

Full Length Research Paper

Application of the biosurfactants produced by *Bacillus* spp. (SH 20 and SH 26) and *Pseudomonas aeruginosa* SH 29 isolated from the rhizosphere soil of an Egyptian salt marsh plant for the cleaning of oil - contaminated vessels and enhancing the biodegradation of oily sludge

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During the screening for biosurfactant-producing bacteria isolated from the rhizosphere soil of an Egyptian salt marsh plant, three bacterial strains (*Bacillus* spp. SH 20 and SH 26, and *Pseudomonas aeruginosa* SH 29) were most active strains. Biosurfactants produced by the three bacterial strains were stable at wide range of pH (1 to 14), wide range of temperature (0 to 121°C) and salinity (5 to 15% NaCl). The two *Bacillus* species were able to produce high E24 values for petroleum oil (75 to 84.4%), while *P. aeruginosa* was able to produce E24 value of 62%. Accordingly, the sterilized broth culture (supernatants) containing the biosurfactants produced by the two *Bacillus* spp. were used for enhancing the bioremediation of oily sludge-contaminated soil. On the other hand, the sterilized supernatant of *P. aeruginosa* was applied for cleaning oil-contaminated vessels. The results show that addition of the supernatant of *Bacillus* sp. SH 26 stimulated the biodegradation of the oily sludge (34.0% w/w); this is in contrast to biodegradation of 28.0 and 22.2% of the oil in presence of uninoculated medium and the supernatant of *Bacillus* sp. SH 20. When the contaminated soil was treated with the mixture of the two supernatants of the two *Bacillus* spp., no biodegradation of the oil above 26.3% was observed. This may indicate inhibitory effect on some of the oil degraders present in this system, due to the presence of the biosurfactant produced by *Bacillus* sp. SH 20. When the phytogenic biosurfactant was applied as a comparison, it was of interest to find that this biosurfactant was able to degrade 53.8% of the oil, and this represent excellent candidate for enhancing bioremediation of oil contaminated sites. This was followed by the microbial biosurfactant (34%) produced by *Bacillus* sp. SH 26. The results of cleaning oil-contaminated vessels by applying the supernatant of *P. aeruginosa* SH 29 show that after 15 min of the addition of the supernatant, the oil was recovered from the bottom and walls of the vessels and floated on the supernatant as a distinct phase. This indicates that the sterilized supernatant of *P. aeruginosa* SH 29 can be used directly for cleaning oil storage tanks and other vessels used for transportation and storage of crude petroleum oil.

Key words: Biosurfactants, *Pseudomonas aeruginosa*, *Bacillus* sp., bioremediation, cleaning oil-contaminated vessels.

INTRODUCTION

The petroleum industry generates large amounts of solid and semisolid wastes known as oily sludge. The composition of this sludge is variable due to the variation and quality of crude oil. Oily sludge includes oil, coarse solids and water. During petroleum oil storage, large amounts of sludge are developed and accumulated at the bottom of the storage tanks causing reduction in the capacity of the tanks and corrosion may be developed. For these reasons, it is necessary to remove these wastes through periodical cleaning of the tanks. The removal and disposal of the sludge from the bottom of the tanks by using traditional methods is expensive and time consuming process that needs additional processes, specialized equipments and skilled personnel. As an alternative, removal of oil sludge from storage tanks may be carried out by the application of biosurfactants which reduce viscosity and may develop emulsification that are able to facilitate sludge removal from tank, this making sludge pumping easier and causes crude oil recovery after breaking the emulsion. This method is simple, cost-effective and less hazardous to persons involved in the cleaning process, at the same time this strategy is environmentally acceptable and leading to less sludge disposal in environment (Banat, 1995; Lima et al., 2011).

Most of the oil sludge generated during oil production processes is piled outdoors without any treatment causing serious environmental problems because it contains toxic materials such as Polycyclic Aromatic Hydrocarbons (PAHs), many of which are carcinogenic and/or mutagenic. Therefore, the oil sludge should be treated to prevent harm to the environment. Although, burning the sludge is a simple and easy adaptable, it has undesirable hazard in air pollution. An alternative technique is the biodegradation of the oily sludge which is safe, efficient, cost - effective method and versatile alternative to physicochemical treatment (Li, 2006). On the other hand, the major factor limiting the biodegradation of hydrocarbons is their low availability to the microbial cells of the oil - degraders. Since bacteria are known to degrade pollutants only when they are dissolved in water, addition of biosurfactants can enhance solubilization of the pollutants and increase their solubility which in turn improves bioavailability for biodegradation (Cameotra and Makkar, 2010). Various experiments have been conducted on the effects of biosurfactants on enhancing the availability and the biodegradation of hydrocarbons. Mulligan and Gibbs (2004) reported that biosurfactants have the ability to enhance petroleum hydrocarbon (PHC) biodegradation by two mechanisms:

- i) Increasing the bioavailability of PHC, for microorganisms.
- ii) Interaction with the cell surface of the hydrocarbonde-

graders and increase the hydrophobicity of the surface, allowing the hydrophobic hydrocarbons to associate more easily to the bacterial cell.

Helmy et al. (2010) studied the application of biosurfactants in the recovery and enhancing the biodegradation of oil sludge. They found that addition of biosurfactant in presence of a consortium of oil - degraders increased the biodegradation rate to 70% after 70 days. This is in contrast to only 55% in the absence of the biosurfactant.

The aim of the present work was to investigate the effect of the broth culture, supernatants containing the biosurfactant produced by *Pseudomonas aeruginosa* for cleaning oil - contaminated vessels, and those produced by *Bacillus* spp. SH 20 and SH 26 for enhancing biodegradation of oil found in the oily sludge.

MATERIALS AND METHODS

Bacterial strains

The three bacterial strains used in this study were previously isolated from the rhizosphere soil of an Egyptian salt marsh plant (*Urospermum picroides*). The three strains were found to produce active biosurfactant, and were identified as *Pseudomonas aeruginosa* SH 20 and *Bacillus* spp. SH 20 and SH 26.

Production and detection of the biosurfactant activities

Each of the aforementioned three bacterial strains was grown on two different media (nutrient broth- NB and inorganic salt medium - ISM). The ISM consisted from 1 g/l NaNO₃, 0.5 NaCl₂, 2g/l K₂HPO₄, 1g/l KH₂PO₄, 0.5g/l MgSO₄.7H₂O, 0.01g/l FeCl₃ and 0.5g/l yeast extract. Each of the two media was supplemented with molasses or waste frying oil (2% w/w) and adjusted at pH 7. The incubation periods were 48 h for NB and seven days for ISM at 30°C on a shaker operated at 150 rpm. At the end of the incubation periods, the cultures were centrifuged at 6000 rpm for 20 min for the removal of the cells. The cell free broth cultures (supernatants) were autoclaved at 121°C for 15 min and tested for the activity of the produced biosurfactants using oil displacement area (ODA) as described by Priya and Usharani (2009) and Techaoei et al. (2011) and the emulsification activities (E24) were estimated based on Tabatabaee et al. (2005) and Techaoei et al. (2011).

Stability of the biosurfactants

Thermostability, effect of salinity and pH were carried out according to Haddad et al. (2009). Application of the biosurfactant produced by the bacterial strains for cleaning oil-contaminated vessels. A preliminary experiment was carried out using a glass funnel (250 ml), the arm of the funnel was closed by rubber stopper, filled with crude oil, left for 24 h and then emptied from the oil. Residual oil was remained on the wall surfaces of the funnel. Sterilized cell free culture broth (supernatant) containing the biosurfactants produced by *P. aeruginosa* SH 29 and *Bacillus* spp. SH 20 and SH 26, each

Table 1. Different treatments of the sludge polluted soil samples.

Treatment	Amendment						
	NH ₄ NO ₃	K ₂ HPO ₄	ISM (medium)	BS 20SH	BS 26SH	Mix 20+26	PBS
1	-	-	-	-	-	-	-
2	+	+	-	-	-	-	-
3	+	+	+	-	-	-	-
4	+	+	-	+	-	-	-
5	+	+	-	-	+	-	-
6	+	+	-	-	-	+	-
7	+	+	-	-	-	-	+

Table 2. Significant differences (P <0.05) among the means between the different treatments.

Treatment	Treatment						
	C	NP	M	BS 20	BS 26	BS20 + BS26	PBS
C		S	S	S	S	S	S
NP	S		S	NS	S	NS	S
M	S	S		S	S	NS	S
BS 20	S	NS	S		S	NS	S
BS 26	S	S	S	S		S	S
BS 20 + BS 26	S	NS	NS	NS	S		S
PBS	S	S	S	S	S	S	

S = Significant. NS = non significant.

was gently applied as small drops on the wall surfaces and left to seep slowly downward. As a control water was applied. After few minutes the results were observed and reported.

The most active strain in the preliminary test was *P. aeruginosa* SH 29. It was selected and applied for the cleaning of oil - contaminated vessels as follows:

i) A microcosm test was designed consisting of 2 beakers (250 ml) and 3 large tubes to behave as oil tanks and pipes. The beakers and the tubes were filled with crude petroleum oil and left stagnant. After 24 h, the microcosms were emptied and their walls and bottoms were left contaminated with the residual oil.

A known volume of the sterilized supernatant of *P. aeruginosa* SH 29 was gently introduced to one of the beakers and one of the tubes covering the bottoms and part of the walls. The same volume of water was used as control. A tube without water and without supernatant was left for comparison. After 3 to 15 min, the results were observed and reported. Application of the microbial biosurfactants (produced by *Bacillus* spp. SH 20 and SH 26) and a phytogetic biosurfactant for the biodegradation of oily sludge contaminated soil. Oily sludge samples were obtained from sludge generated during the periodical cleaning of oil storage tanks at Abu-Dhabi were kindly provided. Analysis of this sludge in the Laboratory of the Faculty of Biotechnology-MSA University revealed the presence of 18 to 20% crude petroleum oil. For successful bioremediation process of contaminated soil, the concentration of oil must be reduced to 4 to 5%. Accordingly, non-polluted desert soil sample was collected and thoroughly mixed with the oil sludge so as to give 4 to 5% w/w oil. The polluted soil was treated as follows:

i) Soil microcosm test was designed to include 7 treatments in duplicates. Each consisting of 500 ml beaker containing 100 g of the sludge polluted soil and treated as shown in Table 1.

ii) The fertilizer used was NH₄NO₃ (100 mg/100 g soil) and K₂HPO₄ (50 mg/100 g soil). Biosurfactants were added as 10 ml of supernatant of each of 20 BS or 26 BS. As a control, 10 ml of the un-inoculated medium (ISM) was added. For comparison, a phytogetic surfactant of plant origin (soya bean seed meal) was used in a concentration of 1 g/100 g soil.

Statistical analysis

All values were averages of three readings, and have been shown as mean ± SD. For determining significance of differences among the means, data were analyzed for significant differences (P<0.05) between treatments (Table 2).

RESULTS and DISCUSSION

P. aeruginosa SH 29 was able to produce active biosurfactant when grown on inorganic salt medium (ISM) and nutrient broth medium (NB), supplemented with waste frying oil (2% w/w), a cheap and easily available substrate. The two *Bacillus* spp. were able to produce active biosurfactant when grown on ISM medium supplemented with 2% (w/w) molasses. All of the produced biosurfactants were stable at wide range of

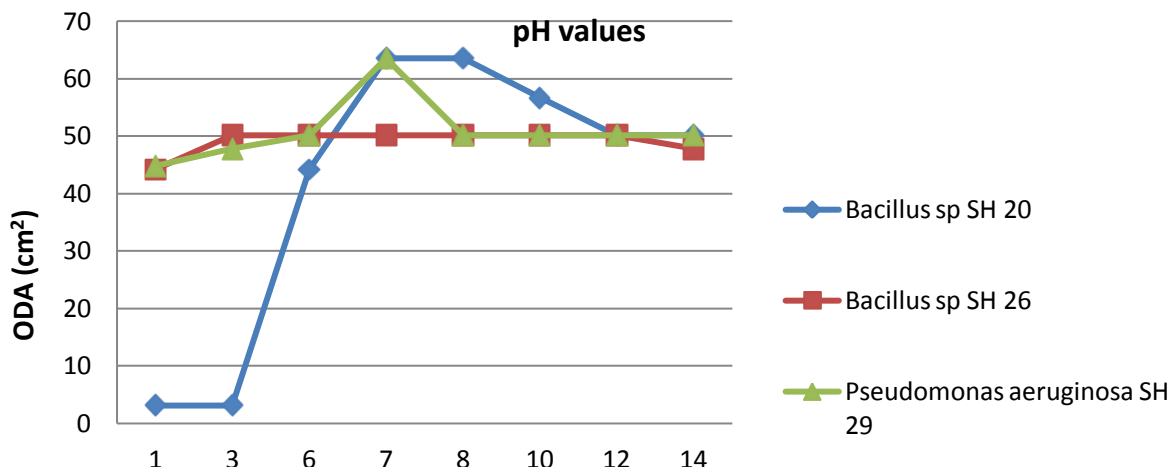


Figure 1. Effect of different pH values on the activities of the biosurfactants produced by the three bacterial strains, as measured by the ODA method.

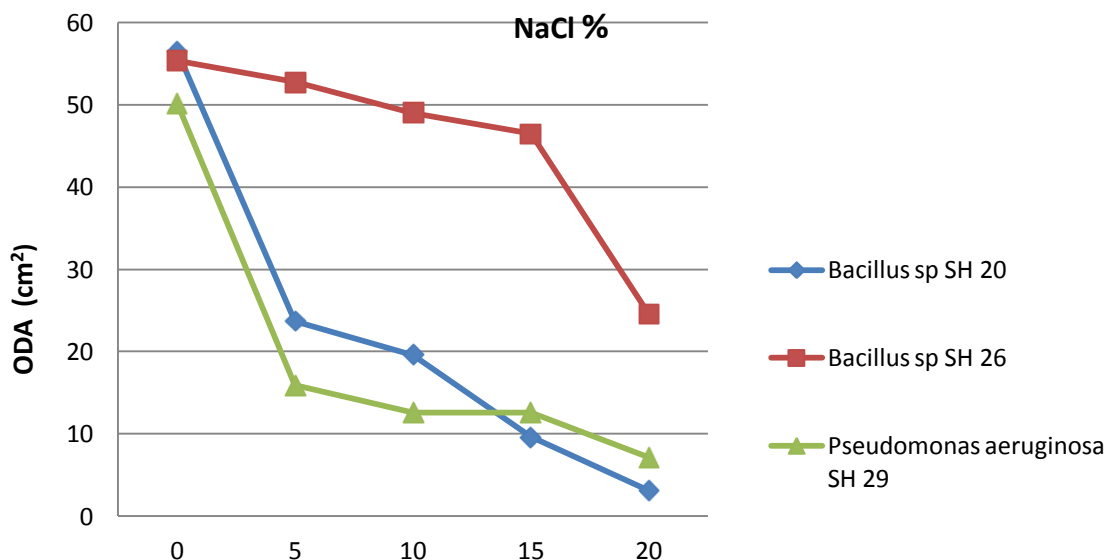


Figure 2. Effect of different concentrations of NaCl on the activities of the biosurfactants produced by the three bacterial strains as measured by the ODA method.

temperature (0 to 121°C) and pH values 1 to 14 (Figure 1). *Bacillus* sp. SH 26 produced biosurfactant of 52.8 ± 3.3 , 49 ± 2.8 , 46.5 ± 2.1 and 24.6 ± 2.3 ODA cm² activities in presence of 5, 10, 15 and 20% (w/v) NaCl respectively (Figure 2), while *Bacillus* sp. SH 20 and *P. aeruginosa* SH 29 showed less activity (23.7 ± 2.4 to 3.1 ± 0.7 and 15.9 ± 2 to 7.1 ± 0.6 ODA cm², respectively). The emulsification activity as measured by the E24 index showed that *Bacillus* sp. SH 20 was able to produce higher E24 values for petroleum oil (84.4%) and kerosene (55.4%). This was followed by *Bacillus* sp. SH 26 which gave E24 of 75% for petroleum oil and 54.4% for kerosene (Table 3). On the other hand, *P. aeruginosa*

SH 29 also had emulsification property (E24) for petroleum oil (62%) and kerosene (57.1%). Willumsen and Kosaric (1997) and Lima et al. (2011) proposed that an emulsion is considered stable if its E24 corresponds to 50% or more.

Accordingly, the three bacterial strains were grown in the appropriate media, temperatures and incubation period of each strain for the production of the biosurfactants (as described in materials and methods). After the incubation period, the cell free broth culture containing the biosurfactant (supernatants) of *P. aeruginosa* SH 29 was sterilized and applied for the cleaning of oil-contaminated vessels, while the sterilized

Table 3. Emulsification activities (E24%) of the three bacterial strains when hydrocarbon oils were used.

Strain	Petroleum oil	Kerosene	Paraffin oil
<i>Bacillus</i> SH 20	84.4	55.3	48.8
<i>Bacillus</i> SH 26	75	54.4	53.6
<i>Pseudomonas aeruginosa</i> SH 29	62.2	57.1	42.9

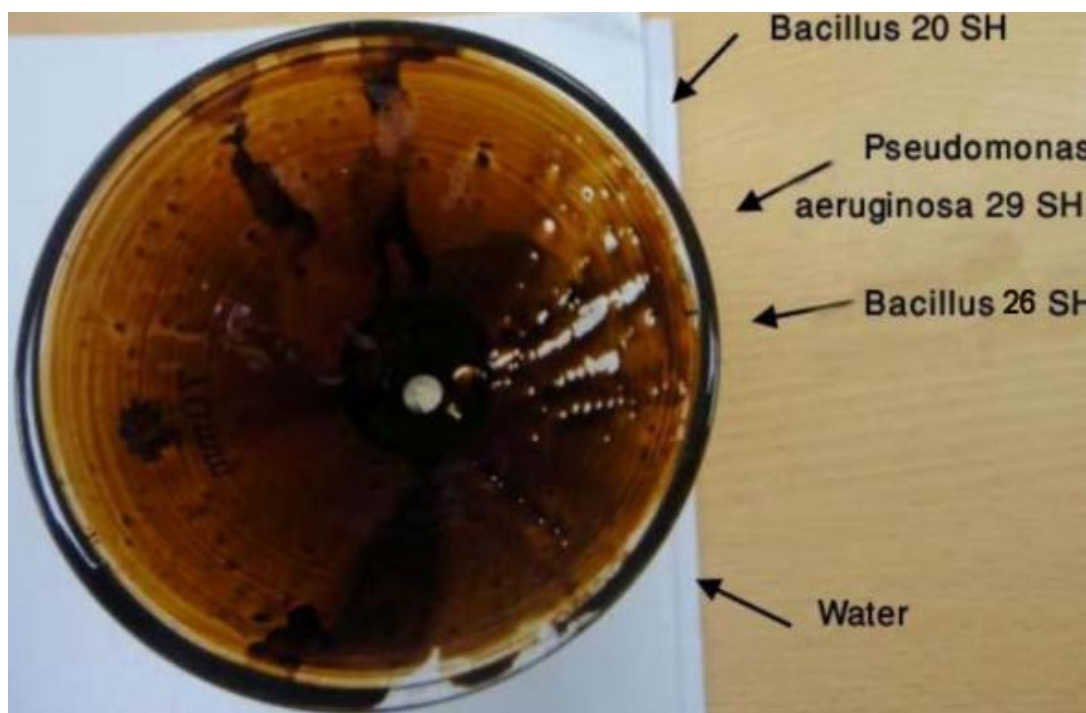


Figure 3. A photograph showing the effect of biosurfactants produced by *Bacillus* sp. SH 20, *Bacillus* sp. SH 26 and *P. aeruginosa* SH 29 on cleaning the wall surfaces of oil - contaminated funnel (a preliminary experiment).

supernatants of the two *Bacillus* spp. were applied for enhancing the biodegradation of oil sludge, this is in addition to a phyto-genic biosurfactant as a comparison. For cleaning oil - contaminated vessels, a preliminary experiment was carried out using glass funnel that was flooded with crude petroleum oil, left for 24 h and then emptied (as described in materials and methods). When the supernatants of the bacterial strains were applied to the surface wall of the funnel, the results show that within few seconds after the application of the three biosurfactant, small clear circles free from oil were developed in vertical row (Figure 3). From these results, it was observed that the supernatant of *P. aeruginosa* SH 20 was able to develop the wide area of clear circles. Accordingly, this bacterial strain was selected and applied for cleaning of the oil contaminated vessels as described in materials and methods. The results after 15 min of the supernatant addition, the oil was recovered

from the bottoms of the contaminated beaker and floated on the top of the supernatant (Figure 4B). When water was used, negligible result was observed (Figure 4A). When the tubes were used as microcosm, the results showed that after 1 to 3 min of supernatant addition, large amount of oil was removed from the bottoms and the walls of the contaminated tube and floated over the supernatant (Figure 5a). After 15 min approximately, more than 97% of the oil was recovered and floated as distinct phase over the supernatant (Figure 5d to e).

The aforementioned results clearly show that cell free culture broth (supernatant) of *P. aeruginosa* SH 29 represents a good candidate for cleaning and recovery of oil remaining in oil - contaminated vessels and tanks. Such a clean process represents economically less hazardous process as compared to the confidential (phy-sicochemical) methods. The results also confirm that the cell free culture broth (supernatant) of *P. aeruginosa* con-

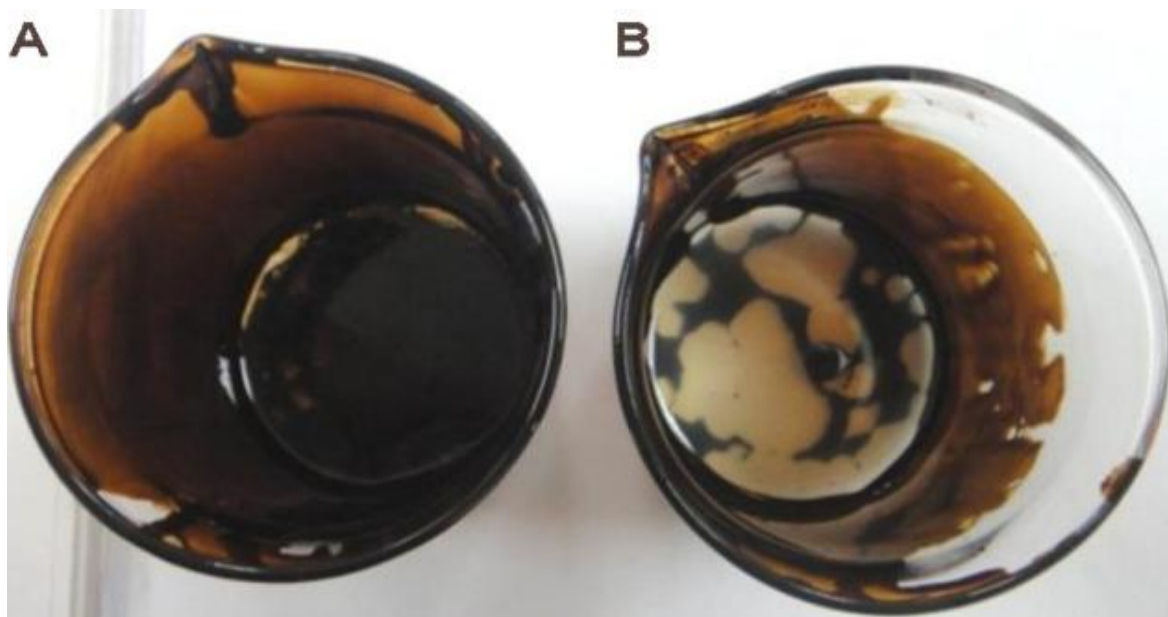


Figure 4. Photographs (top view) showing the effect of the application of a biosurfactant produced by *P. aeruginosa* SH 29 on cleaning oil - contaminated vessels (B) as compared to the effect of water alone (A).

containing the biosurfactant can be used directly to recover oil remaining in the oil - contaminated vessels, oil tanks, storage oil tanks, tank cars, pipelines and other containers used to transport or store crude oil. During petroleum oil storage, large amount of oily sludge are developed at the bottom of the storage tanks, causing reduction of the tank capacity. Lima et al. (2011) reported that reduction in the capacity of storage tanks and development of corrosion makes it necessary to dispose this oily sludge through periodic cleaning of the tanks. Traditionally, methods may be used for cleaning such tanks and recovery of the sludge from the tank bottoms. These methods are expensive, time consuming and need specialized and skilled personnel. As an alternative, removal of oil sludge and cleaning the storage tanks may be carried out using biosurfactants that are able to reduce the interfacial tension and the viscosity in order to facilitate the oily sludge removal from tanks. Chamanrokh et al. (2010) found that washing the oil - contaminated vessels with 10 to 20 mg/ml of biosurfactant solution readily forms an oil-water emulsion. Banat (1995) cleaned oil storage tanks and recovered oil from the sludge by using a biosurfactant produced by bacterial strains.

In the present work, when the supernatant was applied for the cleaning process, the oil was mobilized and formed no suspension; this is through replacement technique (that is, release of oil droplets from the system due to reduction in the interfacial tension). As a result, the oil was floated and remained as distinct phase. Mobilization mechanism includes reduction of surface and interfacial tension, reduction of the capillary force

and reduction of the contact angle. Biosurfactant monomers may accumulate at the solid-oil interface and reduce the capillary force holding oil and solid due to reduction in the interfacial tension. Oil will undergo displacement if the interfacial tension between oil-solid is highly reduced to overcome the capillary force (Urum, 2004). Results of the effects of the microbial biosurfactants (20 BS and 26 BS) produced by *Bacillus* spp. (SH 20 and SH 26) and a phyto-genic biosurfactant (PBS) on the biodegradation of oily sludge are found in Table 4. The results after 40 days incubation period could be summarized in the following points:

- i) In the control experiment (C) without any treatment, the biodegradation of the oil was $17.0 \pm 3.6\%$ (w/w).
- ii) Addition of NP fertilizers only (treatment 2) significantly increased the biodegradation of the oil to $23.6 \pm 0.6\%$, and the increasing factor was (F) 1.4.
- iii) Addition of 10 ml of ISM supplemented with molasses and without bacterial inoculation increased the biodegradation to $28.0 \pm 2.8\%$, and (F) was 1.6
- iv) When 10 ml of ISM medium containing the biosurfactant (26 BS) produced by *Bacillus* sp. SH 26 was added, the biodegradation rate significantly increased to reach $34.0 \pm 1.4\%$ and increasing factor (F) was 2.0. This value represents a high biodegradation activity compared with the short period of incubation (40 days). It is well established that bioremediation process needs several months or years (Kosaric, 2001).

Pacwa-Plociniczak et al. (2011) reported that the effective microbiological method in bioremediation of oil-polluted

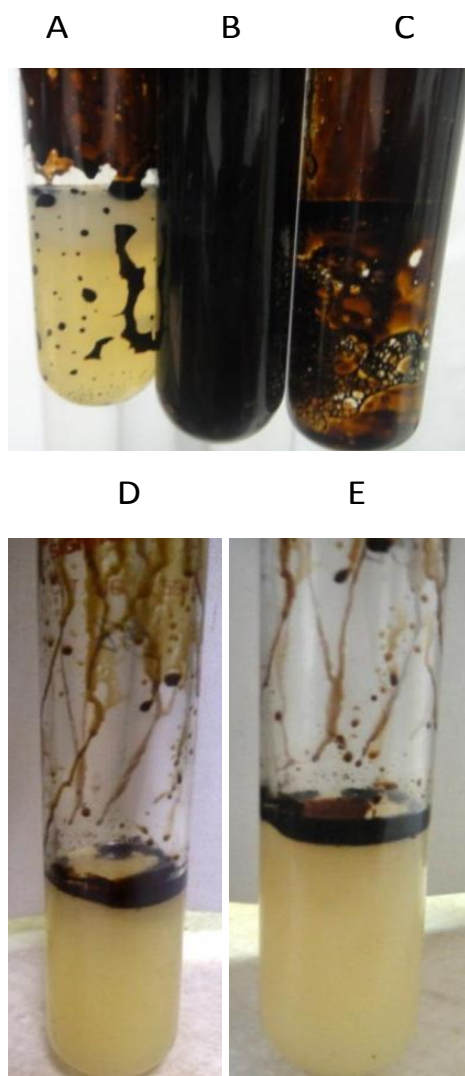


Figure 5. Photographs showing the effect of the application of a biosurfactant produced by *P. aeruginosa* SH29 on cleaning oil - contaminated tubes. Note the floating of oil on the top of the biosurfactant solution (d to e), (b) tube containing no biosurfactant and no water and (c) tube containing water only.

Table 4. Effects of the microbial biosurfactants (BS 20 and BS 26) produced by *Bacillus* spp. SH 20 and SH 26 on the biodegradation (loss % w/w) of the oil of the sludge, as compared to the effect of the phytogetic surfactant (PBS). Increasing factors (F) (data of the different treatment relative to the control- C) are also given.

Treatment	Biodegradation (%loss)	F
Control (C)	17.8 ± 3.6	-
N, P nutrients (NP)	23.6 ± 0.6 ^a	1.4
Culture medium only (M)	28.0 ± 0.0 ^b	1.6
Broth culture medium containing BS 20	22.2 ± 0.0 ^a	1.3
Broth culture medium containing BS 26	34.0 ± 1.4	2.0
Mixture of both culture media containing BS 20 + BS 26	26.3 ± 7.5 ^b	1.5
Phytogetic biosurfactant (PBS)	53.8 ± 3.6	3.2

(±) = standard deviation, (n) = 3. Averages followed by the same letter are not significantly different.

polluted sites is the use of biosurfactants-producing bacteria without necessary characterization of the chemical structure of the biosurfactants. The cell free culture broth containing the biosurfactant can be applied directly or diluting it appropriately to the contaminated site.

The other benefit of this approach is that bio-surfactants are very stable and effective in the culture medium that was used for their synthesis. Das and Mukherjee (2007) inoculated an oil-polluted soil sample with mineral salt medium inoculated with *Bacillus subtilis* or *P. aeruginosa* M and NM strains.

As a control, a polluted soil sample was inoculated with un-inoculated medium. They found that the oil contents in soil inoculated with *P. aeruginosa* M and NM consortium and *B. subtilis* were reduced from 84 to 21 and 39 g/kg soil respectively after 120 days. In the control, the oil was decreased to 83 g/kg. Helmy et al. (2010) studied the application of a biosurfactant for the recovery of oil and enhancing the biodegradation rate. They found that addition of biosurfactant to a mixed culture (consortium) of oil-degrading bacteria increased the biodegradation rate to 70% after 70 days; this is in contrast to only 55% in the absence of biosurfactant. They then suggested that the addition of both oil-degraders and biosurfactant may increase the biodegradation rate.

Cameotra and Makkar (2010) explained that biosurfactants can enhance the solubilization of the hydrocarbon contaminants from the polluted soil which in turn enhances the biodegradation process. These authors explained that there are two mechanisms of surfactant enhanced soil washing, depending on the concentration of the biosurfactant:

- i) Below critical micelle concentration (CMC), the biosurfactant monomers increase the contact angle between soil and oil, this promote the separation of the oil from soil particles, and then displace the oil from the soil.
- ii) Above CMC solubilization of the oil occurs, and pollutants are partitioned from the soil into the hydrophobic core of the micelles of the biosurfactants.

As for the effect of the other microbial biosurfactant (BS 20) produced by *Bacillus* sp. SH 20, it can be seen that the addition of 10 ml ISM medium in which *Bacillus* sp. SH 20 was grown and produced the biosurfactant gave low biodegradation rate ($22.2 \pm 2.5\%$) as compared to BS 26 ($34.0 \pm 1.4\%$) and the un-inoculated ISM medium (28.0 ± 2.6). When the soil was treated with a mixture of the two 20 BS and 26 BS supernatants (treatment 6), no increase in the biodegradation of the oil above 26.3% was observed.

This may indicate an inhibitory effect on some of the oil-degraders present in this system as a result of the presence of the biosurfactant 20 BS. In a preliminary test carried out by Diab and Shereen, it was found that *Bacillus* sp. SH 20 (producing BS 20) and *Pseudomonas*

strain SH 29 were able to produce biosurfactants of antibacterial activities against certain Gram positive bacteria. Cameotra and Makkar (2010) stated that in some cases the inhibition of the biodegradation of pollutants in soil at biosurfactant concentration above CMC was reported, and a variety of factors and mechanisms have been reported for the explanation of the inhibition process. Muthusamy et al. (2008) discussed the therapeutic and biomedical application of bio-surfactant and reported that a rhamnolipid biosurfactant from *P. aeruginosa* in a concentration of 32 mg/ml was active against *E. coli*, *Micrococcus luteus* and *Alkaligenes faecalis*, while in a concentration of 16 mg/ml, it was active against *Serratia marcescens* and *Mycobacterium phlei*. In 8 mg/ml, it was active against *Staphylococcus epidermidis*.

As for the phytogetic biosurfactant, it is of interest to find that this biosurfactant represents excellent candidate for enhancing the biodegradation and removal of higher amount of the oily sludge. It was succeeded to enhance the biodegradation process to reach a removal of $53.8 \pm 3.6\%$ of the oil found in this type of sludge at the end of 40 days. Xu et al. (2011) reported that the European surfactant market in 2004 estimated 2.5 M tons of which 25% were surfactants of plant origin such as saponin, lecithin, soyprotein and soybean oil.

The effects of the phytogetic surfactant soy lecithin on the aerobic biodegradation of polychlorinated biphenyls (PCBs) were studied by Fava and Gioia (2001) and Soeder et al. (1996) who found that in the presence of this biosurfactant, higher availability of biphenyls and cloro-benzoic acid-degrading bacteria, high PCB biodegradation and dechlorination yields were observed. Oleszczuk et al. (2007) found that some phytogetic biosurfactants were able to improve the bioavailability of polycyclic aromatic hydrocarbons (PAHs) in the rhizosphere soil of some plants. Cohen et al. (2004) used the entire plant of water fern *Azola* and seed meal of *Brasica napus* as promising amendments for the bioremediation of contaminated soil. Diab and Sandouka (2012) were the first to use the seed meal of soya bean as a biosurfactant. They found that the application of this cost effective phytogetic biosurfactant in a concentration of 1% (w/w) to a highly oil-polluted desert soil (8% w/w) clearly stimulated the development of high counts of total heterotrophic bacteria and oil-degrading microorganisms which resulted in the enhancement of the biodegradation rate of the pollutant.

The present results show that the cell free culture broth (supernatant) containing the biosurfactant BS 26 produced by *Bacillus* sp. SH 26 may be applied directly to the contaminated site without necessary isolation and characterization of the chemical structure of the biosurfactant. This approach represent a promising, simple and cost effective tool for the treatment of oily sludge generated during the periodical cleaning of oil storage tanks and other petroleum industries. On the

other hand, the phytogetic surfactant used in the present work represented also a promising tool for the detoxification of soil polluted with oily sludge.

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