M. (2002). Towards efficient crude oil degradation by amixed bacterial consortium. Bioreso. Technol. 85: 257–261.
27. Ron E.S and Rosenberg E. (2002). Biosurfactants and oil bioremediation, current Opinion Biotechnol. 13: 249 – 252.
28. Saieb F. M and Elghazawani A. H. (2008). Isolation and Identification of

Bacteria in Sarir Refinary Wastewater in Libya. Delta Journal of Science.33:1-13.

29. Scott M. (2000). The biodegradation of surfactants in the environment , Biochimica et Biophysica Acta (BBA) – Biomembranes. 1508: 235-251.
30. Si-Zhong Y. , Hui-Jun J. , Zhi W. , Rui-Xia H. , Yan-Jun J. , Xiu-Mei L and ShaoPeng Y.(2009). Bioremediation of Oil Spills in Cold Envioronments . Pedosphere . 19: 371–381.
31. Smith C. B. , Johnson C. N and King G. M. (2012). Assessment of polyaromatic hydrocarbon degradation by potentially pathogenic environmental Vibrio parahaemolyticus isolates from coastal Louisiana, USA . Marine Pollution Bulletin 64:138–143
32. Stephens E. L., Molina V. , Cole K. M., Laws E and Johnson C. N. (2013). In situ and in vitro impacts of the Deepwater Horizon oil spill on Vibrio parahaemolyticus, Marine Pollution Bulletin 75: 90–97.
33. Willey T.M , Sherwood L. M. and Woolverton C. J.(2008). Prescott Harley and Kleins Microbiology . Mccraw Hill, New York.