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# Enhancement the Bioremediation of Crude Oil by Nanoparticle and Biosurfactants

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THE PRESENT study concerns the bioremediation process of oil contaminated soil from Suez Oil Processing Company. Nineteen crude oil-degrading bacterial isolates were isolated from this oil-polluted area. Four bacterial species showed the highest growth rate on crude oil hydrocarbons. The bioremediation process was studied by pure and bacterial consortium. The oil contents in the different microcosms were reduced up to 80% via bioremediation after 7 days. The Gas Chromatographic analysis of the crude oil remaining in different culture media after one week was determined. The nanoparticle and biosurfactant showed selective bioremediation enhancement for n- and iso- paraffins. The percentage biodegradation was reached the maximum value in microcosm containing biosurfactant, nanoparticle and bacterial consortium of the best four bacterial strains up to 90% after 7 days. The complete degradation of some different polyaromatics and the percentage biodegradation of other polyaromatics increased in different microcosms were investigated by HPLC analysis.

**Key words:** Oil pollution; Biodegradation; Biosurfactants; Nanoparticles.

## Introduction

Petroleum hydrocarbons are typically a complex mixture of aliphatic and aromatic organic compounds. They can be fractionated by distillation into saturates, aromatics, asphaltenes, and resins [1]. The saturates include n-alkanes, branched alkanes, and cyclo-alkanes. Polycyclic aromatic hydrocarbons (PAHs) are organic molecules with two or more benzene rings in which the number and arrangement of the rings result in diverse physical and chemical properties.

The alkanes and PAHs pollutants in the environment cause serious pollution to the water ecosystems and are harmful to the health of the living creatures and human bodies (Fig. 1). Moreover, polycyclic aromatic hydrocarbons (PAHs) are persistent in the environment and can cause long-term adverse effects. The majority of PAHs have strong toxicity, carcinogenicity, teratogenicity and mutagenicity [2, 3].

The spilled oil had dramatic negative ecological and economic impacts to the coastal ecosystem. The physicochemical properties of the oil, with high density and viscosity, as well as a high content of resins and asphaltenes, prompted the formation of stable emulsions with seawater [4].

Biosurfactants (BS) can emulsify hydrocarbons via enhancing their water solubility, decreasing surface tension and increasing the displacement of oily substances from soil particles [5]. Surfactants can also increase the rate of the biodegradation of slightly soluble contaminants by increasing their bioavailability [6].

In the last two decades, nanotechnology has attracted a great interest due to its expected impact on areas of catalysis and/or water treatment [7]. Nanoparticles have been widely used to improve various reactions as reductants and/or catalysts in chemistry field due to their high specific surface areas and characters [8]. On the other hand, nanoparticles effect on microbes has also caught a great attention. Nanoparticles are capable of assisting microbe activities however, so far, very limited studies have been reported on nanoparticle effect on the microbiological reaction rates [9]. The higher activity of nanoparticles is usually referred to their unique properties and

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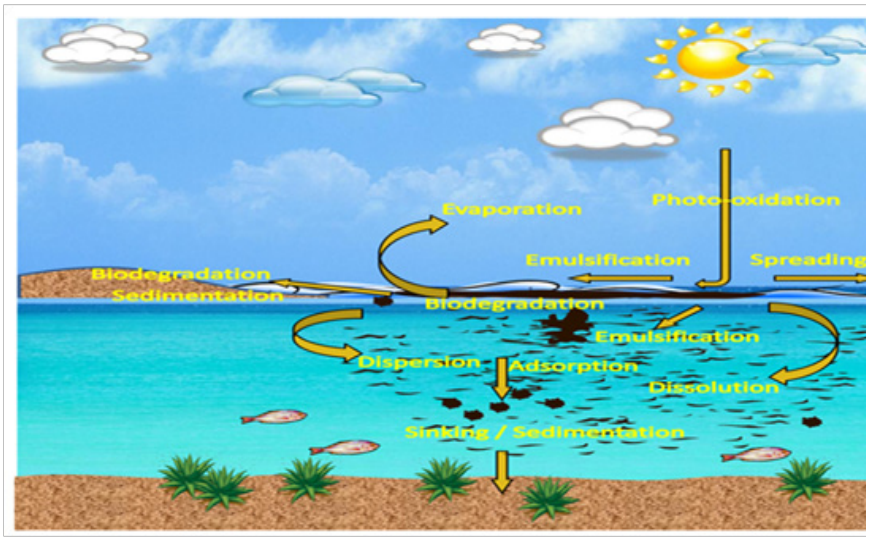


**Fig. 1. Fate of a marine oil spill.**

high available active specific surface areas [10]. Generally, nanoparticle catalysts increase the microbiological reaction rates by locating on the cells to stimulate the activity of microbes [11].

Water contamination by petroleum hydrocarbons in Suez oil processing company, Egypt has caused environmental and health defects. So, the present study increased attention for cleaning up of this contamination by bioremediation process. The degradation capacity of crude oil sample by pure and mixed bacterial cultures isolated from contaminated water sample is studied. Also, the aim of this research is to evaluate the enhancement of crude oil biodegradation (bioremediation) by using of synthetic nanoparticle and biosurfactants.

## Experimental

*Samples collection*

The Arabian heavy crude oil and soil sample used for this study was collected from Suez Oil Processing Company (SOPC), Egypt. Suez Oil Processing Company (SOPC) is uniquely located in Suez, Egypt, on the Gulf of Suez, at the entrance of Suez Canal, which connects the Red Sea with the Mediterranean Sea (Fig.2).

*Physico–chemical characteristics of the tested crude oil*

The general physico-chemical characteristics of the crude oil; has been determined according to the ASTM, IP and UOP standard methods. The density of the crude oil was determined according to the IP190 capillary stoppered pyknometer


**Fig.2. Represeant Position of Suez Oil Processing Company (SOPC).**

method. Also, the kinematic viscosity and pour point of the crude oil were evaluated according to the ASTM D445-IP71 glass capillary viscometer method and ASTM D9-IP 15 methods respectively. The sulfur content was determined by the quartz tube method according to the ASTM-D1551 and IP 63 methods. Wax content of the crude oil sample was calculated according to UOP 46-85. Molecular weight of crude oil and its fractions was determined according to ASTM-D 2505. While, the carbon residue content of the crude oil was investigated according to the ASTM D524-IP 14 Rams bottom carbon residue method.

*Deasphalting of crude oil sample*

Deasphalting for crude oil sample was carried out using the (standard test method IP 143).

*Separation of maltene into its hydrocarbons*

Separation of the obtained maltene into its components saturates, mono-aromatics, Diaromatics, polyaromatics and resins, was carried out using liquid column chromatography [12].

*Analysis of fractionation components hydrocarbon types extracted from crude oil sample*

Saturate compounds that were extracted from the studied crude sample was chromatographically analyzed and was monitored using Agilent 6890 plus, Gas chromatograph attached to computerized system with chemstation software condition of operation according to the standard test method IP 318/ 75 (standard test method IP 318). The component separation was completed on HP-1 capillary column (100 % methyl silicone siloxane, 30 m length, 0.35mm internal diameter and 0.25 mm thickness film).

Extracted saturate has been studied by GC for a typical chromatogram, a 0.5 µl saturate sample was introduced into a splitter injector which was previously heated at 350°C. The oven temperature was programmed 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-paraffin was used as standard. The peak area of each resolved component (consisting of either n- and iso-paraffin) was 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 [13].

*High performance liquid chromatographic analysis (HPLC)*

The aromatic fraction extracted from the studied heavy crude oil sample, used as 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 are as follows: Column: Supelcosil. LC-PAH, 5 lm particles, 15 cm length and 4.6 mm ID, Mobile phase: gradient acetonitrile: water 60–100% acetonitrile (v/v) over 45 min. Flow rate: 0–2 min. 0.2 ml/min., 2–45 min. 1.0 ml/min. Detector set at 254 nm [14].

*Synthesis of nanoparticle*

The analytical grade $Ni(NiO_3).6H_2O$ (Sigma-Aldrich) was used to produce the investigated NiO nanoparticle. The nanoparticle was prepared using the precipitation method. For preparation, a 0.1 M solution of nickel nitrate was prepared in distilled water. The solution was stirred for 30 min. and heated up to 35 °C. The amm. solution as precipitating agent was then added dropwise till the pH 10 is reached and the solution was further stirred for 60 min. the precipitate was then filtered, washed 5 times with distilled water and dried at 120 °C overnight. The dried powder is then grounded for fine powder and calcined at 600°C for 2 h [15].

*Characterization of nanoparticle*

The prepared nanoparticles phase and crystalinity were firstly determined by the X-ray analysis. The prepared sample was analysed using X'Pert PRO PANalytical apparatus which equipped with Cu (Kα = 0.15418 nm) radiation source. The patterns were recorded in Brugge configuration (2θ degree) in range of 5-90 degree. The recording rate was 0.05 s-1. The NiO nanoparticles morphology and structure were investigated by the high resolution transmission electron microscopy (HRTEM). The samples were investigated using the JEM-200CX model (JEOL, Japan) at an accelerating voltage of 200 kV. For analysis, a 0.05g NiO sample was firstly suspended in distilled water and subjected to ultrasonic bath for 30 min. the ultrasonic equipment was adjusted at 70% power and 0.5 frequency. A few droplets of the suspension were then loaded to the Cu grid to investigate the sample [16].

*Isolation of crude oil degrading bacterial isolates*

Bushnell Hass Mineral Salts medium (BHMS) contains the following (g/l): $KH_2PO_4$, 1; $K_2HPO_4$, 0.2; $MgSO_47H_2O$, 0.2; $CaCl_2$, 0.02; $NH_4NO_3$, 1; NaCl, 2; and 2 droplets of 60 % $FeCl_3$, the pH was adjusted 7. This synthetic medium was used for the isolation of crude oil degrading bacteria. The BHMS medium was supplemented with 1 % (v/v) crude oil (Arabian heavy crude oil) as the sole source of carbon and energy. soil sample1) g) was added to 250 ml Erlenmeyer flasks containing 100 ml of the BHMS medium; the flasks were incubated for 7 days at 30°C on a rotary shaker operating at 150 rpm. Then, 10 ml aliquots were transferred to fresh BHMS medium. After a series of four further subcultures, inoculums from the flask were streaked out, and phenotypically different colonies were purified on BHMS agar medium for 3 days of incubation period. The procedure was repeated and only isolates that exhibited pronounced growth on crude oil were stored for further characterization [17].

*Selection of the most predominate bacterial isolates*

The four bacterial isolates ($I_1$- $I_4$) were selected for further studies due to most predominated growth on BHMS medium with crude oil as carbon source. An analysis of 16S rRNA was performed to taxonomically characterize the isolated strains (Sigma Scientific Services Co., Egypt).

*Crude oil degradation trial*

100 ml of mineral salt medium into 250 ml flasks supplemented with 1 % crude oil was prepared. The medium contained (g/l): $Na_2HPO_4$ 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, yeast extract, 3%, using crude oil as carbon source and 1ml of a trace element solution. Trace element solution contained (mg/l): $ZnSO_4.7H_2O$, 525; $MnSO_4.4H_2O$, 200; $CuSO_4.5H_2O$ 705; $NaMnO_4.2H_2O$, 15; $CoCl_2.6H_2O$, 200; $H_3BO_3$, 15; $NiSO_4.6H_2O$, 27 [18]. The pure bacterial isolate ($I_1$-$I_4$) was inoculated into the MSM. Also, mixture of these bacterial isolates (1ml of each isolate) was inoculated into the same type of medium. Hence, the flasks were incubated at 30°C, 150 rpm, pH 7.5 for 7 days. Total viable count (TVC) of cells was determined by agar plate every 3 days, modified method [19]. The remaining crude oil samples were extracted from different microcosm and gravimetric analysis was also performed**.**

*Analysis of produced biosurfactant*

Synthesis of biosurfactant by the most promising bacterial isolates ($I_1$ – $I_4$) was cultured on MSM (the constituent of medium as mentioned above). The carbohydrate (glucose) was added to make a final concentration 2%. Cultivation studies have been done in 250 ml flasks containing 100 ml medium at 30 °C for 72 h.

*Emulsification activity ($E_{24}$)*

$E_{24}$ of the produced biosurfactant 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 [20].

($E_{24}$) = 100 (height of the emulsion layer/ total height) *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 biosurfactant [21]. The value of surface tension was expressed as $mNm^{-1}$.

*Selection of the best bacterial isolates*

Two bacterial isolates ($I_1$ and $I_4$) were selected for further studies, which was giving the best surface properties and biodegradation percentage.

*Biodegradation of crude oil in presence of nanoparticle and biosurfactants*

The bacterial strain was inoculated into MSM (100 ml) in a 250 ml Erlenmeyer flask. The cultures were incubated in a temperature controlled shaker incubator at 150rpm at 30°C for ٧ days, using (1g) crude petroleum oil as a sole carbon source and /or added (0.1 g) of nanoparticle. The biosurfactant production using the medium MSM with (1g) glucose as a sole carbon source for 72 h followed by addition of (1 g) crude oil and / or added (0.1 g) of nanoparticle separately until the end of incubation period. A sample without inoculum was taken as a control. Hence, the flasks were incubated at 30°C, 150 rpm, pH7.5 at seven days [22]. The bacterial count, surface tension and emulsification power were determined. The crude oil samples were extracted from different microcosms and were gravimetrically analyzed after the seven days.

*Extraction of crude oil after treatment by bacterial strains and gravimetric estimation*

The polluted bacterial broth (100 ml) was thoroughly shaken with carbon tetrachloride (50 ml 3 times) in a separating funnel and the three fractions were collected in case of crude oil sample. The collected organic layer was dried over anhydrous sodium sulphate. The solvent was removed using rotary evaporator until a constant weight [23]. The oil sample was accurately weighed, percentage of the biodegraded oil was calculated and alterations in its chemical composition were studied by chromatographic analysis (GC and HPLC) [24].

## Results and Discussion

*Physico-chemical characterization of crude oil sample*

The heavy crude sample has a high specific gravity of 0.9524 at 15.6o°C, low API gravity of 17.07 at @ 6o°F, viscosity 52.52 cSt at 140°F, a pour point 15°C, the pour point represents a consistent temperature at which oil will pour very slowly and therefore has limited use as an indicator of state of the crude. Low wax content 2.07%, and high asphaltene 12.23% as shown in (Table, 1). Its characterization was conducted to more closely approximate the weathered state of crude oil after a spill occurs.

*Analysis of fractionation components hydrocarbon types extracted from crude oil sample*

The results of fractionation components hydrocarbon types extracted from crude oil sample by liquid column chromatography indicated the percentage of maltene components at 87.80, while the asphaltene content at 12.23%. The maltene components were fractionated (%) into saturate 43.05; aromatics 36.25 and resin 20.40.

*Gas chromatography analysis of crude oil sample*

The GC analysis of saturate crude oil exhibits many peaks over hump; these peaks represent the paraffinic hydrocarbon (iso- and n-alkane). The hump represents the heavy non eluted compounds which unresolved complex mixture (UCM). Our crude oil studied sample has iso-component beside the normal one starting from $C_{13}$ to max carbon number $C_{45}$.

The hydrocarbon distribution of the saturated fraction of the crude oil as illustrated in (Fig. 3) showed that there is a gradual increase in paraffinic hydrocarbons and range of carbon number ($C_{13}$- $C_{45}$).

*Characterization of nanoparticle*

The x-ray diffraction of the prepared NiO sample patterns was recorded and represented in Fig. 4. the figure showed a patterns at 2θ of 37.28, 43.3, 62.9, 75.5 and 79.6° which assigned to the 111, 200, 220, 311 and 222 cell orientation respectively. The recorded peaks are slightly wide which reflect the small size crystals of NiO. Also, the peaks arrangements reflect the structure of face-centered cubic particles.

The crystal size of the calcined particles was estimated using the Scherer equation using the dominant diffraction peak at 43.3°. The needed data was represented in Table 2. The calculations showed that the dominant NiO crystal size was around 12 nm.

**TABLE 1. Physicochemical properties of the studied crude oil.**

| Experiments analysis | Method | Result |
|---|---|---|
| Density g/ml, at 15.56°C | ASTMD- 1298 | 0.9515 |
| Specific gravity | | 0.9524 |
| API gravity @ 6o°F | | 17.07 |
| Kinematic Viscosity, cSt @ 140°F | ASTMD-445 | 52.52 |
| Asphaltene Content wt.% | IP-143 | 12.23 |
| Wax content % | According to UOP method | 2.07 |
| Pour Point, °C | ASTMD-97 | 15 |
| Ash content, wt.% | ASTMD-482 | 0.3 |
| Carbon residue, wt.% | ASTMD-189 | 8.71 |
| Total Sulphur, wt% | ASTMD-4296 | 3.35 |

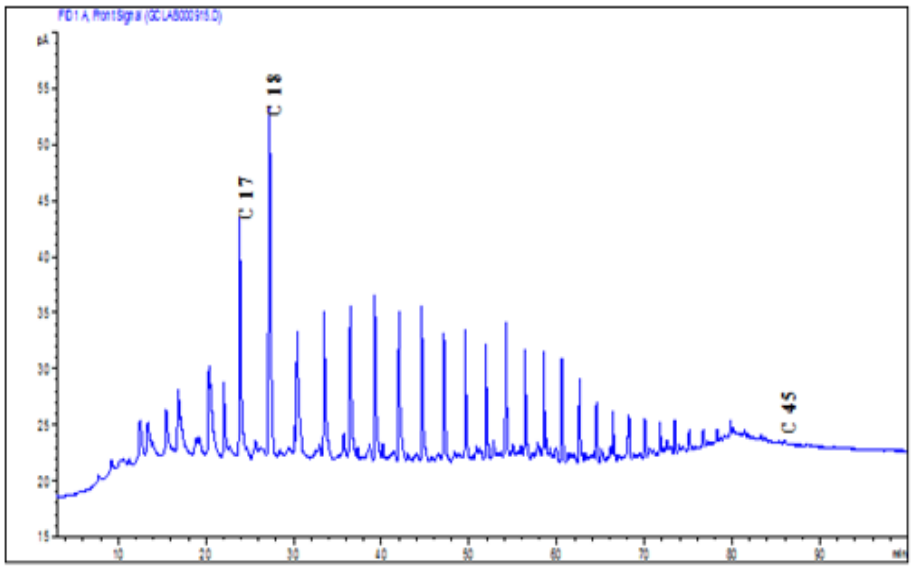


**Fig. 3. Gas chromatography analysis for heavy crude oil sample.**

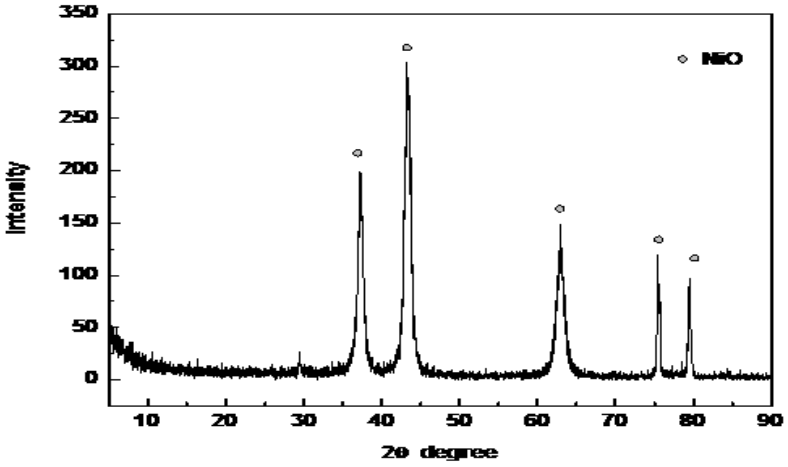


**Fig. 4. The XRD pattern for the as prepared NiO**

**TABLE 2. The Scherer equation parameters and particle size of the prepared NiO.**

| Sample | FWHM | Height | d-spacing | Particle size/ nm |
|---|---|---|---|---|
| NiO | 0.8659 | 260.31 | 2.08642 | 12.49 |

*Isolation and identification of crude oil degrading bacteria*

Eighteen bacterial strains were isolated from BHMS medium that was established at 30°C for 4 weeks. Four of the isolated strains ($I_1$- $I_4$) that showed a higher growth rate on crude oil were selected from the eighteen isolates for further study. The results of the identification procedure showed that the four isolated bacteria belong to the *Halomonas xianhensis* strain A-1 ($I_1$), *Halomonas zincidurans* strain B6 ($I_2$), *Pseudomonas stutzeri* ATCC 17588 ($I_3$) and *Halomonas salifodinae* strain BC7 ($I_4$).

*Evaluation of growth rate and biosurfactant surface properties after biodegradation process*

Biosurfactants, a group of surface active molecules synthesized by diverse microorganisms, can reduce both surface and interfacial tension, making these molecules useful in emulsification processes. The use of biosurfactants has been found to enhance the degradation of crude oil and other hydrocarbons [25].

In the present study, four bacterial strains ($I_1$- $I_4$) were grown together or separately on MSM medium. Data are shown in Table 3; the growth capacity of the mixed bacterial strains together with biosurfactants was greater than that of four bacterial strains separately with/ without biosurfactants. Biosurfactant production by the bacterial isolate was associated with higher values of growth, which proved that the biosurfactant production by *Bacillus* sp. was associated with cellular growth.

Biosurfactants enhance hydrocarbon degradation in two ways. First, by increasing substrate accessibility to microbes, while the second mechanism is increasing in the interaction of hydrophobic substrate and microbial cell surface due to increased cellular surface hydrophobicity, allowing the bacterial cells to associate easily with the hydrophobic contaminants [26].

*Biodegradation of crude oil by bacterial strains*

*Gravimetric analysis*

The percentage biodegradation of crude oil by the four bacterial isolates ($I_1$ – $I_4$) was estimated by gravimetric analysis and listed in Table 3. The Table showed that the bacterial strains were able to degrade in the range from 60 to 80 % of the crude oil. Shahian et al. [17] found a relationship between the levels of biosurfactant production and crude oil biodegradation; the strains that produce high levels of biosurfactant can better degrade crude oil.

**TABLE 3. Evaluation the growth count, some surface properties of biosurfactants and percentage biodegradation of petroleum hydrocarbons after treatment by four different bacterial isolates as pure culture and mixture.**

| Carbon source | CFU/ml | Surface tension (S.T.) mN/m | Emulsification power ($E_{24}$) (%) | Percentage biodegradation (%) |
|---|---|---|---|---|
| 0 (inoculation or zero time) | $2\times10^6$ | 59 | 0 | 0 |
| Crude oil +$I_1$ | $5\times10^7$ | 40 | 50 | 60 |
| Crude oil + $I_1$+ biosurfactant | $1.8\times10^8$ | 37 | 65 | 75 |
| Crude oil + $I_2$ | $7\times10^7$ | 42 | 52 | 65 |
| Crude oil + $I_2$+ biosurfactant | $1.4\times10^8$ | 38 | 60 | 72 |
| Crude oil +$I_3$ | $9\times10^7$ | 38 | 60 | 70 |
| Crude oil + $I_3$+ biosurfactant | $1\times10^8$ | 36 | 67 | 70 |
| Crude oil + $I_4$ | $8\times10^7$ | 40 | 60 | 65 |
| Crude oil + $I_4$+ biosurfactant | $1.6\times10^8$ | 37 | 70 | 77 |
| Crude oil + bacterial consortium | $9.3\times10^7$ | 40 | 65 | 70 |
| Crude oil + bacterial consortium + biosurfactant | $2.5\times10^8$ | 35 | 72 | 80 |

*Gas chromatographic analysis*

The first step in the aerobic degradation of alkanes by bacteria is catalyzed by oxygenases. These enzymes, which introduce oxygen atoms derived from molecular oxygen into the alkane substrate, play an important role in oil bioremediation and in the co-metabolic degradation of compounds [27].

In this study, the biodegradation of crude oil was studied after seven days of incubation period using the GC for aliphatic compounds. Table 4 shows the results of Gas Chromatographic analysis (GC) of the residual crude oil samples of different microcosms, and the negative control (crude oil sample without treatment by microorganisms).

The percentage of residual n-paraffins and iso-paraffins present in crude oil after biodegradation was calculated by comparing with the undegraded control. The obtained data showed better degradation of n-paraffins than iso-paraffins in microcosms containing biosurfactants, $I_1$, $I_4$ and bacterial consortium separately.

Hydrocarbons differ in their susceptibility to microbial attack and in the past they had generally been ranked in the following order of decreasing susceptibility: n-alkanes> branched alkanes> low molecular weight aromatics> cycloalkanes [28].

*High performance liquid chromatographic analysis*

The biodegradation potential of the bacterial strains on polyaromatics was detected by HPLC analysis as given in Table 5. The data in this table showed that, some different membered rings of polyaromatics were fully utilized in all microcosms of bacterial isolates. Also, showed there is an increasing the percentage biodegradation of other membered rings of polyaromatics by different bacterial isolates.

Meanwhile, the best biodegradation of different polyaromatics was appeared in microcosms containing biosurfactants, $I_1$, $I_4$ and bacterial consortium of four bacterial isolates separately. It is necessary to mention that, the increased degradation percentage of 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 the case of lower polyaromatics) or the other probability is the enhanced consumption of other compounds leading to a relative accumulation of such polyaromatic hydrocarbon [2, 29].

**TABLE 4. Evaluation the percentage of residual crude oil after biodegradation by gas chromatogram with/ withoutbiosurfactant.**

| Sample | % paraffins | |
|---|---|---|
| | **n-paraffins** | **iso-paraffins** |
| Control | 84.00 | 16.00 |
| *Halomonas xianhensis*($I_1$)+ crude oil | 52.82 | 47.18 |
| *Halomonas zincidurans*($I_2$)+ crude oil | 60.27 | 39.75 |
| *Pseudomonas stutzeri* ($I_3$)+ crude oil | 50.25 | 49.75 |
| *Halomonas salifodinae*($I_4$) + crude oil | 59.9 | 40.1 |
| *Bacterial consortium* + crude oil | 62.06 | 37.94 |
| *Halomonas xianhensis*($I_1$)+ crude oil + biosurfactant | 46.09 | 53.91 |
| *Halomonas zincidurans* ($I_2$)+ crude oil + biosurfactant | 54.94 | 45.06 |
| *Pseudomonas stutzeri* ($I_3$)+ crude oil + biosurfactant | 61 | 39 |
| *Halomonas salifodinae*($I_4$)+ crude oil + biosurfactant | 50.91 | 49.09 |
| Bacterial consortium + crude oil + biosurfactant | 29.11 | 70.89 |

**TABLE 5. Polyaromatics distribution in crude oil after treatment by different bacterial strains with/without biosurfactants using HPLC analysis.**

| Number of rings | Compound of polyaromatics | Control | Bacterial strains ($I_1$) | | ($I_2$) | |
|---|---|---|---|---|---|---|
| | | | Crude oil | Crude oil + biosurfactant | Crude oil | Crude oil + biosurfactant |
| 2 | Naphthalene | 0.83 | 0 | 51.7 | 0 | 18.27 |
| | **Total concentration (%)** | **0.83** | **0** | **51.7** | **0** | **18.27** |
| 3 | Acenaphthylene | 3.21 | 0 | 0 | 0 | 0 |
| | Acenaphthene | 67.65 | 0 | 0 | 0 | 28.87 |
| | Fluorine | - | 0 | 0 | 0 | 0 |
| | Phenanthrene | - | 0 | 0 | 44.02 | 4.78 |
| | Anthracene | 2.14 | 0 | 0 | 0 | 1.4 |
| | **Total concentration (%)** | **73.00** | **0** | **0** | **44.02** | **35.05** |
| 4 | Fluoranthene | 9.34 | 0 | 0 | 0 | 42.53 |
| | Pyrene | 0.66 | 0 | 0 | 0 | 1.95 |
| | Benzo (a) anthracene | - | 5.18 | 1.57 | 8.37 | 0.93 |
| | Chrysene | - | 0 | 0 | 0 | 0 |
| | **Total concentration (%)** | **10.00** | **5.18** | **1.57** | **8.37** | **45.41** |
| 5 | Bezno (b) fluoranthene | 7.0 | 6.37 | 0.63 | 0.71 | 0.43 |
| | Bezno (k) fluoranthene | - | 0 | 0 | 0 | 0 |
| | Dibenzo(a,h)anthracene | - | 0 | 0 | 0 | 0 |
| | **Total concentration (%)** | **7.00** | **6.37** | **0.63** | **0.71** | **0.43** |
| 6 | Benzo(g,h,i) perylene | 4.17 | 55.03 | 46.10 | 0 | 0 |
| | Indeno(1,2,3-cd)pyrene | 5.0 | 33.42 | 0 | 46.9 | 0.84 |
| | **Total concentration (%)** | **9.17** | **88.45** | **46.10** | **46.9** | **0.84** |

**TABLE 5. Continue**

| Number of rings | Compound of polyaromatics | ($I_3$) | | ($I_4$) | | Bacterial consortium | |
|---|---|---|---|---|---|---|---|
| | | Crude oil | Crude oil+ bio. | Crude oil | Crude oil+ bio. | Crude oil | Crude oil +bio. |
| 2 | Naphthalene | 0 | 0 | 2.09 | 0 | 35.4 | 0 |
| | **Total concentration (%)** | **0** | **0** | **2.09** | **0** | **35.4** | **0** |
| 3 | Acenaphthylene | 0 | 30.77 | 0 | 0 | 0 | 18.03 |
| | Acenaphthene | 93.97 | 61.81 | 0 | 84.91 | 0 | 69.23 |
| | Fluorine | 0 | 0 | 9.12 | 9.3 | 0.41 | 1.46 |
| | Phenanthrene | 1.33 | 3.42 | 0 | 1.33 | 2.58 | 1.26 |
| | Anthracene | 0 | 0 | 15.88 | 0.36 | 0 | 0.02 |
| | **Total concentration (%)** | **95.3** | **96.0** | **25.00** | **95.90** | **2.99** | **90.00** |
| 4 | Fluoranthene | 0 | 0 | 0 | 0 | 0 | 0.27 |
| | Pyrene | 0 | 0 | 68.73 | 4.1 | 57.47 | 9.71 |
| | Benzo (a) anthracene | 0 | 0 | 1.2 | 0 | 0.22 | 0.02 |
| | Chrysene | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Total concentration (%)** | **0** | **0** | **69.93** | **4.1** | **57.69** | **10.00** |
| 5 | Bezno (b) fluoranthene | 0.13 | 0.29 | 0 | 0 | 1.86 | 0 |
| | Bezno (k) fluoranthene | 0.69 | 2.88 | 0 | 0 | 0 | 0 |
| | Dibenzo(a,h)anthracene | 1.06 | 0 | 0 | 0 | 0 | 0 |
| | **Total concentration (%)** | **1.88** | **3.17** | **0** | **0** | **1.86** | **0** |
| 6 | Benzo(g,h,i) perylene | 0.57 | 0 | 0 | 0 | 0 | 0 |
| | Indeno(1,2,3-cd)pyrene | 2.25 | 0.83 | 2.98 | 0 | 2.06 | 0 |
| | **Total concentration (%)** | **2.82** | **0.83** | **2.98** | **0** | **2.06** | **0** |

From the last results in Tables 3, 4 and 5 the bacterial strains ($I_1$ and $I_4$) and bacterium consortium of four bacterial isolates were selected for further studies, giving the best surface properties and biodegradation percentage. Also, these bacteria were considered the best stains for degradation of paraffins and polyaromatics. These strains ($I_1$ and $I_4$) were belonging to the *Halomonas xianhensis* strain A-1 ($I_1$) and *Halomonas salifodinae* strain BC7 ($I_4$).

The data in Table 6 illustrated that the percentage biodegradation of crude oil by the two bacterial isolates ($I_1$ and $I_4$) was estimated by gravimetric analysis. The medium containing biosurfactant with nanoparticle and two bacterial strains separately and bacterial consortium gave higher percentage of degradation. The percentage biodegradation was reached into the maximum value in microcosm containing biosurfactant, nanoparticle and bacterial consortium of four the best bacterial strains into 90% after 7 days.

*High performance liquid chromatographic analysis in the presence of nanoparticle*

The microcosms containing bacterial isolate ($I_1$), nanoparticle and biosurfactant, was enhancing the biodegradation of 2- , 4-, 5- and 6-membered rings polyaromatics Table (7). Meanwhile, the microcosms containing the bacterial isolate ($I_4$), biosurfactants and NiO was promoted the isolate for increasing degradation of 3- , 4- membered rings polyaromatics Table (8). While the medium containing biosurfactant with nanoparticle and bacterial consortium gave the complete degradation of 2- membered rings polyaromatics and higher percentage of degradation of 4-membered rings polyaromatics (Table 9). It is necessary to mention that, the increased degradation percentage of 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 the case of lower polyaromatics) or the other probability is the enhanced consumption of other compounds (such as resins compounds) leading to a relative accumulation of such ployaromatic hydrocarbon [30].

From the results obtained in this study, it can be concluded that, the complete degradation of some different membered rings of polyaromatics and the percentage biodegradation of other polyaromatics increased in microcosms containing nanoparticle with biosurfactant at seven days. However, so far, very limited studies have been reported on nanoparticles effect on the biodegradation of crude oil contaminated sites in the presence of biosurfactants. Thus, there are plenty of aspects required to be studied. Each variety of nanoparticles has their own characters. Meanwhile, the selection of the optimal band of nanoparticles and microorganisms is considered a great help of the reaction rates. Proper nanoparticle concentration should be explored referring to that the excessive concentration can be toxic to microorganism, thus, it may reduce the reaction rate. The reaction conditions in the presence of nanoparticle and microorganisms should be studied to find out the optimal conditions for reaction. The combination of nanoparticle and coated with the microorganism or independently dispersed with microorganisms in the solution, is also an important aspect of the reaction rate effect [31].

**TABLE 6. Percentage biodegradation of crude oil after treatment by two bacterial isolates and bacterial consortium.**

| Carbon source | Percentage biodegradation* (%) |
|---|---|
| 0 (inoculation or zero time) | 0 |
| Crude oil + Biosurfactants + $I_1$ | 75 |
| Crude oil +$I_1$ + Biosurfactants + nanoparticle | 85 |
| Crude oil + Biosurfactants + $I_4$ | 77 |
| Crude oil + $I_4$ + Biosurfactants + nanoparticle | 84 |
| Crude oil + Biosurfactants + Bacterial consortium (Mix.) | 80 |
| Crude oil + Biosurfactants + Bacterial consortium (Mix.) + nanoparticle | 90 |

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

**TABLE 7. Biodegradation of polyaromatics in crude oil by bacterial strain ($I_1$) with/without nanoparticle using HPLC analysis.**

| Number of rings | Compound of polyaromatics | Negative control | *Halomonasxianhensis* | |
|---|---|---|---|---|
| | | | Crude oil + biosurfactant | Crude oil+ nanoparticle |
| | Naphthalene | 0.83 | 51.7 | 0.41 |
| **2** | **Total concentration (%)** | **0.83** | **51.7** | **0.41** |
| | Acenaphthylene | 3.21 | 0 | 98.02 |
| | Acenaphthene | 67.65 | 0 | 0 |
| **3** | Fluorine | - | 0 | 0 |
| | Phenanthrene | - | 0 | 0.65 |
| | Anthracene | 2.14 | 0 | 0 |
| | **Total concentration (%)** | **73.00** | **0** | **98.67** |
| | Fluoranthene | 9.34 | 0 | 0 |
| | Pyrene | 0.66 | 0 | 0.33 |
| **4** | Benzo (a) anthracene | - | 1.57 | 0 |
| | Chrysene | - | 0 | 0 |
| | **Total concentration (%)** | **10.00** | **1.57** | **0.33** |
| | Bezno (b) fluoranthene | 7.0 | 0.63 | 0 |
| **5** | Bezno (k) fluoranthene | - | 0 | 0 |
| | Dibenzo(a,h)anthracene | - | 0 | 0 |
| | **Total concentration (%)** | **7.00** | **0.63** | **0** |
| | Benzo(g,h,i) perylene | 4.17 | 46.10 | 0.29 |
| **6** | Indeno(1,2,3-cd)pyrene | 5.0 | 0 | 0.30 |
| | **Total concentration (%)** | **9.17** | **46.10** | **0.59** |

**TABLE 8. Biodegradation of polyaromatics in crude oil by bacterial strain ($I_4$) with/without nanoparticle using HPLC analysis.**

| Number of rings | Compound of polyaromatics | Negative control | *Halomonassalifodinae* | |
|---|---|---|---|---|
| | | | Crude oil + biosurfactant | Crude oil + nanoparticle |
| | Naphthalene | 0.83 | 0 | 18.1 |
| **2** | **Total concentration (%)** | 0.83 | 0 | 18.1 |
| | Acenaphthylene | **3.21** | **0** | **0** |
| | Acenaphthene | 67.65 | 84.91 | 0 |
| **3** | Fluorine | - | 9.3 | 7.71 |
| | Phenanthrene | - | 1.33 | 3.29 |
| | Anthracene | 2.14 | 0.36 | 0 |
| | **Total concentration (%)** | **73.00** | **95.90** | **11.00** |
| | Fluoranthene | 9.34 | 0 | 0 |
| | Pyrene | 0.66 | 4.1 | 1.62 |
| **4** | Benzo (a) anthracene | - | 0 | 0.41 |
| | Chrysene | - | 0 | 0 |
| | **Total concentration (%)** | **10.00** | **4.1** | **2.03** |
| | Bezno (b) fluoranthene | 7.0 | 0 | 0 |
| **5** | Bezno (k) fluoranthene | - | 0 | 0 |
| | Dibenzo(a,h)anthracene | - | 0 | 0 |
| | **Total concentration (%)** | **7.00** | **0** | **0** |
| | Benzo(g,h,i) perylene | 4.17 | 0 | 0 |
| **6** | Indeno(1,2,3-cd)pyrene | 5.0 | 0 | 68.87 |
| | **Total concentration (%)** | **9.17** | **0** | **68.87** |

**TABLE 9. Biodegradation of polyaromatics in crude oil by bacterial consortium with/without nanoparticle using HPLC analysis.**

| Number of rings | Compound of polyaromatics | Negative control | Bacterialconsortium | |
|---|---|---|---|---|
| | | | Crude oil | Crude oil + nanoparticle |
| 2 | Naphthalene | 0.83 | 0 | 0 |
| | **Total concentration (%)** | **0.83** | **0** | **0** |
| 3 | Acenaphthylene | 3.21 | 18.03 | 7.01 |
| | Acenaphthene | 67.65 | 69.23 | 85.16 |
| | Fluorine | - | 1.46 | 0.83 |
| | Phenanthrene | - | 1.26 | 0 |
| | Anthracene | 2.14 | 0.02 | 0 |
| | **Total concentration (%)** | **73.00** | **90.00** | **93.00** |
| 4 | Fluoranthene | 9.34 | 0.27 | 0 |
| | Pyrene | 0.66 | 9.71 | 0.79 |
| | Benzo (a) anthracene | - | 0.02 | 0.19 |
| | Chrysene | - | 0 | 0 |
| | **Total concentration (%)** | **10.00** | **10.00** | **0.98** |
| 5 | Bezno (b) fluoranthene | 7.0 | 0 | 0 |
| | Bezno (k) fluoranthene | - | 0 | 0 |
| | Dibenzo(a,h)anthracene | - | 0 | 5.52 |
| | **Total concentration (%)** | **7.00** | **0** | **5.52** |
| 6 | Benzo(g,h,i) perylene | 4.17 | 0 | 0 |
| | Indeno(1,2,3-cd)pyrene | 5.0 | 0 | 0.5 |
| | **Total concentration (%)** | **9.17** | **0** | **0.5** |

## Conclusion

In this study, four crude oil-degrading bacterial strains were isolated from Suez Oil Processing Company (SOPC), Egypt. There is a direct relationship between both the emulsification activity ($E_{24}$) and the decrease in surface tension with increasing the growth rate on hydrocarbons. The percentage biodegradation was reached to themaximum value in microcosm containing biosurfactant, nanoparticle and bacterial consortium of the best four bacterial strains into 90% after 7 days. The presence of biosurfactant with nanoparticle helps the bacterial isolates to complete degradation of some different membered rings of polyaromatics and the percentage biodegradation of other polyaromatics was increased after 7 days. This result seems to be newly and valuable biodegradation trend. Thus, these bacterial isolates have a potential to be applied in the bioremediation of petroleum contaminated sites using biosurfactant and specific concentration of $Fe_2O_3$ nanoparticle.

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## تحسين المعالجة البيولوجية للزيت الخام بواسطة المواد النانومترية والمواد ذات النشاط السطحي الحيوي

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تتعلق الدراسة الحالية بعملية المعالجة البيولوجية للتربة الملوثة بالزيت البترولي من شركة السويس لتصنيع البترول. تم عزل تسعة عشرعزلة بكتيرية من هذه التربة الملوثة. وأظهرت النتائج ان أربعة أنواع بكتيرية أعطت أعلى معدل نمو على المركبات الهيدروكربونية الموجودة في الزيت الخام. تمت دراسة عملية المعالجة بالتكسيرالحيوي بواسطة السلالات البكتيرية كلا علي حدي او متحدة مع بعضها البعض. تم تخفيض المحتوي الزيتي في الأوساط الغذائية المختلفة تصل إلى ٨٠٪ عن طريق المعالجة البيولوجية بعد ٧ أيام. تم تحليل الزيت الخام المتبقي في الأوساط الغذائية المختلفة بعد أسبوع واحد من فترة التحضين بواسطة جهاز الكروماتوجرافي الغاز. وأظهرت النتائج دور الجسيمات النانومترية المتناهية في الصغر والمواد ذات النشاط السطحي الحيوية في تعزيز المعالجة البيولوجية للبارافينات المشبعة المتفرعة والغير متفرعة. وتم التوصل إلى النسبة المئوية للتحلل البيولوجي عند القيمة القصوى في البيئة الغذائية المصغرة التي تحتوي على والمواد ذات النشاط السطحي الحيوية ، الجسيمات النانومترية و الاتحاد البكتيريي من أفضل أربع سلالات بكتيرية تصل إلى ٩٠٪ بعد ٧ أيام. وقد تم التوصل الي التكسير الكامل لبعض المواد الهيدروكربونية متعددة الحلقات المختلفة وزيادة النسبة المئوية للتحلل البيولوجي لبعض المواد الهيدروكربونية متعددة الحلقات الأخرى في مختلف البيئات الغذائية الميكروبية المصغرة عن طريق جهاز كروماتوجرافيا السائل عالي الكثافة.