Short-Term Biodegradation of Crude Petroleum Oil in Water by Photostimulated Janibacter terrae Strain S1N1

Safaa M. Ezzat and Nashwa A. Ahmed*

ABSTRACT: Biodegradation is a sustainable green strategy that gives the opportunity for remediation of water contaminated with petroleum products. In this study, 12 bacterial isolates were recovered from River Nile, Egypt and screened for their potential to degrade a mixture of para-alkanes from crude oil. The most promising isolate was identified according to 16S rRNA sequencing as Janibacter terrae strain S1N1 (GenBank accession No. KX570955.1). In order to boost the biodegradation efficiency, the bacterial suspension was photostimulated by exposure to different irradiation doses using a low-power helium–neon (He–Ne) laser (λ = 632.8 nm). Maximum biodegradation was achieved after 4 min of exposure (134.07 J cm\(^{-2}\)) at optimized pH value (6) and temperature (35 °C). The gas chromatography–mass spectrometry (GC-MS) analysis revealed the biodegradation of 96.5% of the substrate after only 48 h of incubation. The n-C\(_{17}/\)Pr and n-C\(_{18}/\)Ph ratios indicated a preferential biodegradation of iso-para-alkanes over normal ones. Meanwhile, pristane/phytane (Pr/Ph) ratios were indicative of selective biodegradation for pristane. The carbon preference index (CPI) was nearly around unity indicating the ability of Janibacter terrae to attack the odd and even n-alkanes simultaneously. These results support the superiority of irradiated bacteria in optimizing the biodegradation efficiency and shortening the time of treatment, thus proposing an eco-friendly technique in water bioremediation programs.

1. INTRODUCTION

The accidental spillage of crude petroleum oil in the water environment is considered a major threat to ecosystems and human beings worldwide. Incidence of petroleum hydrocarbons in water bodies is deemed crucial to a great extent, as most of these compounds are recalcitrant in nature, toxic, and persistent in the aquatic environment.\(^1\) Rivers, lakes, as well as groundwater experiencing such events are vulnerable to long-term ecological disturbances, and people in affected zones are usually subject to health problems and lost access to clean water.\(^2\)

Crude oil constitutes one of the basic composite mixtures of organic compounds forming petroleum products. It is a heterogeneous liquid of hydrocarbons, basically comprising hydrogen and carbon in the ratio of about 2:1, respectively. Other elements such as oxygen, nitrogen, and sulfur constitute approximately <3%. Based on distillation residues, crude oil could be classified into paraffins, naphthenes, or aromatics. More than 17 000 chemical compounds have been identified as components of crude oil.\(^3\) Considering this complexity, it has become inevitable to adopt an efficient and economic methodology to overcome oil spill contamination.

Chemical and mechanical techniques are conventional methods usually applied for crude oil remediation. They may include mechanical collection, burning, sinking, and dispersion, using sorbent agents, volatilization, and photooxidation.\(^4\) Actually, these methods are inadequate since they predominantly result in imperfect cleanup and/or development of undesirable secondary products; they are considered cost prohibitive as well.

On the other hand, biodegradation has emerged as a safe, effective, and eco-friendly alternative technology having the potential to treat contaminated sites in situ or to act as a polishing step after chemical and mechanical options.\(^5\) It refers to the complete mineralization of organic pollutants by microorganisms into carbon dioxide, water, and biomass or conversion of complicated organics into simpler compounds less toxic or completely harmless.\(^6\) Biodegradation of crude oil can be mediated by several mechanisms, the most important of which involves specific microbial enzyme systems. Oxygenases, peroxidases, dehydrogenases, and hydroxylases are considered enzymatic key reaction catalysts in biodegradation. Other mechanisms include microbial cell attachment to oil spills and production of biosurfactants that enhance solubilization and removal of pollutants.\(^7\)
Among petroleum hydrocarbons, n-alkanes are the most amenable to biodegradation. However, alkanes (C5 to C10) are considered inhibitory to the majority of hydrocarbon degraders. During the degradation process, the longer chain alkanes are converted to alcohol under the action of oxygenase enzymes. The produced alcohol is further oxidized to yield aldehyde and then fatty acid. Furthermore, the utilization of fatty acid occurs by β-oxidation of the aliphatic chain to provide energy for microbial activities.8–10

As noted, the success of bioremediation technology highly depends on the presence of native microorganisms with appropriate metabolic capabilities. It is evident that microbial communities which were exposed to hydrocarbon pollutants become more adapted and can exhibit higher biodegradation rates than other communities. Accordingly, microorganisms isolated from contaminated areas are active oil degraders and can be used in bioremediation techniques. In addition, both physicochemical and biological variables influence the rate of microbial growth and enzymatic activity. Accessibility of nutrients, O2 concentration, pH, salinity, and temperature are important factors usually assessed by researchers to optimize the process outputs.10

Most crude oils are biodegradable; however, the process is often very slow and may last for up to 60 days or more.11 Consequently, time is considered a limiting factor governing successful biodegradation. One way to enhance biodegradation and to shorten the time needed for treatment is to photostimulate the microorganism employed in the process. Recently, low-intensity laser radiation with wavelength of 400–600 nm was studied to enhance the activity of microbial enzymes. It brings about accelerated cell division in various microorganisms and enhances protein synthesis; however, higher doses were usually inhibitory.12

The helium–neon (He–Ne) laser is a type of gas laser in which the gain medium is composed of a mixture of helium and neon incorporated into a small-bore capillary tube. The wavelength 632.8 nm is the best known and most widely used He–Ne laser that operates in the red part of the visible spectrum. As is well-known, laser stands for “light amplification by stimulated emission of radiation”. It can stimulate atoms or molecules to emit light at a certain wavelength and amplify that light, producing a focused and very narrow beam of high energy that does not spread out much. Since they are practical and cost-effective devices, they have been increasingly used in many scientific, military, medical, and commercial applications.13

The present study aims to isolate, screen, and identify a native bacterial strain from River Nile in Egypt capable of utilizing and degrading paraffinic petroleum crude oil that could probably develop either from oil leaks and/or accidental spills. The potential of photostimulation using low-power He–Ne laser radiation will be investigated to optimize the biodegradation efficiency of the selected bacteria in a calculated time interval.

2. MATERIALS AND METHODS

2.1. Water Samples. This study was started in 2018 by collecting water samples (n = 15) from five different points at ship settlement stations along the River Nile at El-Roda (927 Km from Aswan High Dam), Cairo governorate, Egypt. Sampling procedure and water analysis were carried out according to Standard Methods for the Examination of Water and Wastewater.14 All samples were delivered in an iced cooler box to the Central Laboratory for Environmental Quality Monitoring (CLEQM), National Water Research Center (NWRC) and analyzed within 6 h. Physico-chemical analysis included pH, temperature, biochemical oxygen demand (BOD), chemical oxygen demand (COD), oil, and grease. Field measurements were performed for pH and temperature using the Hydralab-Surveyor multiprobe system, BOD was measured using the ORION 890 BOD fast respirometry system (measuring range 0–400 mg L−1) at 20 °C (method 5210B, 5210D), while for COD the spectrophotometer DR/2010 (model 690) with COD reactor (HACH) was used (method 5220D). Oil and grease were determined using the partition-gravimetric technique (method 5520B).

2.2. Isolation of Crude Oil Degrading Bacteria. In this study, light paraffinic petroleum crude oil was obtained from the Egyptian Petroleum Research Institute, Ministry of Scientific Research and Technology, Egypt, and used as a sole carbon source for bacterial growth. Some physicochemical properties of used oil were given as follows; specific gravity at 15 °C (0.901), pour point (27 °C), flash point (209 °C), density at 15 °C (0.913 g mL−1), and viscosity at 70 °C (10.5 mPa.s).

Basal salts medium (BSM) was prepared with the following composition (g L−1): NaCl (10.0); KCl (0.29); MgSO4·7H2O (0.42); KH2PO4 (0.83); NaNO3 (0.42); Na2HPO4 (1.25) according to Liu et al.15 The medium was solidified with purified agar (20.0 g), and the pH was adjusted at 7. After autoclaving, 0.5% (v/v) of paraffinic petroleum crude oil was added. Isolation of oil degrading bacteria was performed by spread inoculation of 0.1 mL of the water samples on the prepared media, and after 24 h of incubation at 37 °C, distinct colonies were picked and purified by streaking on nutrient agar and stored at 4 °C as slant cultures for subsequent investigation.

2.3. Screening of the Most Potent Bacterial Isolate. The pure culture of each isolate was standardized in which a single colony of each isolate is inoculated in 10 mL nutrient broth and grown for 24 h at 37 °C. The cell suspensions were centrifuged at 150 rpm for 20 min to obtain a cell pellet of each isolate. The cell pellets were then washed with BSM three times to remove the traces of nutrient broth and finally suspended in BSM medium. The optical densities of cell suspensions were adjusted to be approximately equivalent to 105 cfu mL−1. The isolated microbial strains were separately tested for their biodegradation potential in 250 mL Erlenmeyer flasks containing 100 mL BSM amended with 0.5% (v/v) paraffinic petroleum crude oil as a sole carbon source. The flasks were inoculated with each purified bacterial isolate (105 cfu mL−1), while the control flasks were left without cultures. After incubation at 37 °C for 48 h with constant shaking at 150 rpm, populations of the resulting cultures were enumerated by plating (100 μL) on the surface of nutrient agar and incubation at 37 °C for 48 h. The colonies of each culture were counted, and the best degrader bacterial isolate was selected on the basis of its highest growth as indicated by bacterial count.16

2.4. Identification of the most potent bacterial isolate. Microscopic and biochemical examinations were carried out for the selected pure isolate showing maximum potential for crude oil biodegradation. Microscopic examinations included Gram staining, spore formation, and motility using a light microscope (100× and 40×) objectives. Biochemical tests included Indole, Vogas-Proskauer, methyl red, catalase, oxidase, and urease reactions. Production of H2S
and reduction of nitrates as well as hydrolysis of starch, gelatin, and Tween 80 were also examined. Acid produced from several carbohydrates, viz., D-glucose, D-fructose, D-mannose, D-galactose, D-mannitol, lactose, and maltose were also tested. Molecular characterization involved extraction of the genomic DNA using the protocol of GeneJET genomic DNA purification kit (Fermentas). The extracted DNA was then used as a template for 16S rRNA gene amplification through polymerase chain reaction (PCR) using Maxima Hot Start PCR Master Mix (Fermentas). The universal primers used for the amplification were 8F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and U1492R (5′-GGT TAC CTT GGT ACG ACT T-3′). The PCR reaction was performed according to the recommended thermal cycling conditions as follows: initial denaturing at 95 °C for 10 min (1 cycle), denaturing at 95 °C for 10 s, annealing at 65 °C for 1 min, and extension at 72 °C for 2 min. The previous three cycles were repeated 35 times. The final extension was done at 72 °C for 10 min (1 cycle). The PCR product was cleaned up using GeneJET PCR purification kit (Fermentas) and sequenced using the ABI 3730xl DNA sequencer following the manufacturer’s instructions. The sequence was submitted to the database of the National Center for Biotechnology Information (NCBI) GenBank (http://www.ncbi.nlm.nih.gov/BLAST) and compared to other available sequences, and a phylogenetic tree was constructed by the MEGA software version 6.

2.5. Optimization of Biodegradation Process. Duplicate screw-capped bottles containing 100 mL BSM seeded with 0.5% (v/v) petroleum crude oil were adjusted at different pH values (5, 6, 7, 8, 9) using 1 mol L⁻¹ HCl and NaOH solutions. The media were inoculated (10⁸ cfu mL⁻¹) and incubated in a shaking incubator (150 rpm) at 37 °C. After 48 h, 100 μL from each bottle was withdrawn and spread on BSM. Bacterial growth measurement was recorded as cfu mL⁻¹ after 24 h of incubation at 37 °C, and the optimum pH value was determined. For temperature, duplicate screw-capped bottles containing 100 mL BSM amended at 0.5% (v/v) of the substrate were inoculated (10⁶ cfu mL⁻¹) and incubated at 20, 30, 35, 37, and 40 °C. After 48 h, the optimum incubation temperature was determined as in the previous step.

2.6. He–Ne Laser Irradiation Procedure. Irradiation of the selected bacterial isolate was performed in the physics department at the Faculty of Science, Cairo University, Egypt using a He–Ne laser tube (NEC Corporation, Japan) at a wavelength of 632.8 nm. The laser irradiation was transmitted through an 800 mm optical fiber, which was fixed with a delivery arm permitting precise positioning of the fiber tip at 20 mm height, while the area of the focal spot was 0.013 cm². The bacterial suspension (at optimum pH and temperature) was subjected to different radiation exposure durations (1–5 min), and the nonirradiated suspension was used as control. The equivalent laser irradiation dose (Q) was calculated according to the following equation:

\[ Q = W \times T/S \]  

(1)

W is the output power of the laser radiation (Watt); T is the time (seconds); S is the spot area of the laser beam (cm²).

The bacterial suspensions (10⁸ cfu mL⁻¹) before and after irradiation were inoculated into triplicate screw-capped bottles containing 100 mL BSM and seeded with 0.5% (v/v) of petroleum crude oil. After 48 h of incubation, 100 μL from each bottle was withdrawn and spread on BSM. The bacterial count was recorded as cfu mL⁻¹ after 24 h of incubation at 37 °C, and the optimum radiation dose was determined.

2.7. Determination of the Crude Oil Biodegradation Efficiency. Triplicate screw-capped bottles containing 100 mL BSM were inoculated with the selected isolate before and after its exposure to He–Ne laser at different time of exposure (1–5 min). After incubation for 48 h under shaking conditions (150 rpm), the residual substrates were extracted twice with petroleum ether. 20 mL of petroleum ether was mixed with 50 mL culture broth in a separating funnel and shaken vigorously for 30 min to form two distinguish layers. The top layer contained a mixture of residual crude petroleum oil and petroleum ether, while the bottom contained the culture broth. After 30 min, the bottom layer was drained and the top layer was collected in a beaker. Anhydrous sodium sulfate was applied to remove the moisture content in the petroleum ether/residual crude oil mixture. The mixture was then decanted into a preweighed beaker and oven-dried for about 30 min. After cooling at room temperature, the beaker was reweighed and the gravimetric estimation of the residual crude oil percentage was calculated according to the following equation:

\[ F = W_{res} - W_{res}/W_{ocs} \times 100 \]  

(2)

F is biodegradation efficiency percentage, W_res is weight of residual crude oil in control; W_res is weight of residual crude oil in sample, and W_ocs is original weight of crude oil in sample.

2.8. Assessment of Biodegradation Potential. In order to follow up the biodegradation route of the paraffinic crude petroleum oil, samples were prepared in screw-capped bottles containing 100 mL BSM seeded with 0.5% (v/v) of the oil and the media were inoculated (10⁶ cfu mL⁻¹) with the selected isolate before and after irradiation at optimum conditions of pH and temperature. After 24 and 48 h of incubation under shaking conditions (150 rpm), the residual substrates were extracted with petroleum ether and chromatographically analyzed using gas chromatography–mass spectrometry (GC–MS). Analysis was run on Agilent 19091S-433 equipped with HP-SMS column 30 m (length) × 0.35 mm (internal diameter) × 0.25 μm (film thickness). The oven temperature program was set from 80 to 300 °C at a fixed rate of 3 °C min⁻¹. The GC injector was held isothermally at 280 °C with a split-less period of 3 min, and the solvent delay time was set at 3.5 min. It was used as a carrier gas at a flow rate of 1 mL min⁻¹. The GC-MS interface temperature was maintained at 280 °C, and the scan ranged from 35 to 300 amu (atomic mass unit). The MS ion source and mass filter (quad) temperatures were held at 230 and 150 °C, respectively. The extracted oil samples were analyzed to determine the degree of biodegradation of paraffinic hydrocarbons. The percentage loss of biodegraded n- and isoparaffins was calculated by comparing the areas of the peaks obtained in the case of treated samples with those of crude oil used, taking into consideration that the amounts of injected samples were exactly the same in all cases.

2.9. Statistical Analysis. Data were presented as mean ± standard deviation of three separate experiments. The data was then exposed to one-way ANOVA and Dunnett’s test with GraphPad in Stat software in order to determine statistical significance at p < 0.05.
3. RESULTS AND DISCUSSION

3.1. Quantitative Assessment for Oxygen Demanding Substances in Water Samples. Physico-chemical characteristics of water samples collected for isolation of bacteria capable of crude oil biodegradation are listed in Table 1. As given from the obtained results, mean temperature (24.2 ± 0.325) and pH (8.2 ± 0.246) values were in accordance with the permissible limits of the Egyptian Law 48/1982 Decree No. 49 amended in 2013 for surface water. In addition, both BOD and COD were evaluated to reflect the oxygen burden possibly present due to organic pollutants in investigated water. BOD determines the quantity of dissolved oxygen consumed by indigenous bacteria to decompose the organic matter, while COD represents the amount of oxygen required for complete oxidation of organic matter chemically into CO₂ and H₂O using strong oxidizing agents. The recorded mean values (28.7 ± 0.154 mg L⁻¹ for BOD) and (53.8 ± 0.248 mg L⁻¹ for COD) were higher than recommended permissible limits (<6 mg L⁻¹ and <10 mg L⁻¹ for BOD and COD, respectively). The increased levels of BOD and COD indicate susceptibility of water in the area of study to possible organic pollution. In the same context, the COD/BOD ratio is considered a crucial factor that could describe the extent of biodegradability by which organic matter is readily broken down in aquatic systems. A ratio of 2.0–2.1 was considered optimum in biodegradable environments. Meanwhile, higher COD/BOD ratios (3–5-fold) reveal intensive nonbiodegradable and persistent organic substances. As presented in Table 1, the COD/BOD ratio ranged between a minimum of 1.5 and a maximum of 2.0 with a mean value 1.8. These data reveal the expected biodegradation activity of naturally occurring microflora for organic pollutants in water under study and agree with those reported by Xu et al.²⁴

Table 1. Physico-Chemical Characteristics of Water Samples

| Parameters       | Units     | Min   | Max    | Mean ± SD  |
|------------------|-----------|-------|--------|------------|
| Temp             | °C        | 23.5  | 24.7   | 24.2 ± 0.325 |
| pH               | -         | 7.9   | 8.3    | 8.2 ± 0.246  |
| BOD              | mg L⁻¹    | 22.0  | 32.0   | 28.7 ± 0.154 |
| COD              | mg L⁻¹    | 45.0  | 60.0   | 53.8 ± 0.248 |
| COD/BOD          |           | 1.5   | 2.0    | 1.8 ± 0.183  |
| Oil and grease   | mg L⁻¹    | 38.6  | 50.7   | 44.25 ± 0.175 |

Values are means of replicate samples (n = 15).

Table 2. Morphological and Biochemical Characteristics of Janibacter terrae

| Characteristics                                      | Results |
|------------------------------------------------------|---------|
| Growth temperature range                              | 10–40 °C |
| Growth pH range                                       | 5–10    |
| Growth in presence of NaCl                            | Up to 8% |
| Color of colony                                       | Pale cream |
| Shape of colony                                       | Circular, opaque, and convex |
| Gram staining                                         | +²⁺      |
| Cell morphology                                       | Cocci |
| Cell arrangement                                      | Single |
| Motility                                              | -ᵇ      |
| Spore formation                                       | –       |
| Indole, Vogas-Proskauer, methyl red, and urease reactions | –       |
| Catalase reaction                                     | +       |
| Oxidase reaction                                      | W⁻      |
| Nitrate reduced to nitrite                            | +       |
| Gelatin and tween 80 hydrolysis                       | +       |
| Starch hydrolysis                                     | –       |
| H₂S production                                       | +       |
| Acid produced from D-glucose, D-fructose, D-mannose, D-galactose, D-mannitol, lactose, and malrose | – |

Values are means of replicate samples (n = 15).

As presented in Table 2, the phenotypic properties obtained showed coincidence with a description in the literature for Janibacter terrae given by Yoon et al.¹⁷ Furthermore, the 16S rRNA gene sequence of the strain S1N1 was analyzed and compared with strains from other related taxa. The BLAST search revealed that this strain is located in the evolutionary position occupied by the genus Janibacter. Figure 1 demonstrates the results of phylogenetic characterization and shows that strain S1N1 is most likely related to a type strain Janibacter terrae, and it was given the accession No. KX570955.1 from GenBank.

3.3. Optimum Conditions for Biodegradation Process. Successful biodegradation requires the development of metabolic activities by microorganisms that are capable of converting the pollutants to a more simple and safe form. When conditions are favorable, the biodegradation process will reach an optimum level. Microbial activities have been recognized as affected by a number of limiting factors, the most important of which are pH and temperature. In this work, the optimum conditions studied for Janibacter terrae were detected by determination of the viable bacterial count. Its increase reflects the ability of utilizing the substrate as a sole carbon and energy source for growth. The pH value is considered one of the main factors affecting the progress of the bioremediation process. pH of the medium can affect the electrical charges on the various chemical groups present in enzyme molecules leading to uncoiling of the protein chain. Any slight change in pH may lead to denaturation of the enzymes related to biodegradation.²⁶ As shown in Figure 2, the maximum biomass (4.4 × 10⁵ cfu mL⁻¹) for Janibacter terrae was recorded at pH 6 which was reported as the optimum pH value at which maximum biodegradation could be achieved. Similar results were obtained by Kumar et al.²⁷ who indicated that the neutral range is optimal for biodegradation, and extremes in pH values
(either acidic or alkaline) may have a negative influence on the ability of the bacterial population to degrade hydrocarbons. pH 6 was considered in further studies.

Temperature is among the factors that influence the biodegradation process through its direct effect on the physical and chemical nature of the pollutants as well as the physiology and diversity of the microbial flora. In the present study, the effect of incubation temperature on the growth of *Janibacter terrae* was investigated. It was evident from results that the optimum incubation temperature at which the maximum population was obtained (4.8 × 10⁵ cfu mL⁻¹) was at 35 °C (Figure 2). These results are consistent with those reported by Sihag et al.²⁸ who indicated that most hydrocarbon degraders are active in the mesophilic range of 25 to 40 °C. Callaghan²⁹ reported that at low temperatures the viscosity of the oil increases while the volatility of the toxic low-molecular-weight hydrocarbons reduces, thus delaying the onset of the biodegradation process. Meanwhile, at high temperatures the toxicity of some compounds may inhibit the microbial metabolism. Temperature 35 °C was considered in further studies.

### 3.4. Effect of He–Ne Laser Radiation on Biodegradation Efficiency of *Janibacter terrae*

As our target has turned to enhancing the potential of the biodegradation process and to decrease the time needed for bioremediation, it was decided to use low-intensity laser radiation to accelerate cell division and intensify protein synthesis in *Janibacter terrae*. Similar attempts have been reported in other microorganisms.³⁰ In this regard, the bacterial cells were subjected to a wide range of low-power He–Ne laser doses (33.69–168.45 J cm⁻²) performed by varying the exposure time from 1 to 5 min. Quantitative results demonstrated in Tables 3 and 4 revealed that irradiation had induced notable improvement in both the bacterial growth and the oil biodegradation efficiency in comparison to the control (nonirradiated). Depending on the exposure time and dose, the He–Ne laser had variable potential on the growth response of *Janibacter terrae*. Extending the irradiation time from 1 to 4 min (134.07 J cm⁻²) induced a significant maximum increase in the bacterial population (12.8 × 10⁵ cfu mL⁻¹). Increasing the count of bacterial colonies indicates that the bacteria are capable of utilizing the crude oil as a sole source of carbon and energy and can proliferate well. In this context, enhancement of cell proliferation could be a clear indicator for increasing both enzyme quantity and activity, thus leading to improvement in biodegradation efficiency.³¹ Data obtained through this study showed that the biodegradation efficiency of the studied substrate was enhanced by about 4.45-fold reaching a maximum value of 96.5% after 4 min of irradiation compared to nonirradiated cells (21.7%). Our results are in harmony with those reported by El Naggar et al.¹²,¹³ By contrast, extending the exposure duration to 5 min (168.45 J cm⁻²) resulted in a decrease in the bacterial count.

---

**Figure 1.** Phylogenetic tree of *Janibacter terrae* constructed with the neighbor joining method.

**Figure 2.** Determination of the optimum pH and temperature values for biodegradation.
under low laser radiation on survival and cell morphology, a study on the effects of low-level monochromatic laser radiation considering the oil as the sole carbon and energy source. Based on earlier investigations, laser irradiation retains an optimum dose that can modulate maximum enzymatic activity. Higher or lower doses usually induce tangible depletion in activity.33 In all cases, the extent of observed enhancement in cell growth rate is time- and dose-dependent, and strictly parallels the trend of biodegradation performance.

Regarding the in vitro effects of low-level monochromatic laser radiation on survival and cell morphology, a study on Pantoea agglomerans under low fluencies of laser radiation showed increased bacterial survival with no changes in morphology or cell aggregation.34

In another study, high fluences of low-intensity red and IR lasers were reported as being lethal, inducing filamentation, and alteration in the cell morphology of E.coli.35 In earlier investigations, phototreatment of E. coli (632.8 nm, 4 J cm⁻²) stimulated the cells to exhibit both biochemical and morphological changes, including intensified cytoplasmic membrane protein synthesis and increase in cell volume and content of ribosomes. These changes were suggestive of enhanced cell metabolism.36 In this respect, both proliferative and inhibitory effects are species-dependent. The exposure parameters (fluency, directionality, emission mode, and high monochromaticity) are also considered key factors controlling the net result effect.

In order to present a more detailed explanation regarding the response of bacterial cells to the He–Ne laser effect, three mechanisms are proposed.37 The first mechanism deals with stimulation effects in which it is suggested that low power lasers can promote cell proliferation through a "photochemical mechanism". In this mechanism, certain cellular component molecules, the so-called photoacceptors belonging to the bacterial respiratory chain, absorb the emitted photons, changing their ground state to excited state. They become more adapted to pump protons across the plasma membrane, thus increasing the motive force of the protons. An increase in ATP synthesis follows which enables the bacterial cells to increase the synthesis of DNA, RNA, and proteins. In turn, bacterial cells proliferate and divide at higher rates. The second mechanism explains the inhibition effect. Bacterial inhibition could result from activating endogenous photosensitizers. Subsequently, photosensitizers transfer their excess energy to cell molecules leading to an increase in free radical production. These free radicals are highly reactive at the cytotoxic level leading to damage in the cellular structure. On the other hand, the third mechanism suggests a useless response in which activation of endogenous photoacceptors and photosensitizers occurs at the same level. Under such a condition, ATP synthesis and free radicals production will be balanced. Figure 3 demonstrates the proposed photochemical mechanism of laser-induced activation suggested in the current study.

It is worth mentioning that the improvement attained in crude oil biodegradation through this investigation was achieved within only 48 h of incubation under the influence of He–Ne laser radiation. Other related studies involving conventional biodegradation reported longer treatment periods of time ranging 14–60 days.11,31 Exposure times of 4 min and laser energy dose of 134.07 J cm⁻² were considered in further studies.

3.5. Biodegradation Followup. As revealed from GC-MS analysis given in Table 5 and illustrated by Figure 4, it was evident that there was a gradual decrease in the weight percentages of the residual normal and iso-paraffinic hydrocarbons ranging from C₁₇ to C₉₀. This decrease was detected after bacterial inoculation compared to the control (without bacteria) and was more pronounced in samples treated with irradiated bacteria (24 and 48 h incubation).

Results showed a relatively slight reduction in the weight percentage of the total paraffinic fraction after inoculation with experimental conditions.

Table 3. Effect of He–Ne Laser Radiation on Bacterial Growth

| Time of exposure (min) | Equivalent laser energy dose (J cm⁻²) | Bacterial count (cfu mL⁻¹) × 10⁵ |
|------------------------|--------------------------------------|----------------------------------|
|                        |                                      | Mean ± SD | Min ± Max | COV | P     |
| 0                      | 0.00                                 | 4.7 ± 0.15 | 4.6 ± 4.9 | 0.0322 | 0.0326   |
| 1                      | 33.69                                | 5.1 ± 0.15 | 5.0 ± 5.3 | 0.0297 | 0.0000   |
| 2                      | 67.38                                | 5.3 ± 0.21 | 5.1 ± 5.5 | 0.0390 | 0.0079   |
| 3                      | 101.07                               | 7.0 ± 0.20 | 6.8 ± 7.2 | 0.0285 | 0.0026   |
| 4                      | 134.07                               | 12.6 ± 0.15| 12.5 ± 12.8| 0.0120 | 0.0001   |
| 5                      | 168.45                               | 3.1 ± 0.26 | 2.9 ± 3.4 | 0.0853 | 0.0003   |

“Values are means of three replicate samples.

Table 4. Effect of He–Ne Laser Radiation on Oil Biodegradation Efficiency

| Time of exposure (min) | Equivalent laser energy dose (J cm⁻²) | Biodegradation efficiency of the crude oil (%) |
|------------------------|--------------------------------------|-----------------------------------------------|
|                        |                                      | Mean ± SD | Min ± Max | COV | P     |
| 0                      | 0.00                                 | 21.5 ± 0.26 | 21.2 ± 21.7 | 0.0172925 | 3.093 × 10⁻⁰⁰ |
| 1                      | 33.69                                | 34.3 ± 0.20 | 34.1 ± 34.5 | 0.005830904 | 4.61674 × 10⁻⁰⁶ |
| 2                      | 67.38                                | 36.4 ± 0.20 | 36.2 ± 36.6 | 0.00549505 | 2.03374 × 10⁻⁰⁸ |
| 3                      | 101.07                               | 47.5 ± 0.20 | 47.3 ± 47.7 | 0.004210526 | 1.60744 × 10⁻⁰⁶ |
| 4                      | 134.07                               | 96.3 ± 0.20 | 96.1 ± 96.5 | 0.0002076843 | 1.87386 × 10⁻¹⁰ |
| 5                      | 168.45                               | 11.7 ± 0.26 | 11.5 ± 12.0 | 0.00226 | 0.000385 |

“Values are means of three replicate samples.
the non-irradiated bacterial isolate (78.28% left). On the other hand, inoculation of the oil sample with the bacterial culture subjected to He−Ne laser radiation dose equivalent to 134.07 J cm$^{-2}$ had led to enhancement of the biodegradation process, and the percentage of total residual compounds did not exceed 11.34% after only 24 h of incubation. It was noted that there was a complete degradation of some low (C$_{11}$−C$_{15}$) and heavy (C$_{34}$−C$_{36}$) fractions which can be attributed to their low concentrations that make them easily bioavailable. Increasing the incubation time to 48 h promotes complete biodegradation of more heavy components (C$_{31}$−C$_{36}$) and the total residual compounds were decreased to 3.5%. These results agreed with those of Popov et al. who reported that application of low

Figure 3. Schematic diagram showing the photochemical mechanism of laser-induced activation.

Table 5. Weight Percentages of Residual Compounds Obtained from GC-MS Analysis

| Residual compounds | Carbon number | Weight percentage of residual compounds (%) |
|--------------------|---------------|---------------------------------------------|
|                    |               | Control | Nonirradiated | Irradiated 24 h | Irradiated 48 h |
| Undecane           | C$_{11}$      | 0.58    | 0.22          | 0.00            | 0.00            |
| Dodecane           | C$_{12}$      | 0.20    | 0.21          | 0.00            | 0.00            |
| Tridecane          | C$_{13}$      | 1.93    | 0.86          | 0.00            | 0.00            |
| Tetradecane        | C$_{14}$      | 7.81    | 4.31          | 0.00            | 0.00            |
| Pentadecane        | C$_{15}$      | 1.18    | 1.04          | 0.00            | 0.00            |
| Hexadecane         | C$_{16}$      | 1.83    | 1.35          | 0.05            | 0.02            |
| Heptadecane        | C$_{17}$      | 15.11   | 14.63         | 1.06            | 0.19            |
| Pristane           | Pr            | 1.26    | 1.22          | 0.02            | 0.002           |
| Octadecane         | C$_{18}$      | 6.22    | 5.91          | 0.65            | 0.32            |
| Phytane            | Ph            | 1.17    | 1.10          | 0.03            | 0.01            |
| Nonadecane         | C$_{19}$      | 15.10   | 13.30         | 1.18            | 0.54            |
| Eicosane           | C$_{20}$      | 1.04    | 0.78          | 0.33            | 0.15            |
| Heneicosane        | C$_{21}$      | 3.01    | 2.65          | 1.27            | 0.12            |
| Docosane           | C$_{22}$      | 13.90   | 9.13          | 0.45            | 0.32            |
| Tricosane          | C$_{23}$      | 1.21    | 1.04          | 0.41            | 0.35            |
| Tetracosane        | C$_{24}$      | 1.20    | 0.94          | 2.13            | 0.05            |
| Pentacosane        | C$_{25}$      | 1.50    | 0.99          | 0.20            | 0.07            |
| Hexacosane         | C$_{26}$      | 13.19   | 10.09         | 1.28            | 0.62            |
| Heptacosane        | C$_{27}$      | 1.22    | 0.85          | 0.20            | 0.11            |
| Octacosane         | C$_{28}$      | 6.10    | 4.08          | 1.01            | 0.30            |
| Nonacosane         | C$_{29}$      | 0.44    | 0.41          | 0.36            | 0.26            |
| Tricontane         | C$_{30}$      | 2.42    | 1.74          | 0.20            | 0.07            |
| Hentriacontane     | C$_{31}$      | 1.21    | 0.71          | 0.30            | 0.00            |
| Dotriacontane      | C$_{32}$      | 0.61    | 0.35          | 0.10            | 0.00            |
| Trittiacontane     | C$_{33}$      | 0.31    | 0.23          | 0.11            | 0.00            |
| Tetratriacontane   | C$_{34}$      | 0.13    | 0.07          | 0.00            | 0.00            |
| Pentatriacontane   | C$_{35}$      | 0.08    | 0.04          | 0.00            | 0.00            |
| Hexatriacontane    | C$_{36}$      | 0.04    | 0.03          | 0.00            | 0.00            |
| Total              |               | 100     | 78.28         | 11.34           | 3.50            |
power He–Ne laser supports cells proliferation, activates the oxygen consumption, and increase the enzymatic activities. All of these conditions favor the bacterial potential for biodegradation.

The iso-paraffines pristane Pr (2,6,10,14-tetramethyl pentadecane) and phytane Ph (2,6,10,14-tetramethyl hexadecane) have been considered as important conservative biomarkers in oil biodegradation studies. In spite of their known resistance to biodegradation than n-alkanes, it was evident from the results given in Table 6 that the new isolate *Janibacter terrae* was able to attack these compounds successfully. Irradiation stimulated the substantial depletion in the weight percentages of the two iso-paraffines compared to the control and the nonirradiated samples. The degradation percentages reached approximately 99.8% and 99.1% for Pr and Ph, respectively, after 48 h of incubation.

Additionally, the ratios n-C<sub>17</sub>/Pr, n-C<sub>18</sub>/Ph, and Pr/Ph were calculated for residual hydrocarbon compounds and used as indices to follow up the pathway of biodegradation. The calculated ratios over the prescribed time intervals (24 and 48 h) showed a significant increase in n-C<sub>17</sub>/Pr and n-C<sub>18</sub>/Ph ratios after treatment with the irradiated isolate which revealed a preferential degradation of iso-paraffines over normal ones. This could be attributed to the nature of the microorganism employed in the biodegradation process. On the other hand, the decreased values of Pr/Ph ratios after irradiation compared to the control indicate selective biodegradation for pristane. Similar findings were reported by Christova et al.6

Carbon preference index (CPI) which represents the relative abundance of odd-numbered linear alkanes versus even-numbered linear alkanes was calculated for residual hydrocarbon mixture before and after irradiation. Results in Table 6 showed that in all samples CPI was nearly constant and around unity, indicating that *Janibacter terrae* has the ability to attack the odd and even n-alkanes simultaneously and nearly by the same ratio.

Overall, it is noted from the literature that investigating the biodegradation activity of *Janibacter terrae* is somehow limited. *Janibacter terrae* strain XJ-1 was reported as a degrader of paraffins, it was evident from the results given in Table 6 that the new isolate *Janibacter terrae* was able to attack these compounds successfully. Irradiation stimulated the substantial depletion in the weight percentages of the two iso-paraffines compared to the control and the nonirradiated samples. The degradation percentages reached approximately 99.8% and 99.1% for Pr and Ph, respectively, after 48 h of incubation.

Additionally, the ratios n-C<sub>17</sub>/Pr, n-C<sub>18</sub>/Ph, and Pr/Ph were calculated for residual hydrocarbon compounds and used as indices to follow up the pathway of biodegradation. The calculated ratios over the prescribed time intervals (24 and 48 h) showed a significant increase in n-C<sub>17</sub>/Pr and n-C<sub>18</sub>/Ph ratios after treatment with the irradiated isolate which revealed a preferential degradation of iso-paraffines over normal ones. This could be attributed to the nature of the microorganism employed in the biodegradation process. On the other hand, the decreased values of Pr/Ph ratios after irradiation compared to the control indicate selective biodegradation for pristane. Similar findings were reported by Christova et al.6

Carbon preference index (CPI) which represents the relative abundance of odd-numbered linear alkanes versus even-numbered linear alkanes was calculated for residual hydrocarbon mixture before and after irradiation. Results in Table 6 showed that in all samples CPI was nearly constant and around unity, indicating that *Janibacter terrae* has the ability to attack the odd and even n-alkanes simultaneously and nearly by the same ratio.

Overall, it is noted from the literature that investigating the biodegradation activity of *Janibacter terrae* is somehow limited. *Janibacter terrae* strain XJ-1 was reported as a degrader of Dibenzo furan. Dibenzo furan was degraded to 2,2′,3-trihydroxyphenyl, salicylic acid, gentisic acid, and other metabolites.39

Degradation of polycyclic aromatic hydrocarbons by another dibenzo furan-utilizing *Janibacter* sp. strain YY-1 was also reported.40 Another *Janibacter* sp. isolated from saline sediment of an arid land was studied for its biodegradation ability of pentachlorophenol.41

4. CONCLUSIONS

The main contribution of this paper is expansion of the existing attitude toward microbial biodegradation of crude petroleum oil contaminating vital water resources. Low power He–Ne laser irradiation was utilized to boost the biodegradation ability of the isolated strain *Janibacter terrae* S1N1 and shorten the time needed for treatment. The maximum biodegradation efficiency of the irradiated strain reached 96.5% after only 48 h of incubation leaving traces. We expect the approach we lead to offer an eco-friendly and new technology in water bioremediation using photostimulated bacteria by low power He–Ne laser radiation. In view of the laser irradiation potential reported in this work, the following topics are planned to be investigated in future studies under laser influence: in-depth understanding of enzymatic mechanism associated with biodegradation, the ability of *Janibacter terrae* to produce biosurfactants, and testing mixed bacterial consortium of known composition to optimize the degradative performance.

■ AUTHOR INFORMATION

**Corresponding Author**
Nashwa A. Ahmed — Microbiology Department, Faculty of Applied Medical Sciences, October 6 University, Giza 12858, Egypt; orcid: 0000-0003-4938-6082; Phone: +2.01004868776; Email: nashwa.abbas.ams@o6u.edu.eg; Fax: (+202)38362498

**Author**
Safaa M. Ezzat — Microbiology Department, Central Laboratory for Environmental Quality Monitoring (CLEQM), National Water Research Center (NWRC), El-Kanater 13621/6, Egypt

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c00460

**Notes**
Data Availability. All data generated or analyzed during this study are available upon request from the author at dr-safaa-ezzat@hotmail.com.

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors wish to thank the microbiology department at CLEQM-NWRC, and the physics department at Faculty of Science, Cairo University, Egypt for supporting this work.

■ REFERENCES

1. Varjani, S. J. Microbial degradation of petroleum hydrocarbons. *Bioresour. Technol.* 2017, 223, 277–86.

2. Lawniczak, L.; Zniak-Karczewska, M. W.; Loibner, A. P.; Heipieper, H. J.; Chrzanowski, I. Microbial degradation of hydrocarbons-basic principles for bioremediation: A Review. *Molecules.* 2020, 25, 856.

3. Marshall, A. G.; Rodgers, R. P. Petrolemics: the next grand challenge for chemical analysis. *Acc. Chem. Res.* 2004, 37, 53–59.

4. Fingas, M. F. *The Basics of Oil Spill Cleanup*; CRC Press/Taylor and Francis: Boca Raton, FL, 2013.

5. Medic, A.; Ljesevic, M.; Inui, H.; Beskoski, V.; Kojic, I.; Stojanovic, K.; Karadzic, I. Efficient biodegradation of petroleum n-alkanes and polycyclic aromatic hydrocarbons by polyextremophilic *Pseudomonas aeruginosa* with multiderivative capacity. *RSC Adv.* 2020, 10, 14060–70.
(6) Christova, N.; Kabaivanova, L.; Nacheva, L.; Petrov, P.; Stoineva, I. Biodegradation of crude oil hydrocarbons by a newly isolated biosurfactant producing strain. *Biotech Biotech Equip.* 2019, 33, 863–72.

(7) Souza, E. C.; Vessoni-Penna, T. C.; de Souza Oliveira, R. P. Biosurfactant-enhanced hydrocarbon bioremediation: An overview. *Int. Biodeterior. Biodegrad.* 2014, 90, 88–94.

(8) Victor, I. A.; Iwok, E. O.; Archibong, I. A.; Effiom, O. E.; Andem, A. B. The biochemical mechanisms of petroleum degradation by bacteria. *Int. J. Eng. Res.* 2020, 11, 1258–75.

(9) Mostafa, N. A.; Tayeb, A. M.; Mohamed, O. E.; Farouq, R. Biodegradation of petroleum oil effluents and production of biosurfactants: Effect of initial oil concentration. *J. Surfactant Deterg.* 2019, 22, 385–94.

(10) Gao, C. Experiences of microbial enhanced oil recovery in Chinese oil fields. *J. Pet. Sci. Eng.* 2018, 166, 55–62.

(11) Ichor, T.; Okerentugh, P.O.; Okpokwasil, G.C. Biodegradation of total petroleum hydrocarbons by consortium of cyanobacteria isolated from crude oil polluted brackish waters of Bodo Creeks in Ogoniland, Rivers State. *Res. J. Environ. Toxicol.* 2016, 10, 16–27.

(12) El-Naggar, A. Y.; Shtetaia, Y. M.; Youssef, K. A.; Ismail, N. A. Stimulation of the hydrocarbon compounds degrading *Saccharomyces rossini* by low power laser radiation. *Der Pharma Chemica* 2012, 4, 1424–34.

(13) Singh, S. C.; Zeng, H.; Guo, C.; Cai, W. Lasers: Fundamentals, Types, and Operations. In *Nanomaterials: Processing and Characterization with Lasers*; Wiley-VCH Verlag & Co. KGaA, 2012; pp 1–34.

(14) APHA. *Standard methods for the examination of water and wastewater*, 3rd ed.; American Public Health Association: Washington, DC, 2017.

(15) Liu, Z.; Jacobson, A. M.; Luthy, R. G. Biodegradation of naphthalene in aqueous nonionic surfactant systems. *J. Amer Sci.* 2010, 6, 211–27.

(16) Lily, M. K.; Bahuguna, A.; Dangwal, K.; Garg, V. Degradation of benzo (a) pyrene by a novel strain *Bacillus subtilis* BMT74 (MTCC 9447). *Baraz J. Microbiol.* 2009, 40, 884–92.

(17) Yoon, J. H.; Lee, K. C.; Kang, S. S.; Kho, Y. H.; Kang, K. H.; Park, Y. H. *Janibacter terrae* sp. nov., a bacterium isolated from soil around a wastewater treatment plant. *Inter J. Syst. Evol. Microbiol.* 2010, 6, 661–70.

(18) Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A.; Kumar, S. MEGA 6: Molecular Evolutionary Genetics Analysis (MEGA) software version 6.0. *Mol. Biol. Evol.* 2013, 30, 2725–29.

(19) El-Naggar, A. Y.; Kamel, M. M.; Aladly, A. A.; Ismail, N. A. Bioremediation of paraffinic and polynuclear aromatic hydrocarbons using laser irradiated *Bacillus amyloliquefaciens*. *J. Amer Sci.* 2010, 6, 651–70.

(20) Lu, W.; Wen, J.; Jia, X.; Sun, B.; Chen, Y.; Liu, M. Effect of He–Ne laser irradiation on hydrogen production by *Enterobacter aerogenes*. *Int. J. Hydrog Energy.* 2008, 33, 34–42.

(21) Ra, T.; Zhao, Y.; Zheng, M. Comparative study on the petroleum crude oil degradation potential of microbes from petroleum-contaminated soil and non-contaminated soil. *Int. J. Environ. Sci. Technol.* 2019, 16, 7127–36.

(22) Sanyaolu, A. A.; Sanyaolu, V. T.; Kolawole-Joseph, O. S.; Jawando, S. S. Biodeterioration of premium motor spirit (PMS) by fungal species. *Int. J. Sci. Nat.* 2012, 3, 276–85.

(23) Ni, J.; Shi, H.; Xu, Y.; Wang, Q. A comparison of the mechanism of TOC and COD degradation in rhodamine B wastewater by a recycling-flow two- and three-dimensional electro-reactor system. *Water.* 2020, 12, 1853.

(24) Xu, X.; Liu, W.; Tian, S.; Wang, W.; Qi, Q.; Jiang, P.; et al. Petroleum hydrocarbon-degrading bacteria for the remediation of oil pollution under aerobic conditions: a perspective analysis. *Front Microbiol.* 2018, 9, 2885.

(25) Chen, Q.; Li, J.; Liu, M. Study on the biodegradation of crude oil by free and immobilized bacterial consortium in marine environment. *PLoS ONE* 2017, 12, e0174445.

(26) Wang, J.; Zhang, M. Y.; Chen, T.; Zhu, Y.; Teng, Y.; Luo, Y. M.; Christie, P. Isolation and identification of a Di-(2-Ethylhexyl) phthalate-degrading bacterium and its role in the bioremediation of a contaminated soil. *Pedosphere.* 2015, 25, 202–11.

(27) Kumar, K. S.; Sridevi, D. T.; Thamaraiselvi, K. Utilization of petroleum hydrocarbons by *Micrococcus and Streptococcus* spp isolated from contaminated site. *J. Microbiol Biotechnol.* 2013, 3, 71–78.

(28) Siyah, S.; Patkik, H.; Jaroli, D. Factors affecting the rate of biodegradation of polyaromatic hydrocarbons. *Int. J. Pure App Biosci.* 2014, 2, 185–202.

(29) Callaghan, A. V. Enzymes involved in the anaerobic oxidation of n-alkanes: from methane to long chain paraffins. *Front Microbiol.* 2013, 4, 89–97.

(30) Jiang, Y.; Wen, J.; Jia, X.; Caiyin, Q.; Hu, Z. Mutation of Candida tropicalis by irradiation with a He-Ne laser to increase its ability to degrade phenol. *Appl. Environ. Microbiol.* 2007, 73, 226–31.

(31) Tian, X.; Wang, X.; Peng, S.; Wang, Z.; Zhou, R.; Tian, H. Isolation, screening, and crude oil degradation characteristics of hydrocarbons-degrading bacteria for treatment of oily wastewater. *Water Sci. Technol.* 2018, 78, 2626–38.

(32) Mussttaf, R. A.; Jenkins, D. F.; Jha, A. N. Assessing the impact of low-level laser therapy (LLLT) on biological systems: a review. *Int. J. Radiat. Biol.* 2019, 95, 120–43.

(33) Da Silva, N. S.; Potrich, J. W. Effect of GaAlAs laser irradiation on enzyme activity. *Photomed Laser Surg.* 2010, 28, 431–34.

(34) Thomé, A. M. C.; Souza, B. P.; Mendes, J. P. M.; Soares, L. C.; Trajano, E. T. L.; Fonseca, A. S. Dichromatic and monochromatic laser radiation effects on survival and morphology of *Pantoea agglomerans*. *Laser Phys.* 2017, 27, 055602.

(35) Barboza, L. L.; Campos, V. M. A.; Magalhães, L. A. G.; Paoli, F.; Fonseca, A. S. Low-intensity red and infrared laser effects at high fluencies on *Escherichia coli* cultures. *Braz. J. Med. Biol. Res.* 2015, 48, 945–52.

(36) Bertoloni, G.; Sacco, R.; Baro, E.; Ceccherelli, F.; Jori, G. Biochemical and morphological changes in *Escherichia coli* irradiated by coherent and non-coherent 632.8 nm light. *J. Photochem. Photobiol. B: Biol.* 1993, 18, 191–96.

(37) De Souza da Fonseca, A.; Da Silva Sergio, L. P.; Mencalha, A. L.; De Paoli, F.; Soares, R. A.; Jha, A. N. Assessing the impact of low-level laser therapy (LLLT) on biological systems: a review. *Int. J. Radiat. Biol.* 2019, 95, 120–43.

(38) Popov, A. Yu.; Popova, N. A.; Tyurin, A. V. A physical model of the action of low-intensity laser radiation on biological objects. *Opt. Spectros.* 2007, 103, 671–77.

(39) Jin, S.; Zhu, T.; Xu, X.; Xu, Y. Biodegradation of dibenzofuran by *Janibacter terrae* strain XJ-1. *Curr. Microbiol.* 2006, 53, 30–36.

(40) Yamazo, A.; Yagi, O.; Oyaizu, H. Degradation of polycyclic aromatic hydrocarbons by a newly isolated dibenzofuran-utilizing *Janibacter* sp. strain YY-1. *Appl. Microbiol. Biotechnol.* 2004, 65, 211–8.

(41) Khessairi, A.; Fhoula, I.; Jaouani, A.; Turki, Y.; Cherif, A.; Boudabous, A.; Hassen, A.; Ouzari, H. Pentachlorophenol degradation by *Janibacter sp.*, a new actinobacterium isolated from saline sediment of arid land. *Biomed Res. Int.* 2014, 2014, 1.