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Application of Biosurfactant Produced by *Bacillus lichneformis* and Chemical Surfactant in Biodegradation of Crude Oil: Part I

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The biodegradation capacity of crude oil in mineral salt medium by a bacterial strain (*Bacillus lichneformis*) was analyzed. The biosurfactant and synthesized nonionic surfactant were used separately to enhance the biodegradation process. The biodegradation percentage was demonstrated at 50% of microcosms containing biosurfactant and chemical surfactant. The biosurfactant and chemical surfactant were deceased surface tension of the culture media to 38 and 42 mN/m, respectively. The bacterial strain consumed iso-paraffins more than n-paraffins in crude oil samples. Additionally, this bacterial strain could utilize the five and six member rings of polyaromatics compounds which consider being more toxic and carcinogenic for soil and aquatic environment. The results indicated that the bacterial strain has a potential to be applied in bioremediation of petroleum contaminated sites.

Key words: Biosurfactants, chemical surfactant, biodegradation, petroleum crude oil, *Bacillus lichneformis*.

Petroleum hydrocarbons are the most common environmental pollutants and oil spills pose a great hazard to terrestrial and marine ecosystems. Oil pollution may arise either accidentally or operationally whenever oil is produced, transported and stored. Oil spills are a major menace to the environment because they severely damage the surrounding ecosystems[1]. Biodegradation by natural populations of microorganisms is the most basic and the most reliable mechanism by which thousands of xenobiotic pollutants, including crude oil, eliminated from the environment[2]. The effects of environmental conditions on the microbial degradation of hydrocarbons and the effects of hydrocarbon contamination on microbial communities are areas of great interest[3]. Bioremediation is a strategy to utilize biological activities to the greatest extent possible for the rapid elimination of environmental pollutants which contain hydrocarbons and their derivatives. Stimulation of the growth of indigenous microorganisms, biostimulation, is based on the ability of microorganisms to increase their biomass growing on these substrates and degrading them to non-toxic products, such as H_2O and CO_2[4]. Inoculation with foreign oil-degrading bacteria is promising means of accelerating the detoxiûcation of a polluted site with minimal impact on the ecological systems[5]. Polycyclic aromatic hydrocarbons (PAHs) extensively occur as pollutants in soil and water. On the other hand, (PAHs) are important environmental contaminants because of their recalcitrance. These compounds also mean a potential risk to human health, as many of them are carcinogens[6]. Their persistence within the ecosystems is due to their low aqueous

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solubility and high sorption to soil, two features that limit their availability for the degrading microorganisms[7]. The growth of microorganisms on hydrocarbons presents particular problems because hydrocarbons are immiscible in water. Many bacteria are able to emulsify hydrocarbons in solution by producing surface active agents such as biosurfactants that increase the adhesion of cells to the substrate. Biosurfactants reduce the surface tension by accumulating at the interface of immiscible fluids, which leads to increased bioavailability and subsequent biodegradation of the hydrocarbons[8].

One way to increase the solubility of PAHs in crude oil is to apply surfactant approaches (synthetic surfactants or biosurfactants) to enhance biodegradation. The effect of a surfactant on the availability of organic compounds to the indigenous microorganisms can be explained by dispersion of nonaqueous phase liquid (NAPL), leading to reduce the interfacial tension between the aqueous phase and the nonaqueous phase; increased apparent solubility of the pollutant[9]. Also, the addition of surfactants changes the cell hydrophobicity, facilitating the direct contact between cells and NAPLs.

The toxicity of surfactants is dependent on its molecular structure. According to the ionizability of the polar head group, surfactant can be classified into nonionic and ionic surfactants. Ionic surfactants could be further categorized into cationic, anionic and zwitterionic surfactants The negatively charged surface of bacterial cells makes the cells more sensitive to the introduction of charged surfactants, especially positively charged cationic surfactants. Generally, nonionic surfactants are less toxic to microorganisms than ionic surfactants[10]. The surfactant molecules at a concentration (near or above CMC) will form mixed micelles with membrane lipids, which may solubilize cell membranes and lead to the necrosis and lysis of cells. At concentrations below the CMC, when the surfactant molecules cannot form mixed micelles with lipid molecules, the incorporation of surfactant into the cell membrane is enough to impair the barrier function of cell membrane[11, 12]. In the present work used nonionic surfactant at CMC because they are very less toxic to bacteria and thus enhance their efficiency in degrading PAHs. Although many studies have reported the abilities of bacteria to degrade hydrocarbons with different carbon numbers, most bacteria can use only a narrow range of hydrocarbons. For example, *Bacillus stearothermophilus* is only capable of growth on C_{15}–C_{17} [13], Geobacillus jurassicus grows only on C_6–C_{16} [14], and Bacillus thermoleovorans degrades n-alkanes up to C_{23} [15]. Degradation of a wide range of hydrocarbons and crude oil is of crucial importance for bioremediation of oil contamination and microbial enhanced oil recovery[16]. Indeed, the strains capable of degrading a wide range of hydrocarbons is scarce. Moreover, the native microbial populations in contaminated sites may have insufficient catabolic capabilities to achieve extensive, rapid, and sustainable biodegradation; especially in soils where no previous exposure to contaminants has occurred and/or contaminant concentrations are low[17]. For these resones, the *Bacillus licheniformis* ATCC 10716 is investigated as a noval biodegrading strain in this work.

The aim of the present study was to enhance the biodegradation process of petroleum crude oil in the presence of biosurfactant and chemical surfactant by *Bacillus licheniformis* ATCC 10716. The biodegradation process was detected by: 1- The gravimetric analysis of crude oil remaining after incubation of Bacillus lichneformis using MSM medium containing biosurfactant and chemical surfactant separately and another microcosm containing MSM medium with crude oil without chemical and biosurfactant. 2- Gas chromatographic analysis (GC) and high performance liquid chromatographic analysis (HPLC) for the crude oil with / without treatment by the bacterial strain. 3- The bacterial count and some surface properties of surfactant in different microcosms were measured.

MATERIALS AND METHODS

Microorganism

Bacillus lichneformis ATCC 10716 was supplied by the microbial resource center (MIECEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Crude oil

The crude oil used in the present work was conducted from Esh El-Malaha, Alexandria, Egypt.

Culture medium and the production of biosurfactant

The bacterial strain was streaked on a nutrient agar slant and incubated for 24 h at 30°C. Two loops of culture were inoculated in 40 ml of nutrient broth in a 100ml Erlenmeyer flask and incubated in a rotary shaker 150 rpm at 30 °C for 8–12 h until cell numbers reached 10^8 CFU/ml. For biosurfactant synthesis, a mineral salt medium with the following composition was utilized: Na_2HPO_4 2.0, KH_2PO_4 2.0, $MgSO_4.7H_2O$ 0.01, $NaNO_3$ 2.5, NaCl 0.8, $CaCl_2$, 0.2, KCl, 0.8, $FeSO_4.7H_2O$, 0.001 and 5 ml of a trace element solution. Trace element solution contained 0.116 g/l of $FeSO_4 \cdot 7H_2O$, 0.232 g/l of H_3BO_3, 0.41 g/l of $CoCl_2 \cdot 6H_2O$, 0.008 g/l of $CuSO_4 \cdot 5H_2O$, 0.008 g/l of $MnSO_4 \cdot H_2O$, 0.022g/l of $[NH_4]_6Mo_7O_{24}$ and 0.174 g/l of $ZnSO_4$ [18]. The carbohydrate (glucose) was added to make a final concentration 2 %. A 3 % concentration of yeast extract was added. Cultivation studies have been done in 500 ml flasks containing 150 ml medium at 30 °C for 72 h.

Biodegradation of crude oil

The crude oil (1g) was added to the mineral salt medium MSM (100 ml) in a 500 ml erlenmeyer flask containing as a sole carbon source with and without (0.1 g) added chemical surfactant separately. The flasks were autoclaved at 120 °C for 20 min. The bacterial strain (1×10^7 CFU/ml) an aliquot of 2 ml of inoculum was inoculated into the mineral medium. The cultures were incubated at a temperature controlled shaking incubator at 150 rpm at 30 °C for 7 days. The biosurfactant production using the medium MSM with (1 g) glucose as a sole carbon source for 72 h was followed by adding sterile (1 g) of crude petroleum oil until the end of the incubation period. A sample without inoculum was taken as a control. Hence, the flasks were incubated at 30 °C, 150 rpm, pH 7.5 at 7 days[19]. After the incubation period, the bacterial count and surface properties were determined. The crude oil samples were then extracted from the different microcosms. Consequently, a gravimetric analysis was then determined.

Extraction of crude oil after treatment by bacterial strain and gravimetric estimation

By the end of the 7 days incubation period, the polluted bacterial broth (100 ml) was thoroughly shaken with carbon tetrachloride)3 times using 50 ml at each) in a separating funnel and the three samples were then collected. The collected organic layer was dried over anhydrous sodium sulphate. The solvent was removed on a rotary evaporator until a constant weight was attained. The oil sample was accurately weighed. Then the percentage of the biodegraded oil was calculated and hence the chemical composition alterations were studied by chromatographic analysis (GC and HPLC)[20,21].

Gas chromatographic analysis

The biodegradation of the crude oil was monitored using an Agilent 6890 gas chromatographic instrument equipped with flame ionization detector according to the testing method IP 318 [22]. The component separation was completed on HP-1 capillary column (100 % methyl silicone siloxane, 30 m length, 0.35 mm internal diameter and 0.25 mm thickness film). For a typical chromatogram, a 0.5 ìl crude oil sample was introduced into a splitter injector which was previously heated at 350 °C. The oven temperature was programed 100-320 °C at a fixed rate of 3 °C/ min. The nitrogen (oxygen-free) was used as a carrier gas with a flow rate of 2 ml/min. A mixture of pure n-paraffins was used as standard. The peak area of each resolved component (consisting of either n- and iso-paraffin) is determined individually. While, the unresolved complex mixtures (humps), composed of non n-paraffins presumably mainly cycloparaffins and aromatics with long side chains, were determined only as a total.

High performance liquid chromatographic (HPLC) analysis

The crude oil remained after the biodegradation and the corresponding control sample was analyzed using a (HPLC) instrument model Waters 600E, equipped with dual UV absorbance detector Waters 2487 and auto sampler Waters 717 plus attached to a computerized system with Millennium 3.2 software. PAHs standards were obtained from Supelco. The conditions of separation[23] are as follows: Column: Supelcosil. LC-PAH, 5 µm particles, 15 cm length and 4.6 mm ID, Mobile phase: gradient acetonitrile: water 60 to 100% Acetonitrile (v/v) over 45 min. Flow rate: 0–2min. 0.2ml/min., 2–45 min. 1.0 ml/min. Detector set at 254 nm.

Synthesis of chemical surfactant

The used nonionic surfactant in this study was synthesized in two steps as follow:

Synthesis of 2-(dodecylcarbamoyl) benzoic acid

A mixture of phthalic anhydride (14.8 g, 0.1 mol) and dodecyl amine (18.5 g, 0.1 mol) in dry toluene, heated under reflux until all the anhydride was dissolved and then refluxed for 4 h to produce 2-(dodecylcarbamoyl) benzoic acid. The product was allowed to cool down and then extracted with dilute sodium bicarbonate solution. The extract was washed out with ether and recrystallized with hot water[24].

Synthesis of 41-hydroxy-3,6,9,12,15,18,21,24 ,27,30,33,36,39-tri decaoxa hentetra contyl 2 -(dodecylcarbamoyl) benzoate

The product from the previous step was esterified with poly ethylene glycol of molecular weight 600 at 150 °C in the presence of xylene as a solvent. The reaction was catalyzed by p-toluene sulphonic acid. The reaction mixture was refluxed with continuous stirring until the theoretical amount of water was collected in the dean stark trap. The reaction was completed when no further water was formed. The mixture was allowed to cool down and was neutralized by the addition of 5 % aqueous sodium carbonate solution and then dissolved in petroleum ether (b.p 40-60 °C). The organic layer was separated and the solvent was distilled off. The chemical structure of the synthesized compound was confirmed by FTIR, mass and ^{1}HMNR spectroscopy analysis[25].

Surface properties

Surface properties including surface tension, Emulsification index (E_{24}) and Foaming as indicator for the production of biosurfactant were determined.

Surface tension

Surface tension was measured by a Du Nouy platinum ring method with Krüss K6 tensiometer. The bacterial supernatant solution (50 ml) was tested at 25 °C to evaluate the surface tension of bio and chemical surfactant[26].

Emulsification index (E_{24})

E_{24} of the produced biosurfactant and chemical surfactant in the supernatant was measured by adding kerosene (6 ml) (Dearomatized kerosene was supplied by Alexandria Petroleum Refining Company, Alexandria, Egypt) to the aqueous phase (supernatant culture) and vortexing for 2 min, after 24 h the emulsion index (E_{24}) was calculated according to the following equation [27].

$$(E_{24}) = 100 \text{ (height of the emulsion layer/ the total height)}$$

RESULTS AND DISCUSSION

Physicochemical properties of Esh El-Malaha crude oil

The different physicochemical parameters of the crude oil sample obtained from Esh El-Malaha, Egypt was determined. Density, specific gravity and API gravity were calculated according to ASTM D-1298 [28] at 0.900 g /cm^3, 0.901 and 25.5 respectively. Pour point following ASTM D-97 [29] was measured at 6 °C. Flash point in conformity with ASTM D-93 [30] at 58 °C and viscosity in accordance with ASTM D-445 [31] was recorded at 23.03 cSt. Sulfur content was measured according to ASTM D-4294 [32] at 1.49%.

Table 1. Evaluation the growth of bacterial strain, some surface properties of bio- and chemical surfactant and percentage biodegradation of petroleum hydrocarbons after treatment with *Bacillus licheniformis* (B.L.)

Carbon source	Log count	Surface tension (S.T.) mN/m	Emulsifiation power (E_{24}) (%)	Weight of residual crude oil (g/l)	Percentage biodegradation* (%)
(inoculation or zero time)**	7.0	59	0	0.98	0
Crude oil + B.L.	7.5	48	0	0.6	40
Crude oil + B.L. + Biosurfactant	7.8	38	62	0.5	50
Crude oil + B.L. + Chemical surfactant	7.9	42	0	0.5	50

Percentage biodegradation* = Weight of original oil – wt. of residual / wt. of original oil × 100

0 (inoculation or zero time)**: The time of inoculation bacteria in MSM medium before the incubation time with adding chemical or biosurfactant

Chemical structure confirmation of the synthesized nonionic surfactant FTIR spectra of 2-(dodecylcarbamoyl) benzoic acid

The schematic diagram of preparation the chemical surfactant was represented in Fig.1. FTIR spectra using ATI mattsonm Infinity series TM, Bench top 961 controlled by Win First TM V2.01 software. FTIR spectra of 2-(dodecylcarbamoyl) benzoic acid compound showed the absorption bands at 2917.8, 2850.4, 1390.6, 1457.04,716.6, 1701.7, 3292.23, 1544.9,3061, 3431.76 cm^{-1} which corresponded to asymmetric and symmetric stretching CH group, CH_3, CH_2 bending, CH_2 rocking, stretching C=O, stretching NH group, C=C ring stretching, =C–H stretching OH, respectively.

Mass spectra of 2-(dodecylcarbamoyl) benzoic acid

Mass spectra were measured by GC MS-OP1000EX (Micro Analytical Center,

Table 2. Distribution of carbon number in gas chromatogram of residual crude oil

Carbon number of paraffins	Negative control		Crude oil + microorganism*		Crude oil + microorganism+ biosurfactant		Crude oil + microorganism+ chemical surfactant	
	Percentage residual of normal and iso paraffins after bio-treatment							
	normal	iso	normal	iso	normal	iso	normal	iso
C_8- C_{20}	32.35	19.12	24.02	11.20	25.86	9.26	23.12	6.66
C_{21}- C_{30}	23.8	4.67	38.94	8.06	31.97	7.96	36.12	10.28
C_{31}- C_{43}	18.06	2.00	18.33	4.25	20.01	4.94	19.5	4.32
Total paraffins	74.21	25.79	81.29	23.51	77.84	22.16	78.74	21.26

microorganism* *Bacillus licheniformis*

Table 3. Polyaromatics distribution in crude petroleum oil after treatment by *Bacillus licheniformis*

Number of rings	Compounds of polyaromatics	Control	Crude oil + microorganism	Crude+ microorganism + biosurfactant	Crude + microorganism + chemical surfactant
2	Naphthalene	5.37	0.49	4.38	0
	Acenaphthylene	54.18	15.09	0	0
	Acenaphthene	4.88	6.57	4.76	0.02
	Fluorine	6.84	10.46	7.68	2.37
	Total concentration (%)	71.27	32.61	16.82	2.39
3	Phenanthrene	1.19	0.49	2.83	0.11
	Anthracene	7.91	36.25	12.68	0
	Total concentration (%)	9.1	36.74	15.51	0.11
4	Fluoranthene	7.45	4.62	4.14	15.11
	Pyrene	7.87	18.49	12.94	28.22
	Benzo (a) anthracene	0.004	0	3.64	10.94
	Chrysene	0.006	0	3.46	26.5
	Total concentration (%)	15.33	23.11	24.18	80.77
5	Bezno (b) fluoranthene	0.09	1.95	3.30	11.41
	Bezno (k) fluoranthene	0	0	0.06	0.63
	Dibenzo(a,h)anthracene	0.21	0	0	4.69
	Total concentration (%)	0.3	1.95	3.36	16.73
6	Benzo(g,h,i) perylene	0	4.62	0	0
	Indeno(1,2,3-cd)pyrene	4.0	0.97	40.13	0
	Total concentration (%)	4.0	5.59	40.13	0

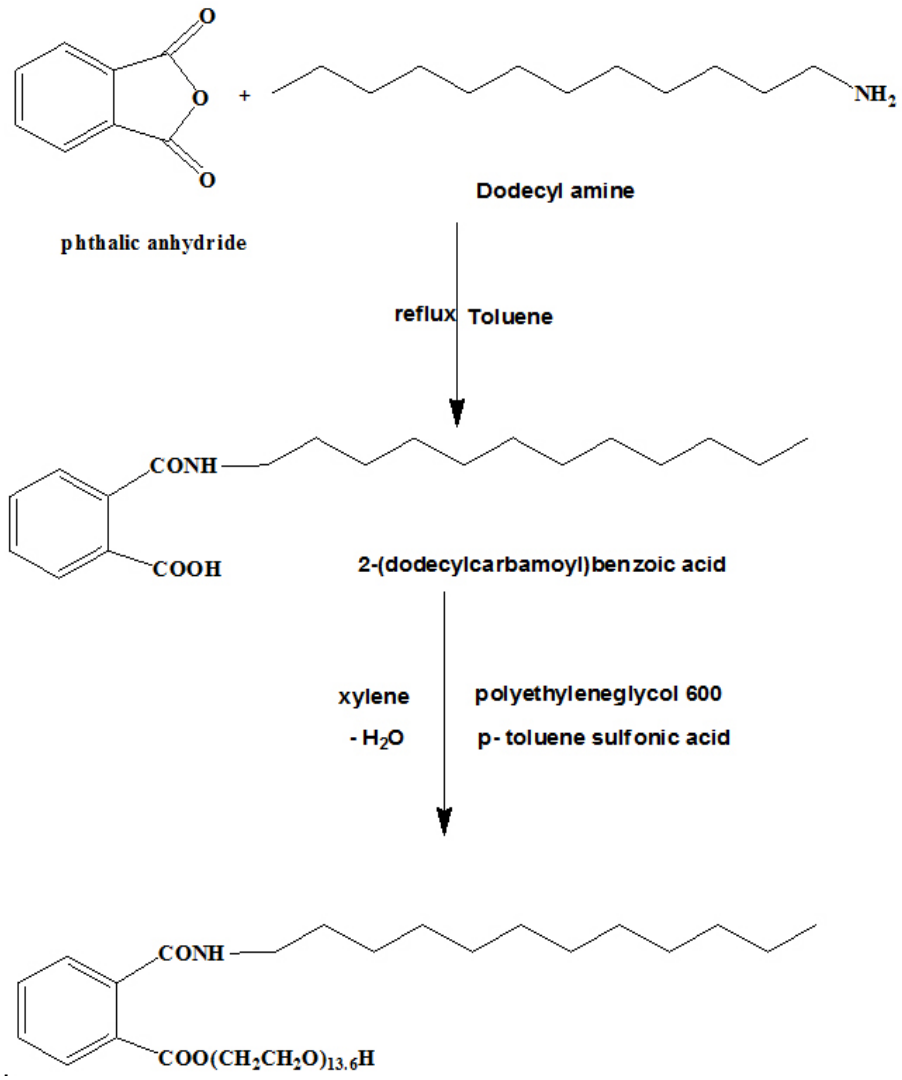

Fig. 1. Schematic diagram of preparation the chemical surfactant

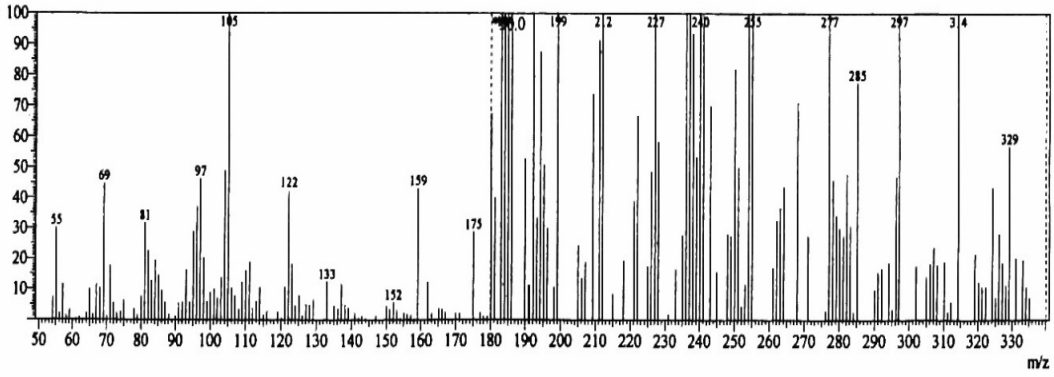

Fig. 2. Mass spectrum of 2-(dodecylcarbamoyl) benzoic acid

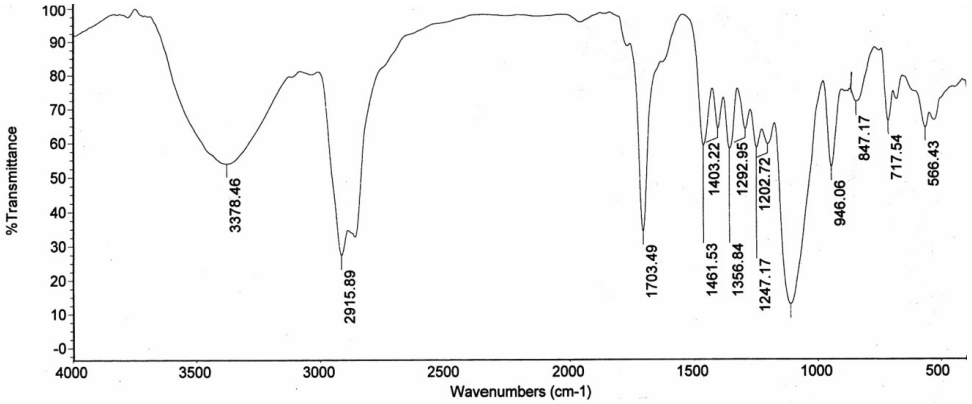

Fig. 3. FTIR spectrum of 41-hydroxyn m 3,6,9,12,15,18,21,24,27,30,33,36,39-tridecaoxahentetracontyl 2-(dodecylcarbamoyl) benzoate

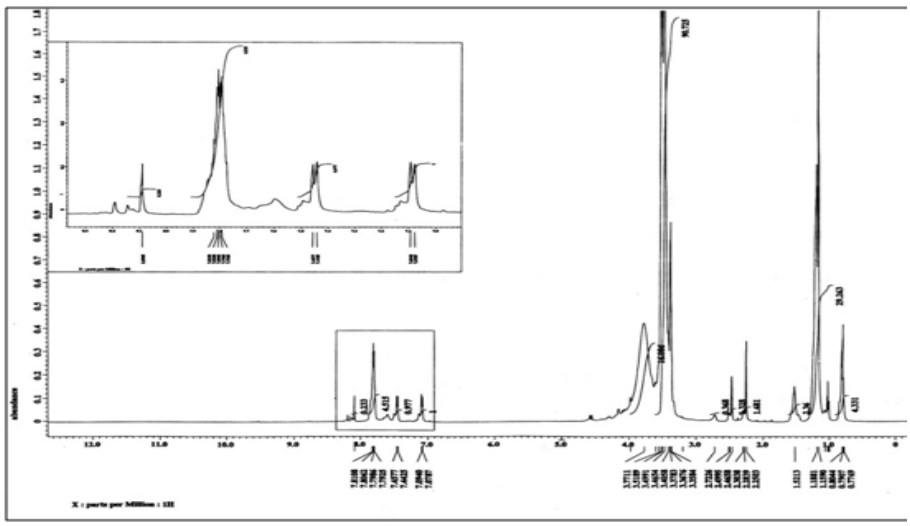

Fig. 4. [1]HNMR spectrum of 41-hydroxy-3,6,9,12,15, 18,21,24 ,27,30, 33, 36,39- tridecaoxahentetracontyl 2-(dodecylcarbamoyl) benzoate

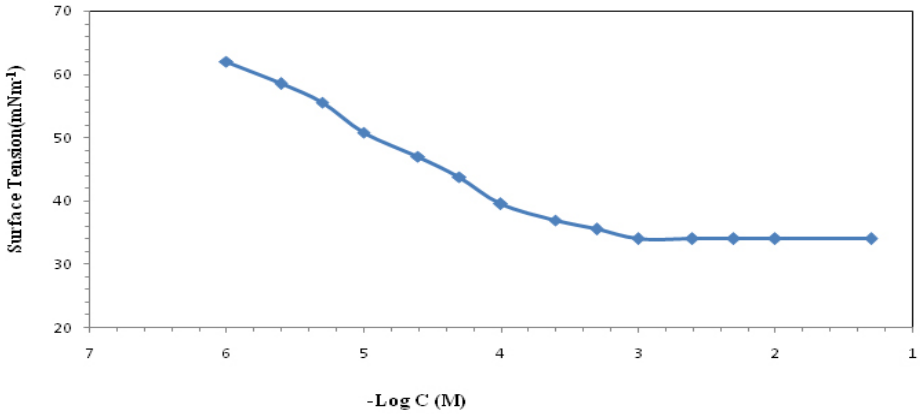

Fig. 5. Variation of the surface tension with the synthesized surfactant concentrations in water at 25 °C

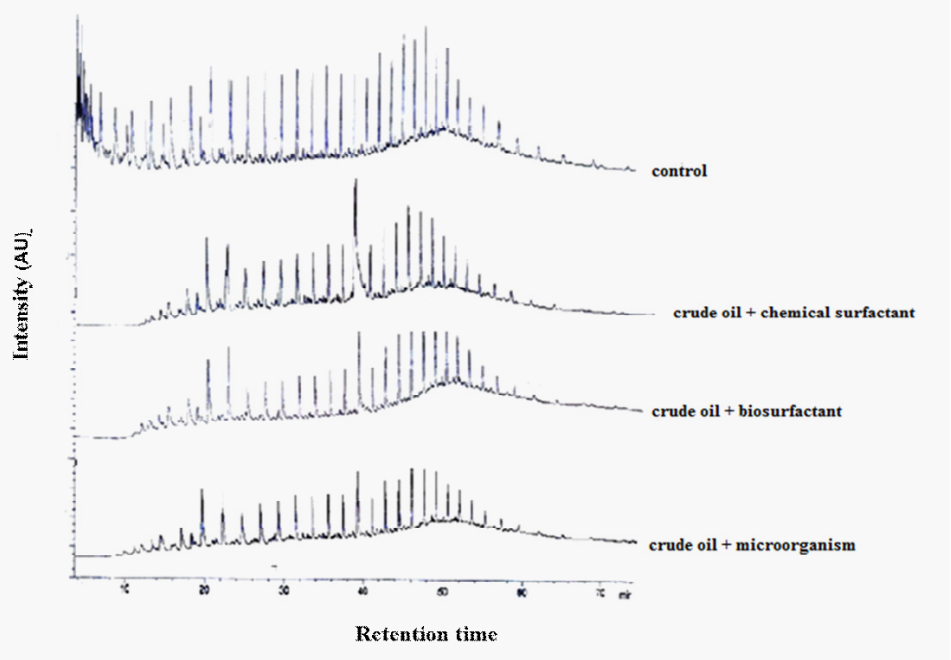

Fig. 6. Gas Chromatography (GC) analysis of residual crude oil under different types of biological treatment

Cairo University). The mass spectrum of 2-(dodecylcarbamoyl) benzoic acid compound in Fig.2 illustrated a molecular ion peak at m/z 333 (0.4%) together with other significant peaks, which were observed at m/z (intensity %): base peak at 105(100%C_6H_5CHO), 122 (42.64 % C_6H_5COOH), 212(5.35% $CH_3(CH_2)_{11}CONH_2$). Data of mass spectrum confirmed the chemical structure of the synthesized compound.

FTIR spectra of 41-hydroxy-3,6,9,12,15,18,21,24,27,30,33,36,39 tridecaoxahentetracontyl 2-(dodecylcarbamoyl) benzoate

FTIR spectrum of 41-hydroxy-3,6,9,12,15,18,21,24,27,30,33,36,39-tridecaoxahentetracontyl 2-(dodecylcarbamoyl) benzoate in Fig. 3 showed the same characteristic bands of 2-(dodecylcarbamoyl) benzoic acid compound besides the appearance of bands at 1109.8, 3378.5cm^{-1} corresponded to C-O and OH stretching vibrations.

^{1}HNMR spectra

^{1}HNMR was measured in DMSO using Jeol ECA 500 MH_Z NMR spectrometer, at 500 MH_Z (National Research Center, Cairo). ^{1}HNMR spectrum of 41-hydroxy3,6,9,12,15,18,21,24,27,30,33,36,39-tridecaoxahentetraconty l2(dodecylc-rbamoyl) benzoate compound in Fig. 4 illustrated that the different peaks at δ=0.79 ppm (t, 3H, $NCH_2CH_2(CH_2)_8CH_2$ $C\underline{H}_3$); δ1.15 ppm (m, 2H, $NCH_2CH_2(CH_2)_8C\underline{H}_2$ CH_3); δ=1.18 ppm (m, 16H, $NCH_2CH_2(C\underline{H}_2)_8$ CH_2CH_3); δ=1.52 ppm (m, 2H, $NCH_2C\underline{H}_2(CH_2)_8CH_2CH_3$); δ=3.35 ppm (2H, $NC\underline{H}_2CH_2(CH_2)_9CH_3$); δ=3.4 ppm (m, 48H, $O(C\underline{H}_2C\underline{H}_2)_{12}O$); δ=3.37 ppm (2H, $OCH_2C\underline{H}_2OH$); δ=3.49 ppm (2H, $OC\underline{H}_2CH_2OCO$); δ=4.3 ppm (2H $OCH_2C\underline{H}_2OCO$); δ=2.28 ppm (s, 1H, $OCH_2CH_2O\underline{H}$);δ=7.07-8.09 ppm (d ,4H, Ar-H); δ=8.2 ppm (s,1H, N-$\underline{H}$).

Critical micelle concentration (CMC)

CMC of the sythesized surfactant was determined by plotting the surface tension α versus –log C as shown in Fig. 5.Value of CMC was determined from the break point in the α–log C plot. The CMC of the prepared surfactant was 9×10^{-4}.

Evaluation of the growth rate and some surface properties of bio- and chemical surfactants after biodegradation process

Bacillus licheniformis used for biodegradation of crude oil by using biosurfactant and/or chemical surfactant separately for the enhancement of biodegradation process. The

bacterial count and measurement of surface tension and emulsification power in different microcosms after 7 days incubation period were determined and listed in Table 1. Surface tension and emulsifying activity measurements were demonstrated that, the bacterial strain was able to produce biosurfactant in mineral medium after 72 h using glucose as co-substrate and added the crude oil as a carbon source. Also, when the chemical surfactant is added to mineral medium the surface tension decreased from 71 for distilled water to 42 mN/m. The greatest surface tension reduction and increasing of emulsification power was achieved in the microcosm containing biosurfactant which reached a value of 38 mN/m and 62 % respectively. Some investigations showed that surface activity of biosurfactant is comparable with surface activity of synthetic surfactants. For example biosurfactants are able to reduce surface tension of water to 29.0 mN/m, while Pluronic F-68 to 42.8 [33]. Moreover, water-in-oil emulsions of palm, crude, soybean, coconut and olive oils with biosurfactants were comparably or even more stable than that with synthetic surfactants[33].

Growth rate and biodegradation of crude oil by bacterial strain

Bacillus licheniformis was grown in 1% crude oil in a medium (MSM) for 7 days with shaking at 150 rpm. After 7 days, the microbial growth was measured, the crude oil biodegradation was analyzed using spectrometry methods. The biodegradation percentage was estimated by gravimetric analysis. The data in Table 1 clear that the bacterial strain was degraded up to 50 % for the crude oil in medium containing biosurfactant or chemical surfactant.

The Gas Chromatographic analysis (GC) of the residual crude oil samples in a medium containing bio- and chemical surfactant separately, with comparison with the control (sample crude oil without treatment by microorganism) and sample (crude oil + microorganism) without adding chemical surfactant were shown in Table 2 and Fig. 5. The components of total paraffins changed in the biodegradation of the crude oil were detected by GC in Fig. 5. It was found that, the biodegradation of total paraffins was observed in different microcosms comparing with the control sample. From the Table 2 the percentage degradation of the n-paraffins and iso-paraffins (C_8–C_{20}) present in crude oil was calculated by comparing with the GC chromatograms of the undegraded control. The obtained data showed that the microcosm containing chemical surfactant given better degradation in shorter chain length of n-paraffins and iso-paraffins (C_8–C_{20}) than the other of different microcosms. But the percentage degradation of medium chain length (C_{21}–C_{30}) n-paraffins and iso-paraffins was increased in all different microcosms and long chain length (C_{31}–C_{43}) was relatively increased in all different microcosms, this may attribute to the consumption of other compounds which leading to a relative accumulation of such compounds, the bacterial actions towards similar compounds agree with the previous works[34]. As generally, the total iso-paraffins concentration decreased more than n-paraffins in all different microcosms, it was indicated that the bacterial strain could be consumed iso-paraffins over n-paraffins in crude oil samples which seems to be unexpected and valuable importance. Hydrocarbons differ in their susceptibility to microbial attack and in the past have generally been ranked in the following order of decreasing susceptibility: n-alkanesÃ branched alkanesÃ low molecular weight aromaticsÃ cyclic alkanes[35]. The biodegradation potential of the bacterial strain on polyaromatics was detected by HPLC analysis in Table 3. The polyaromatics of the control crude oil sample were Naphthalene, Acenaphthylene, Acenaphthene and Fluorine (2-member rings polyaromatics), Phenanthrene and Anthracene (3-member rings polyaromatics), Fluoranthene, Pyrene, Benzo (a) anthracene and Chrysene (4-member rings polyaromatics), Bezno (b) fluoranthene, Bezno (k) fluoranthene and Dibenzo (a,h) anthracene (5-member rings polyaromatics) and Benzo (g,h,i) perylene and Indeno (1,2,3-cd) pyrene (6-member rings polyaromatics). The polyaromatics were fully utilized in microcosm containing biosurfactant including Acenaphthylene as (2-member rings polyaromatics) and Dibenzo (a,h) anthracene (5-member rings polyaromatics). While, in microcosm containing chemical surfactant, Naphthalene, Acenaphthylene, (2-member rings polyaromatics), Anthracene (3-member rings polyaromatics) and Indeno (1,2,3-cd) pyrene (6-member rings polyaromatics) were fully utilized by bacterial strain. Few studies have documented the bacterial degradation of PAHs with five or

more rings in both environmental samples and pure or mixed cultures[36]. It has been documented that, the effects of four non-ionic surfactants on the bioavailability and rates of biodegradation of crystalline naphthalene and phenanthrene[37]. Also, *Mycobacterium* sp. can degrade 95 % fluoranthene if efficient organic nutrients are provided in a mineral medium[36]. It is necessary to explain that, the percentage increase in any compound may be attributed either to a real increase due to the formation of additional amounts of this polyaromatic hydrocarbon, this is only accepted in case of lower polyaromatics, and the other probability is the enhanced consumption of other compounds leading to a relative accumulation of such ployaromatic hydrocarbon[38] (Table 3). In microcosm containing chemical surfactant a remarkable decrease in the 2 and 3 member rings polyaromatics was observed, while the percentage of 4 member rings polyaromatics increased due to the consumption of other compounds. On the other hand, in microcosm containing biosurfactant and positive control separately decreased in 2 member rings polyaromatics was observed and increased in 3,4,5, and 6 member rings polyaromatics. The biodegradation rates of PAHs were increased signiûcantly by surfactant addition. Alasan, a HMW bioemulsiûer complex of an anionic polysaccharide and proteins produced by Acinetobacter radioresistens KA53, enhanced the aqueous solubility and biodegradation rates of PAHs. In the presence of Alasan (500 mg/l), the apparent aqueous solubilities of phenanthrene, fluoranthene, and pyrene were increased from 7 to 26 fold[39].

CONCLUSION

In this present study we can conclude that

1. The biosurfactant produced by the bacterial strain Bacillus licheniformis and chemical surfactant as potential to be used in bioremediation of petroleum crude oil.
2. The bacterial strain could be consuming iso-paraffins over n-paraffins in crude oil samples.
3. This bacterial strain could be fully utilizing the 5 and 6 member rings of polyaromatics compounds in crude oil samples. These compounds are considering more toxic and carcinogenic for soil and aquatic environment.
4. Thus, this bacterium was considered as good candidates for application in bioremediation process of petroleum contaminated sites using biosurfactant and/or nonionic chemical surfactant at CMC concentration.

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