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Screening Bacterial Strains Isolated from Used Motor oil-Polluted Desert Soil for the production of Biosurfactants and the Possibility of Applying the Produced Biosurfactants for Washing and Bioremediation of the Polluted Soil

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Abstract: ***Six bacterial strains were previously isolated as hydrocarbon degraders from spent motor oil polluted desert soil.The six isolates were: Arthrobacter sp (EM2): Bacillus Subtilis (EM6), Bacillus sp (EM10), Corynebacterium sp (EM14) Pseudomonas aeruginosa (EM1) and Pseudomonas sp (EM19). The six bacterial strains were screened for biosurfactant production. Pseudomonas aeruginosa (EM1) and Pseudomonas sp (EM 19) were succeeded to produce biosurfactants of high activities (118.8 ± 8.2 and 89.0 ± 3.6 ODA cm2 respectively); of higher stability under wide range of temperature, pH and NaCl E24 values against petroleum oil. These characters give these biosurfactants potential applications in enhanced bioremediation of polluted sites, cleaning oil storage tanks, enhanced microbial oil recovery (EMOR) and recovery of oil from oily sludge. The sterilized supernatant containing the biosurfactant produced by Pseudomonas aeruginosa (EM1) was selected and applied directly to the spent motor oil- polluted soil. The results show that addition of biosurfactant- NP and biosurfactant alone resulted in higher biodegradation of the pollutant (71.3 ± 5.2% and 68.5 ± 3.2% respectively) with no significant variation between the results. As a conclusion, addition of biosurfactant alone for the bioremediation of polluted sites will reduce the cost of bioremediation processes and minimize the dilution or wash away of the soluble nutrients that may be used for bioremediation of water.***

Keywords: Screen of biosurfactant production, Pseudomonas spp, spent motor oil, polluted soil, biosurfactant, bioremediation

1. Introduction

Soils polluted with crude petroleum oil and its derivatives such as spent motor oil are of concern, some of these pollutants especially polycyclic aromatic hydrocarbons (PAHs) have mutagenic and carcinogenic effects on living organisms including humans. These hydrocarbons are classified as compounds with hazardous effects on human health. They are listed as priority pollutants by the US EPA (Kalf *et al*, 1997; Olezczuk and Baran, 2005).

On the other hand spent motor oil contains in addition to PAHs, more metals such as lead, zinc arsenic, cadmium, chromium, aluminum, copper, iron, manganese, nickel, and tin which come from engine parts as they wear down (Keith and Telliard, 1979; Surajudeen, 2012).

Some of these metals can dissolve in water and contaminate soil and water. These metals can be taken by plants and finally can be accumulated in living tissues and leading to toxicity and the development of cancer diseases.

Prolonged exposure to high concentration of these oils may cause liver and kidney diseases and may damage the bone marrow and the development of cancer diseases. (Mishra *et al*, 2001, Lloyed and Cackette, 2001).

For the protection of the environment, these pollutants must be eliminated. Matvyeyeva *et al* (2014) reported that at present time mechanical, physical and chemical dispersants are used for the treatment of the polluted sites. These methods are expensive and cannot completely remove the pollutants. On the other hand the chemical dispersant or surfactants are non-degradable and remaining toxic to the environment. Joshi and Shekhwat, (2014) explained that chemically synthesized surfactants currently in use are derivative of petroleum oil, and when released to the environment cause potential damage to the environment due to their non-degradability.

Bioremediation of the polluted soil is the alternative technique for the removal of the hydrocarbons, it is a cost-effective strategy, depends on the activity of the hydrocarbon-degrading microorganisms for the removal of the pollutants and converting them to non-toxic and harmless compounds. Helmy *et al* (2010) reported that biodegradation of the pollutants must be stimulated by the addition of the needed nutrients such as N_2 and P compounds. This is a biostimulation process; it needs long time and sometimes may be time consuming. Addition of biosurfactants helps in stimulating the indigenous microorganisms. The hydrocarbon pollutants are hydrophobic and strongly adsorbed to soil particles, which limit the biodegradation of the hydrocarbons due to their poor solubility and poor availability to the hydrocarbon degrades (Helmy *et al*, 2010; Zhang *et al*, 2010). A promising way to overcome this difficulty is the application of biosurfactants (Matvyeyeva *et al* 2014).

Biosurfactants increase the solubilization and the availability of the hydrocarbons to microorganisms due to the reduction of the interfacial tension.

Luna *et al* (2013) reported that in recent years interest in biousurfactants production is increased due to their advantages as compared to the chemically synthesized surfactants.

Biosurfactants are non-toxic, biodegradable and stable at extreme conditions such as wide ranges of temperature, pH and salinity. They have better environmental compatibility. Accordingly the development of these less or non-toxic products is a key strategy for acquiring environmentally friendly compounds.

Marchant and Banat (2012) reported that biosurfactants are applied in different industrial processes as well as possible novel uses in the future and are expected to become known as multifunctional products of the 21th century.

Shoeb *et al* (2013) reported that many types of biosurfactants are used in different industrial activities, but it is important to develop indigenous technology for the production of biosurfactants by microorganisms of local origin in which would be more suitable for application to that specific environment.

The aim of the present work is to screen certain bacterial strains previously isolated from used motor oil-contaminated desert soil, for the production of biosurfactants and to apply the produced products for bioremediation of spent motor oil- polluted sandy soil.

2. Materials and Methods

1. Bacterial Strains

The six bacterial strains used in this study were previously isolated from spent motor oil-contaminated desert soil during the bioremediation of this soil and were identified as : *Arthrobacter* sp (EM2), *Bacillus subtilis* (EM6), *Bacillus* sp (EM10), *Corynebacterium* sp (EM 14), *Pseudomonas aeruginosa* (EM1) and *Pseudomonas* sp (EM19), (Eman, 2015).

All of the above bacterial spp were able to degrade the spent motor oil polluting this type of soil (Eman, 2015). For this reason the six isolates were screened for biosurfactant production and their application in enhanced bioremediation and washing of this contaminated soil.

2. Biosurfactant production:

Each of the above six bacterial species were grown in inorganic salt medium (ISM) of the following composition (gm/L distilled water):

Na NO_3, 2.0; NaCl, 0.5; K_2HPO_4, 2.0; KH_2PO_4, 1.0; Mg SO_4. $7H_20$. 0.5; $FeCl_3$, 0.01 and yeast extract, 1.0.

This culture medium was supplemented by different resources (2%) such as: glucose, sucrose, soybean oil, sunflower oil and waste frying oil. Each bacterium was grown in 250 ml flask containing 50 ml of the supplemented ISM medium. The cultures were incubated at 30 ^{0}C for a period of seven days, on a shaker operated at 140 rpm. At the end of the incubation period, the cultures were autoclaved at 121 ^{0}C for 15 min, and each culture was centrifuged at 6000 rpm for 30 min to remove the bacterial cells. The cell-free broth cultures (supernatants) were tested for the production and activity of the biosurfactants using the following methods:

(2-a) Oil Spread method:
(Oil displacement area. ODA)

This method was carried out according to Techaoei *et al* (2011) and Priya and USharani (2009) as follows:

- 40 ml distilled water or sea water was introduced into a petri dish (15cm diameter) and 40 uL of light crude oil was spread over the water surface. 10 ul of the supernatant was dropped on the center of the oil film.
- The diameter of the clear circle formed was measured, and the area of the clear zone was calculated as ODA cm^2 using the following equation:

$$ODA = 3.14 \times r^2$$

- Bacterial strains showing the highest ODA values were selected and tested for their emulsification activity (E24) and their stability under wide range of temperature, pH values and different concentrations of NaCl (w/v)

(2-b) CTAB agar plate method

This method was developed by Siegmund and Wanger (1991) and was used for detection of anionic surfactants. ISM agar medium (described before) was supplemented with 0.5 mg/ml cetyltrimethylammonium bromide (CTAB) and 0.2 mg/ml methylene blue (Satpute *et al*. 2008). With a sterile cork borer (10 mm) wells were made in the agar plates. Each well was filled with supernatant containing the biosurfactant. The plates were then incubated for 48-72h at 30 ^{0}C, after which the plates were observed for the appearance of bluish/ greenish colour around the wells.

3. Stability of the Biosurfactants

(3-a) Thermostability test

20 ml portions of the supernatant of each culture were exposed to different temperatures (50-121 ^{0}C) for 30 min, and they were left to cool at room temperature. The activity of the biosurfactant of each culture was measured by using the ODA test (Techaoei *et al*, 2011).

(3-b) Effect of different pH values.

The cell free culture broth (supernatant) of each organism was adjusted at different pH values (2-11). The activity of each was measured by using the ODA test (Haddad *et al*, 2009).

(3-c) Effect of salinity

Different concentrations of NaCl (2-20% w/v) were added to the supernatant of each culture and left for 30 min, after which the activity of each culture was measured by the ODA test.

4. Emulsification index

Based on the work of Tabatabaee *et al* (2005) and Techaoei *et al* (2011), this test was carried out as follows :

- In a screw caped tube 3 ml of the supernatant was added to 3 ml of each of the following oils: petroleum oil, Kerosene, petroleum oil- kerosene (1:1), used motor oil, soybean oil and sunflower oil.
- The tubes were vortexed at high speed for 3 minutes, after which the mixture was left for 24h and the emulsification index was measured as follows :

$$E_{24} = \frac{The\ height\ of\ the\ emulsion\ layer}{The\ total\ height\ of\ the\ mixture}\ x\ 100$$

5. Application of Biosurfacant for enhancing bioremediation of spent motor oil – polluted soil

Supernatent showing the higher ODA and E24 values was selected and used for enhancing the bioremediation of the spent motor oil- polluted soil, as follows:

(5-a) Soil treatment

Soil samples were collected from a spent motor oil-polluted desert area. Five samples were collected from different sites of the same area, and thoroughly mixed to form one composite sample. This soil sample was treated as follows :

- Soil microcosm test was designed to indicate 4 treatments. Each microcosm consisting of 500 ml beaker containing 100 g of the polluted soil, and treated as shown in the following table (Table1)

Table 1: Different treatments of the spent motor oil-polluted soil

Treatment	*Amendments*				
	Biosurfacant	*Nutrients*	*Biosurfactant + nutrients*	*Free medium*	*Control*
1	+	-	-	-	-
2	-	+	-	-	-
3	-	-	+	-	-
4	-	-	-	+	-

- The fertilizer used was $NaNO_3$ (80mg/100g soil) and k_2HPO_4 (30mg/100g soil).
- 5 ml of the supernatant containing the active biosurfactant was used to inoculate treatments 1 and 3.
- For treatment No 4, five ml of uninoculated medium was aded.
- A small glass rod was introduced to each beaker for tilling the soil.
- The moisture content of each treatment was adjusted to 5% (v/w).
- All the treatments were covered by thin aluminum foil to reduce loss of water by evaporation.
- The microcosms were incubated at 30°C for a period of 70 days.
- The amount of water lost due to evaporation was added periodically (after 2-3 days).
- From each of the above treatments, samples were taken at the beginning of the experiment (0- time) and at the end of the incubation period (70 days) for extraction and determination of the loss of total hydrocarbons as a result of biodegradation.

  (5-b) Extraction and determination of the residual spent motor oil.

At the beginning of the experiment (0-time) and at the end of 70 days incubation period, four grams of the air-dried soil was mixed with 2 g of anhydrous $(Na)_2SO_4$. The residual oil in the sample was extracted by n-hexane using the shaking method described by Chen *et al* (1996). The extract was pooled and evaporated in a preweighed dish, and the amount of residual oil was determined.

3. Results and Discussion

From a previous work on the bioremediation of spent motor oil. Polluted soil, six bacterial strains were isolated, identified and studied for their ability to degrade spent motor oil (Eman, 2014). The six bacterial strains were : *Arthrobacter* sp (EM2), *Bacillus subtilis* (EM6), *Bacillus* sp (EM10), *Corynebacterium* sp (EM14), *Pseudomonas aeruginosa* (EM11) and *Pseudomona* sp (EM19).

In the present work the six bacterial strains were screened for biosurfactant production when they were grown in 15M medium supplemented with different resources. The production of biosurfactants was detected by the ODA method and CTAB test. Strains showing the higher ODA values were selected and studied for their emulsification activity as indicated by their E24. The results of the production of bisurfactants produced by the six bacterial strains when grown on different resources are found in Table (2) and Fig (1), and can be summarized in the following points:

- Among the six bacterial strains, *Pseudomonas aeruginosa* (EM1) and *Pseudomonas* sp (EM19) were highly positive for ODA test, they succeeded to give the maximum ODA cm^2 values (118.8 ± 8.2 and 89.0 ± 3.6 cm^2 respectively) when grown in presence of waste frying oil (Fig. 1c-b).
- On the other hand *Bacillus sabtilis* (EM6) and *Bacillus* sp (EM10) were able to produce moderately active biosufactants when grown in the pressure of sucrose (31.2 ± 2.8 and 20.9 ± 0.1 ODA cm^2 respectively. It was noted that the two *Bacillus* strains failed to produce bisurfactants in presence of vegetable oils as carbon sources.
- *Arthrobaiter* sp and *corynebacterium* sp are considered in this work as week biosurfactant producers (Table 2).

Hamza *et al* (2013) screened 20 bacteria for biosurfactnt production by using the oil spread method (oil displacement area), microplate method and drop collapse method. They found that 45% of the bacterial isolates were positive for oil spread method. Tambekar *et al* (2013) screened 14 bacterial for biosurfactant production using the oil spread method, the drop collapse method and B-hemolysis test. They considered 5 mm diameter clear zone (0.2 ODA cm^2) and 10 mm diameter clean zone (0.8 ODA cm^2) as positive biosurfactant production. They found that 92.9% of the bacterial strains tested were positive for the oil spread method. Techaoei *et al* (2011) isolated 25 bacteria from garage sites, all of the 25 isolates were positive for the ODA method.

The oil displacement test (ODA) is an indirect measurement of surface activity of a biosurfactant sample tested against oil; a large diameter clear zone represents a higher activity of the biosurfactant (Rodigues *et al*, 2006). The diameter of the clear zone on the oil surface correlated to surface activity. Biosurfactants have a linear correlation between quality of surfactants and clearing zone diameter (Vandana and Peter, 2014).

The production of biosurfactants was tested also by the CTAB agar test. The result show that the two *Pseudomonas* spp and the two *Bacillus* spp were positive for CTRB method. *Pseudomonas* spp gave bluish colour around the inoculated wells (Fig 2a), while *Bacillus* spp were able to from bluish-

green colour (Fig 2b). The formation of blue colour may indicates that the test biosurfactants are of the glycolipid group. While the formation of greenish colour may indicate the presence of lipopeptide biosurfactant (Feinger *et al*, 1995).

CTAB agar can be used for the detection of glycolipid bisurfactant producer strains. The glycolipids are able to react with bromide salt found in CTAB and forming with methylene blue insoluble ion pair CTAB-MB which is blue in colour (Pinzon and Ju, 2009; Abdel-Mawgood *et al*, 2010).

Results of the emulsification activity of the two *Pseudomonas* strains as measure by the E24 test are found in Table (3). The two *Pseudomonas* spp were grown on 15M medium supplemented with hydrocarbon oils as well as vegetable oils. The results show that the two bacterial strains were able to emulsify all the oils studied but with different emulsification activities (E24). *Pseudomonas aeruginosa* (EM1) was able to produce higher E24 values (53-66%) as compared to *Pseudomonas* sp (EM19) (51-62.5% E24). *Pseudomonas aeruginosa* was able to produce the higher E24 values (66.5±2.8%) with crude petroleum oil and (62.5± 2.9%) with petroleum oil- kerosene mixture. On the other hand *Pseudomonas* sp (EM19) produced high E24 value with crude oil (62.5±3.5%) and used motor oil (61.5±4.2%). As for the E24 values of the two *Pseudomonas* sps against the two vegetable oil (soybean oil and sunflower oil), the results (Table 3) show that the bacterial strains were able to produce more than 50% E24 values. Lima *et al* (2011) reported that an emulsification character is considered stable if its E24 correspond to 50%. Anyanwu *et al* (2011) reported that the ability of a biosurfactant to emulsify hydracrbon- water mixture has demonstrated to enhance the biodegradation of the hydrocarbons and is potentially useful in enhanced oil recovery. Gnanamani *et al* (2010) suggested that higher E24 more than 50% give the biosurfactant potential application.

As for the stability of the biosurfactants produced by the two *Pseudomonas* spp (EM1 and EM19) in the presence of different concentrations of Na Cl. The result (Fig 3) show that the activity of the two biosurfactants (as measured by the ODA method) differ according to the different concentrations Na Cl (25% w/v). Biosurfactant produced by *Pseudomonas aeruginosa* (EM1) was more active than that produced by *Pseudomonas* sp (EM19), it showed 75-114 ODA cm^2 at 25-5% NaCl, while biosurfactant produced by *Pseudomonas* sp (EM19) showed 60-82 ODA cm^2 at 25-5% NaCl. These results confirm the stability of the two biosurfactants at wide range of NaCl concentrations.

The results of the stability of the produced biosurfactants at different pH values show variation in the activity of the biosurfactants with the variation of pH values (2-12 pH) as indicated by the variation of the ODA cm^2 values (Fig.4). Optimum activities of the two produced biosurfactants were achieved in pH 7-8. The results also showed that at alkaline pH values (9-12) the biosurfactants were more active than the acidic pH values (2-4).

Results of the thermostability of the two biosurfactants showed that their activities (as measured by the ODA method) were unaffected at wide range of temperature (0-121°C) even after heating at the autoclave temperature (121°C for 30 min). This means that they maintained their full activities at wide range of temperature.

The thermostability character gives the biosurfactant a potential use in food, cosmetics and pharmaceutical industries, where heating to achieve sterility is required (Abouseoud *et al*, 2008). This character also increases the potential application of the biosurfactants in conditions where high temperature prevail as in biological enhanced oil recovery (Khopade *et al*, 2012). The application of the two biosurfactants produced by the two *Pseudomonas* spp. may be useful for the enhancement of bioremediation of contaminated sites; cleaning oil storage tanks enhanced microbial oil recovery from reservoirs and recovery of oil from oily sludge.

The above results show that the biosurfactant produced by *Pseudomonas aeruginosa* (EM1) was more active than that produced by *Pseudomonas* spp (E19).

Accordingly, *Pseudomonas aeruginosa* was selected and was grown in ISM medium supplemented with waste frying oil, and incubated at 30°C on a rotary shaker operated at 140 rpm. After 48h incubation period the cell free culture medium containing the biosurfactant (supernatant) was sterilized and applied for enhancing the biodegradation of the spent motor oil during the bioremediation of the polluted soil.

Pacwa-Plociniczak *et al* (2011) reported that the cell free culture broth (supernatant) containing the biosurfactant can be applied directly to the contaminated site, without necessary characterization of the chemical structure of the biosurfactant.

The above authors also reported that biosurfactants are very stable and effective when they are in the supernatant.

Results of the biodegradation of the spent motor oil pollutant in this type of desert soil under the influence of the addition of biosurfactant alone or biosurfactant – NP combination (Table 4, Fig.5) could be summarized in the following points:

- At the begining of the experiment (0- time), total pollutant was 2.7% (w/w).
- Polluted soil without any treatment (The control) at the end of 70 days showed 35±4.6% biodegradation of the spent motor oil
- Addition of the uninoculated mediums (treatment 4) increased the biodegradation from 35% to reach 42.4±4.0%
- Addition of biosurfactant alone (treatment 1) increased the biodegradation from 42.4 ±4.0 to 68.5 ±3.2%.
- In the presence of NP alone (treatment 2), the biodegradation was 65.0 ± 4.6%. Statistically no significant variation was found between the data obtained in presence of biosurfactants alone and in presence of NP fertilizer (Table 5).
- On the other hand addition of biosurfactant + NP combination (treatment 3) resulted in higher biodegradation of 71.3±5.2% No significant variation between the loss percentage of oil in presence of biosurfactants alone and bisurfactant- NP fertilizer combination. On the otherhand a significant variation was found between the results in

presence of NP fertilizer (treatment 2) and biosurfactant - NP fertilizer combination.

From these results it can be seen that addition of biosurfactant alone had significant spent motor oil biodegradation with little difference compared to the addition of biosurfactant + NP fertilizer combination. This means that biosurfactant alone was able to enhance the biodegradation process without the combination with fertilizers.

Thavasi *et al* (2011) reported that biosurfactants alone are capable of promoting biodegradation to a large extend without added fertilizers, which will reduce the cost of bioremediation process and minimize the dilution or wash away problem encountered when water soluble fertilizers are used during bioremediation of aquatic environment Maki *et al* (2003) reported that fertilizers only stimulate the early stage degradation rate of the oil, and that the final degradation efficiency with fertilizer were not significantly differ from those where no fertilizers were used i.e. with biosurfactant alone.

The presence of biosurfacants may lead to the increase of biodegradation efficiency, in this case, the biosurfactant molecules act as mediators, which increase the mass transfer rate by making the hydrophobic pollutant more bioavailable for microorganisms (Inakallu *et al*, 2004; Whang *et al*, 2009). Cameotra and Singh (2008) found that 73% of the oil pollutants were removed when biosurfactant nutrients were used, while 63% only of the pollutant was biodegraded in presence of nutrients only. Cameotra and Singh (2008) studied the effect of crude biosurfacant and nutrient amendment on the biodegradation of oil sludge of different origins carried out by a mixed culture. Upon addition of the biosurfactant + nutrients, 98% of the oil was biodegraded in 8 weeks, where 52% only was degraded in the absence of additives.

Silva *et al* (2014) reported that biosurfactants play an important role in bioremediation processes due to their efficacy as dispersion and remediation agents as well as their environmentally friendly characteristics such as low toxicity and high biodegradability.

Marchant and Banat (2012) reported that biosurfacatnts have applications in different industrial processes as well as possible novel uses in the future and are expected to become known as multifunctional materials of the 21st century. Sobrinho *et al* (2013) reported that the major market for biosurfactants is the petroleum industry in which these compounds can be used in the clean-up of oil spills, the removal of oil residues from storage tanks, microbial enhanced oil recovery and the bioremediation of soil and water.

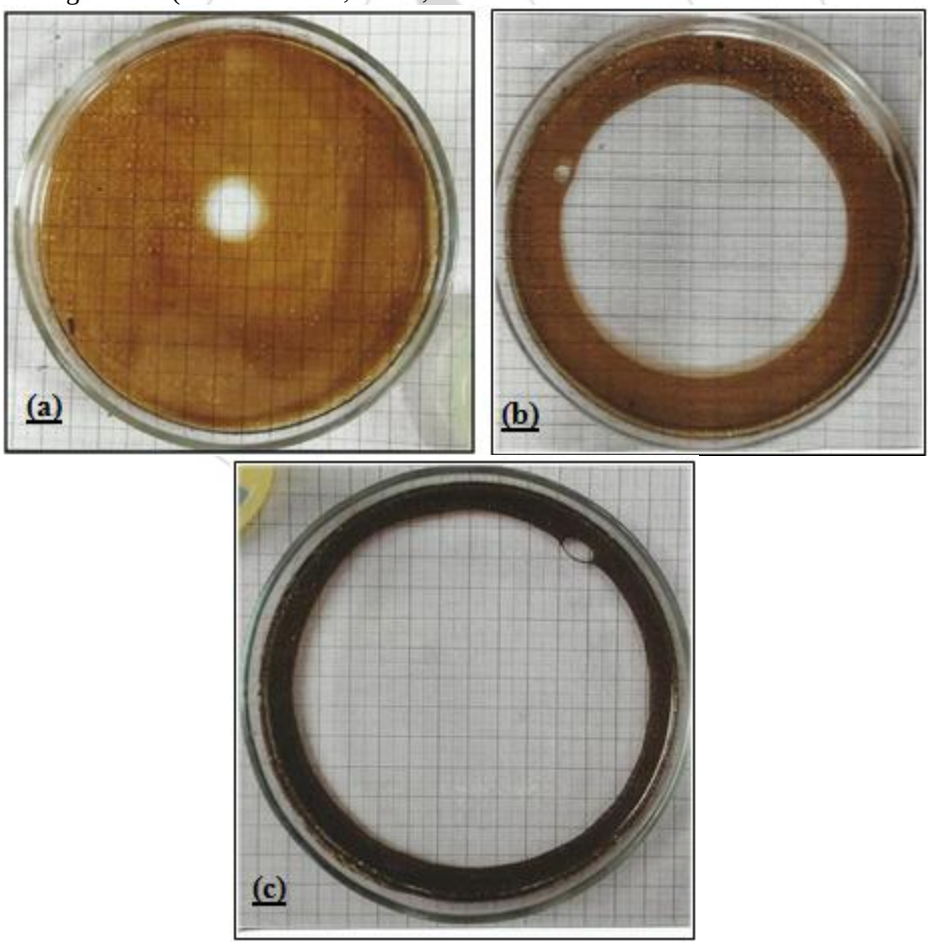


Figure 1: Photomicrographs showing the activity of the biosurfactants produced by the different strains when grown on ISM medium with waste frying oil as measured by ODA
a. *Corynebacterium* sp (EM14).
b. *Pseudomonas* sp.(EM19)
c. *P.aeruginosa* (EM1)).

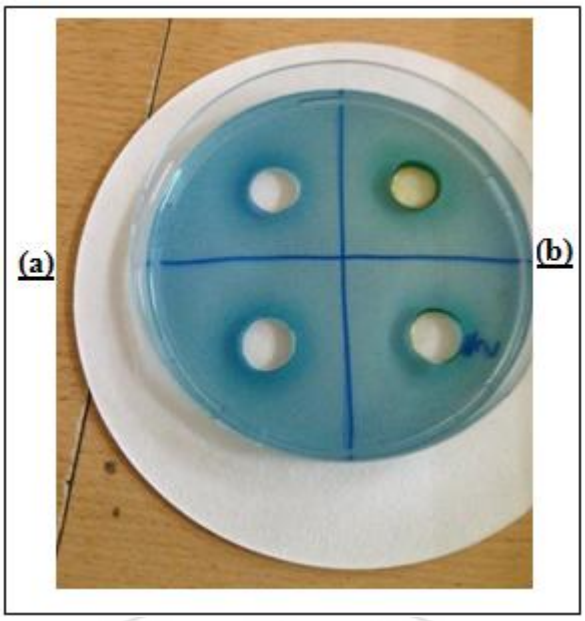
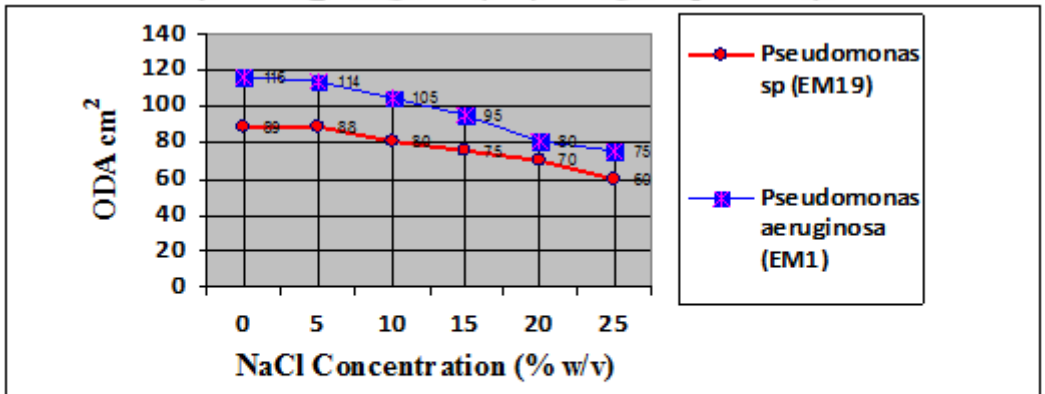
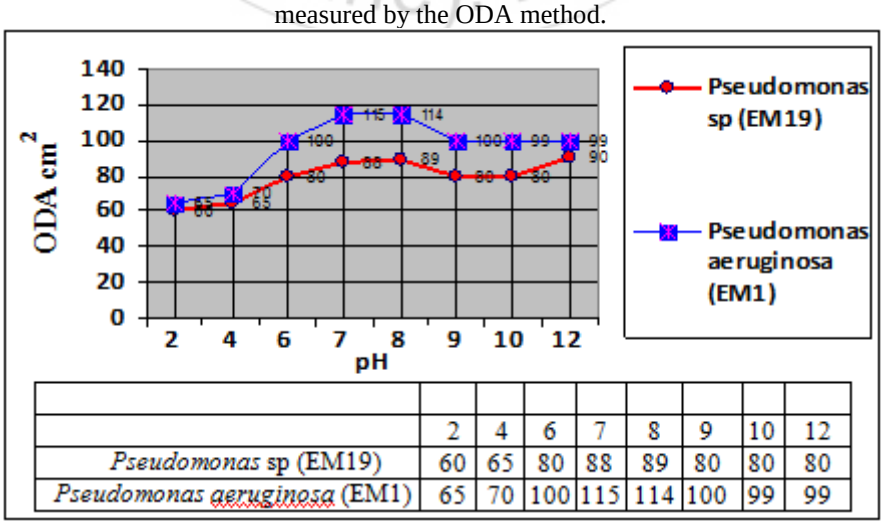


Figure 2: Photomicrographs showing
a. CTAB positive *P. aeruginosa* (EM1) showing bluish colour around each well.
b. CTAB Positive *Bacillus* sp (EM10) showing greenish-blue colour around each well.



	NaCl Concentration (%w/v)					
	0	5	10	15	20	25
Pseudomonas sp (EM19)	89	88	80	75	70	60
Pseudomonas aeruginosa (EM1)	116	114	105	95	80	75

Figure 3: Effect of different concentration of NaCl on the activities of biosurfanctants produced by the two bacterial strains as measured by the ODA method.



	2	4	6	7	8	9	10	12
Pseudomonas sp (EM19)	60	65	80	88	89	80	80	80
Pseudomonas aeruginosa (EM1)	65	70	100	115	114	100	99	99

Figure 4: Effect of different pH values on the activities of biosurfanctants produced by the two bacterial strains as measured by the ODA method.

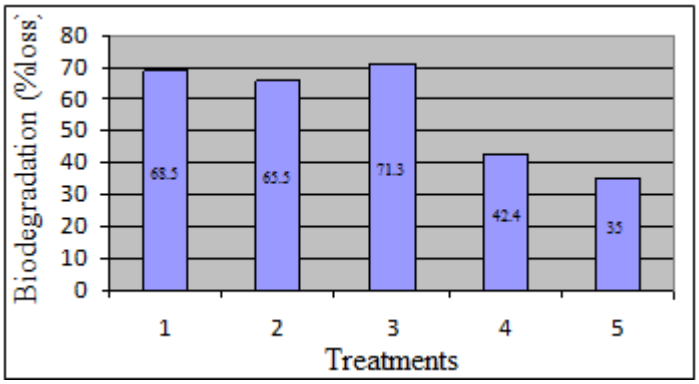


Figure 5: Biodegradation of THC of used motor oil when different treatments were used

Table 2 : Activities of the biosurfactants produced by the six bacterial strains as measured by the ODA test, when grown on ISM medium supplemented by 2% (w/v) of the following : glucose (G), Sucrose (S), Soybean oil (SB), sunflower oil (SF) and waste frying oil (WF)± = standard deviation, n = 3

Bacterial strains	ODA cm^2				
	G	S	SB	SF	WF
Arthrobacter Sp (EM2)	8.1 ± 1.4	3.5± 0.5	-	1.1±0.1	4.0 ± 0.3
Bacillus subtilis (EM6)	13.9± 1.8	31.2± 2.8	-	-	0.9± 0.1
Bacillus sp (EM10)	17.0± 1.6	20.9 ± 1.8	-	-	-
Corynebacterium sp (EM14)	8.1 ±1.3	3.3± 0.1	1.0±0.0	-	3.14± 0.0
Pseudomonas aeruginosa (EM1)	18.9±1.1	7.9±0.9	25.1±2.0	21.7±1.2	118.8±82
Pseudomonas sp (EM19)	8.8±1.1	2.2±0.4	26.8±1.7	24.7±1.9	89.0±3.6

Table 3: Emulsification index (E24) of the biosurfactants produced by *Pseudomonas aeruginosa* (EM1) and *Pseudomonas* sp (EM19) when different hydrocarbons and vegetable oils were used

Oils	E24	
	Pseudomonas aeruginosa	Pseudomnas sp
Petroleum oil	66.5 ±2.8	62.5 ± 3.5
Kerosene	56.5 ± 2.1	51.5 ± 4.2
Petroleum oil – Kerosene (1: 1)	62.5 ± 2.8	56.0 ± 2.8
Used Motor Oil	57.0 ±4.2	61.0 ± 4.2
Soybean oil	53.5 ± 3.5	51.5 ± 3.2
Sunflower oil	53.0 ± 2.2	51.5 ± 2.1

Table 4: Total hydrocarbons (THC) biodegradation (%loss) of motor oil by using different treatments after 70 days period.
At 0 time THC were 2.7% (w/w)

Treatment	*Biodegradation of THC (% loss)*
1. Biosurfactant	68.5 ± 3.2
2. NP nutrients	65.0 ± 4.6
3. NP + biosurfactant	71,3 ± 5.2
4. Medium free	42.4 ± 4.0
5. Control	35.0 ± 4.6

Table 5: Significant differences ($p < 0.05$) among the means between the different treatment. S = significant, NS= Non significant

Treatment	Treatment				
	BS	NP	BS+ NP	M	Cont
1. Biosurfactant (Bs)	-	NS	NS	S	S
2. NP Fertilizer (NP)	NS	-	S	S	S
3. BS + NP	NS	S	-	S	S
4. Medium free (M)	S	S	S	-	S
5. Control	S	S	S	S	-

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