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Biodegradation Study of Used Engine Oil by Free and Immobilized Cells of the *Pseudomonas oleovorans* Strain NMA and Their Growth Kinetics

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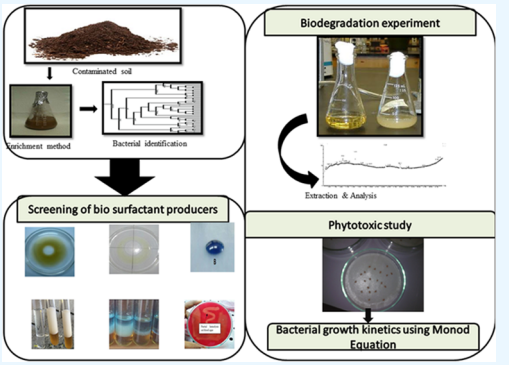
ABSTRACT: Used engine oil is considered to be one of the high-risk pollutants, and if introduced untreated in the environment, it threatens the whole ecosystem. Therefore, there is a need to find some rapid and efficient methods for the remediation of used engine oil. The present study aimed to isolate indigenous bacterial strains having the capability to degrade used engine oil. The enrichment technique was employed for the isolation of bacterial strains, which were identified by the 16S rRNA technique. As biosurfactants play a vital role in the degradation process, the activity was determined by standard protocols. The bacterial strain was isolated by the enrichment technique and identified as the *Pseudomonas oleovorans* strain NMA. The bacterial isolate has the ability to utilize used engine oil as the sole source of energy. The biodegradation experiment revealed that both free and immobilized cells degrade used engine oil, but immobilized cells showed the best biodegradation result, with 98−99% degradation efficiency in 7 days of incubation irrespective of all oil concentrations. For the analysis of degraded products, gas chromatography−mass spectrometry (GC−MS) was performed, which indicates that the treated samples do not carry the major engine components, i.e., methyl hexane, pyrene, and phytane, which confirmed that these were transformed by the bacterial activity. Monod kinetics further confirmed that the isolated bacterium utilizes used engine oil as the sole source of energy. These findings clearly indicate the potential of the bacterium NMA to degrade used engine oil with high kinetics, converting it into nontoxic products, and thus be a potential candidate for remediation at contaminated sites.



INTRODUCTION

The economic and technological developments of the human population boost energy consumption. Among all, the major energy resources come from petroleum and its products. The vehicle pollution contributes approximately 30% of the greenhouse gas emissions worldwide. The transportation industry mainly relies on diesel fuel rather than petrol. Annually, 23,000 million tonnes of oil are used, while the daily utilization reaches up to 11 billion liters.[1] Engine oil is used in vehicles for cleaning and lubricating various engine parts, also inhibiting corrosion and improving its efficiency by cooling and sealing. This oil is a product of petroleum and nonpetroleum compounds. The main components of used engine oil comprise aromatic and aliphatic hydrocarbons, pyrene, benzo anthracene, and fluoranthene.[2] The exploitation of these aromatic hydrocarbons causes ecological hazards, as it is estimated that 28,000 tons of sludge of this waster were generated every year.[3] Similarly, in oceans, about 1.3 million tonnes of oil have been spilled; this not only damages the oceanic ecology and seashore soil but also badly influences economic development.[4] Before disposal, proper treatment is needed to prevent the toxic effects of these pollutants. Commonly, various biological treatments including bioslurry, landfill, and biopiling were used for the treatment of these pollutants. These methods require considerable amounts of area and time. Another alternative method used to remove the hydrocarbons is bioremediation. The major hindrance in the removal of hydrocarbons is their low solubility and bioavailability to microorganisms. To overcome this barrier, biosurfactant-producing microorganisms are capable of lowering the surface tension (ST) of hydrocarbons and enhancing their bioavailability either by solubilization or surface modification.[5,6] Biosurfactants also play a vital role in hydrocarbon degradation, including viscous oil and petroleum

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products.[7] The biodegradation efficiency is significantly enhanced in the presence of biosurfactants.[8,9] Biosurfactant-mediated hydrocarbon degradation can be done by two main processes. In the first process, the biosurfactant reduces surface or interfacial tension depending on various interfaces, i.e., liquid−solid, liquid−liquid, and liquid−air phases. In the water/oil interface, microorganisms create a surfactant monolayer around the hydrocarbons with a hydrophobic end, thus pointing out the liquid phase. As a result, the substrate surface areas increase, which also accelerates their solubility by the emulsification process. This phenomenon enhances the bioavailability of the substrate for microbial degradation through the solubilization of hydrocarbons in water.[10] The second process is microbial cell modification, including changes in cell surface hydrophobicity and membrane permeability.[11]

A number of bacterial strains belonging to different genera have been identified, such as Pseudomonas sp., *Pseudomonas aeruginosa*, Aeromicrobium, Burkholderia, Streptomyces, Micrococcus, Acinetobacter sp., Paenibacillus, Celeribacter indicus Vibrio, Gordonia, Dietzia, Nocardioides, Moraxella, Mycobacterium Thermotogota, Brevibacterium, Sphingomonas, Desulfobacterota, Bacteroidota, and Alcaligenes sp.,[12−17] capable of degrading the recalcitrant oil products. Several studies reported in the past decade revealed that biosurfactant production from Pseudomonas, Bacillus, and Candida species was enhanced by optimizing culture medium.[18,19] The amount of nitrogen and carbon along with metal cations in the media is the most important factor in biosurfactant production shake flasks and large-scale fermenters.[20] Other important factors such as pH, substrate concentration, and available nutrients have major impacts on the degradation process and growth of microorganisms. The growth kinetic studies provide an extensive and advance knowledge about the microbial growth rate using empirical and mathematical models and hence are very useful in designing and controlling the biological process. The bacterial biomass is a general index for calculating the growth rate and metabolic activities.[21] Very few studies have focused on the bacterial growth kinetics of hydrocarbon degradations. The bacterial growth kinetics at various initial concentrations is useful to understand the relationship of microbial cells and substrate concentration.[22] The best model used to study the microbial growth kinetics and substrate consumption or utilization is Monod kinetics.

Furthermore, to obtain higher degradation efficiency and microbial cell density, the bacterial cells are immobilized on a carrier through the immobilized bioremediation process.[23] This technique offers several advantages such as high microbial density and stability, low biomass production, and more tolerance to environmental stress. The selection of an immobilized carrier is also an important factor to enhance the removal efficiency of oil. The carrier should be low cost, nontoxic, and detachable.[24] In the past studies, the immobilization technology is used for the removal of crude oil in the marine environment.[25,26] However, very few studies are available on the crude oil removal efficiency using free and immobilized cells. The aim of this investigation is to isolate and characterize the native biosurfactant-producing bacterial strains capable of degrading crude oil. The present study will be helpful in providing the theoretical and technical basis for the development of the bioremediation process for oil-polluted environments.

MATERIALS AND METHODOLOGY

Chemical and Reagents. All chemicals and reagents used were analytical grade (99%), purchased from Sigma-Aldrich. Synthetic oligonucleotides (universal primers) were obtained from Integrated DNA technologies, and sequencing was done at Macrogen, South Korea.

Sample Collection. Soil samples were collected from contaminated sites from car workshops situated in Abbottabad, KPK, Pakistan. The debris of surface soil is removed, and soil samples were taken (5−10 cm depth) in a sealed bag. Similarly, used engine oil samples was collected in sterile screw-cap bottles in the same workshop. Both samples were transferred to the laboratory within 3 h.[27,28] The culture enrichment technique was adopted for the isolation of oil-degrading strains with slight modification. 5 g of soil was added into 80 mL of distilled water, supplemented with 3% (V/V) waste engine oil, and incubated for 14 days at 37 °C and 220 rpm. After incubation, the samples were serially diluted and spread on nutrient agar plates. These isolates were subcultured and preserved for further studies.[29,30]

Screening of Biosurfactant-Producing Bacteria. For screening of the biosurfactant producers, each bacterial isolate was inoculated in MSM medium and 1−3% engine oil as the sole carbon and energy source and incubated in a rotary shaker at 37 °C and 220 rpm for 5−7 days. The growth of each isolate was measured each day at OD_{600} nm using a UV/vis spectrometer. After 5 days, the cell culture was centrifuged at 8000 rpm for 10−15 min, and the supernatant was used for biosurfactant-screening tests. The biosurfactant producing capacity was measured by using the following screening methods.[19]

Penetration Method. For this assay, the ELISA 96-well microplate was used containing hydrophobic paste (200 μL) filled with oil and silica gel. The paste is covered with 10 μL of oil. Then, the culture supernatant was stained by adding 10 μL of safranin in 90 μL of supernatant, and the activity of the biosurfactant was observed as described earlier.[31]

Oil Displacement Method. In the oil displacement method, the spreading of oil is observed after adding a few drops of biosurfactant solution. In the Petri plate, 25 mL of distilled water was added that approximately covered 15 cm, and the crude oil (100 μL) was spread on the center of the water surface. Then, 10 μL of the cell-free culture supernatant was added to the center of the oil. The transparent zones were observed, and their diameter was measured using a caliper. Distilled water served as a negative control.[32]

Drop Collapse Method. In this method, 2.0 μL of crude oil was coated on a glass slide and allowed to stand for 2 h at room temperature. Then, 10 μL of cell-free culture supernatant was added on the glass slide. The drop shape on the oil surface was observed after 1−2 min. The drops that remained stable were considered negative, while drops that collapsed were taken as positive results. Distilled water was used as a control.[33]

Hemolytic Activity. The overnight bacterial culture was inoculated onto blood agar plates. These were incubated at 37 °C for 48 h. The lysis around colonies was observed.[34]

Emulsification Index (EI %). The isolates were grown overnight and centrifuged at 10,000 rpm for 10 min. The culture supernatant was mixed in oil (1:1) by using a vortex. This mixture was incubated for 24 h at 30 °C.[35] Emulsification index percentages (EI %) were calculated by using this formula.

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ACS Omega 2025, 10, 541−549

$$\text{EI \%} = (\text{total height of the emulsified layer} \div \text{total height of the liquid layer}) \times 100$$

Emulsification Activity. In this assay, the culture supernatant (3 mL) was mixed with 0.5 mL of oil and vortexed for 5 min. This mixture was kept at room temperature for 1 h. The aqueous phase was collected separately and analyzed by a UV spectrophotometer at the wavelength of 400 nm.[36]

Bacterial Adhesion to Hydrocarbons (BATH) Test. The bacterial strains were grown on modified M9 medium. The overnight bacterial culture was centrifuged at 10,000 rpm for 15 min. The supernatant was discarded, and the bacterial pellet was collected and washed with 3 mL of phosphate buffer saline. This mixture was centrifuged at 10,000 rpm for 15 min. The above step was repeated three times in order to remove cell debris. After washing, the cell suspension (2 mL) was added in 100 μL of *n*-hexane. The solution was vortexed and maintained at 30 °C for 1 h. After 1 h, two layers were formed, and the aqueous layer was collected. The optical density (OD) of the aqueous layer was measured at 620 nm via a UV−vis spectrometer. The percentage of cell hydrophobicity was measured using the following formula.

$$\text{hydrophobicity (\%)} = \{-(\text{optical density of original} \div \text{optical density of crude oil})\} \times 100$$

Molecular Identification. The isolated strains were identified by using 16S rRNA sequencing.[37] The PCR amplification was carried out by using universal primers, i.e., 1492R (5′-TACGGYTACCTTGTTACGACTT-3) and 27F (5′ AGAGTTTGATCMTGGCTCAG- 3′). The thermal cycler conditions were initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 90 °C for 30 s, primer annealing at 50 °C for 30 s, extension at 72 °C for 2 min, and final polymerization at 72 °C for 2 min. The amplified product was resolved electrophoretically and purified by a gel extraction kit of Affymetrix Prep Ease obtained from USB Corporation. The amplified product was sequenced and compared to other 16S rRNA sequences available in the NCBI database. The phylogenetic trees of the bacterial isolate are constructed based on the neighbor-joining method using MEGA 11.0 software.

Preparation of NMA-Immobilized Beads. The agar entrapment method was used to prepare the immobilized beads. The overnight bacterial cultured was centrifuged (8000 rpm) for 10 min after measuring the OD at 600 nm by using UV−Vis spectrophotometer. The supernatant was removed and then cells were washed with sterile phosphate buffered solution to ensure that the cell suspension had a concentration of 0.7 AU (UV−visible). The agar beads were prepared by mixing 2% w/v agar with cell suspension of NMA (1×10^6 cfu/g) and were poured into Petri plates. The solidified agar was cut into small pieces (3 mm thickness, surface of 10 mm) and cured by placing in phosphate buffer (100 mM) at 4°C for 1 h. After incubation, the buffer solution was removed, and immobilized cells were preserved until further use.[38]

Biodegradation Experiment. Before the biodegradation experiment, the optimization of bacterial growth was carried out at various temperatures (22, 25, 28, 32, and 37 °C) and pH (6, 6.5, 7, and 7.5). Briefly, bacterial cells were grown in MSM medium supplemented with engine oil as a sole carbon source and incubated at various temperature ranges (22, 25, 28, 32, and 37 °C) for 5−7 days in the shaker incubator. Bacterial growth was monitored after every 24 h through measuring the optical density OD at 600 nm against the sterile control. Each experiment was performed in triplicate. Similarly, the effect of different pH, 6, 6.5, 7, and 7.5, on the growth of selected isolates was also analyzed by inoculating the bacterial isolate in MSM medium supplemented with engine oil as the sole carbon and energy source. These were incubated at 32 °C and 220 rpm. The bacterial growth was observed using a UV−Vis spectrophotometer at the wavelength of 600 nm.[39]

To analyze the biodegradation ability of the free bacterial cells, 1% v/v bacterial cells were added to conical flasks containing MSM medium amended with various concentrations of oil (1, 3, 5, and 7%), thus utilizing the oil as a sole carbon source. The MSM medium containing various initial concentrations of oil, but no inoculum, was used as the negative control. These were incubated in a shake flask incubator at 32 °C while shaking at 220 rpm. The experiment was proceeded for 5 days, and after every 24 h, 3 mL of samples was taken aseptically from each treatment and bacterial growth was measured using a UV−Vis spectrophotometer at 600 nm along with the counting of colony-forming units (CFU) in order to determine the growth of bacteria while using the oil as the sole carbon source. For the immobilization experiment, the NMA immobilized cells, which were equivalent to 1×10^6 cfu/g, were added to the same culture medium and incubated under the same conditions. All experiments were performed in triplicate. The surface tension of oil was measured using the drop weight method before and after the experiment at room temperature.[40] All the experiments were conducted in triplicate with negative controls as mentioned above.

Residual oil concentration was calculated using the formula

$$\text{residual oil concentration} = (\text{peak area of the sample} \div \text{peak area of the control}) \times \text{concentration of the control}$$

Extraction and Analyses of Engine Oil and Biosurfactants. The residual engine oil was extracted by centrifuging the culture medium at 10,000 rpm for 20 min. The supernatant was collected separately and mixed with hexane (1:1), and the extracted samples were concentrated by using a rotary vacuum evaporator. The residual oil and its degrading products were analyzed by using gas chromatography equipped with a mass spectrometer (GC−MS, PerkinElmer Clarus 600). Helium was used as a carrier gas with a flow rate of 1.0 mL/min. The apparatus was set at 60 °C for 2 min, and the temperature was then slowly raised by 6 °C until holding at 300 °C. The working temperature for the injector was 300 °C, and the detector one was 320 °C. Total time for one GC run is 43 min.[41]

The biosurfactant was also extracted by the method described by Chittepu. In this method, the supernatant is also collected by centrifuging the bacterial culture medium at 10,000 rpm for 20 min, and the pH is adjusted by 2.0 using 3 M HCL. Then, the supernatant was precipitated by keeping at 4 °C for 24 h, and the biosurfactant was taken by centrifuging the culture medium at 10,000 rpm for 20 min.[42]

Reusability of Immobilized NMA Cells. For this, immobilized NMA cells were incubated in 100 mL of MSM containing 5% (v/v) used engine oil. The medium was shaken

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ACS Omega 2025, 10, 541−549

at 200 rpm on a rotary shaker for 5 days/cycle at 32 °C. After each incubation cycle, the immobilized NMA cells were washed with phosphate buffer (pH 7.0) and suspended in fresh MSM medium containing 5% (v/v) crude oil. The cells were reused under the same conditions for up to three cycles. MSM medium supplemented with engine oil but without immobilized cells served as the negative control. All experiments were performed in triplicate. The oil removal efficiency was also determined after every 24 h.

Growth Kinetics. Growth kinetics were determined during the batch experiments with various concentrations of engine oil following the method as described by Ray et al.[5] For the determination of μ_{max} and K, the specific growth rate was plotted against various concentrations of engine oil using Hyper32 software. The specific growth rate (μ) of bacterial isolates was determined using the logistic and linear growth model.

$$\mu_{max} = i/XDx/dt = \mu_{max}S/K_s + S$$

$$\mu_{max} = \mu_{max}S/K_s + S$$

$$X = X_o^{\mu t}$$

X (cfu/mL) = microbial cell with respect to time, X_o (cfu/mL) = initial microbial cells (cfu/mL), μ (h^{-1}) = specific growth rate, μ_{max} (h^{-1}) = maximum specific growth rate, K (mg/L) = half saturation, and S (mg/L) = substrate concentration.

Phytotoxicity Effect. The phytotoxic effect of the treated and untreated crude oil was determined on the germination of the *Solanum lycopersicum* L. seed. The seeds were obtained from the local market of Abbottabad, Pakistan. Seeds of the same size were taken and sterilized with 0.2% solution of mercuric chloride for 4−5 min and subsequently washed thoroughly three times with distilled water. The seeds were left in filter paper for drying. The metabolites of used engine oil 5% (v/v) were extracted in *n*-hexane followed by air drying. Afterward, the extracted metabolites were dissolved in 10−15 mL of distilled water. The filter paper was moistened with the crude oil (untreated oil), 5% (v/v) treated crude oil with bacteria and distilled water (control) were used to germinate the seeds placed in sterilized Petri plates. A total of 12−15 seeds were added in each Petri plate and kept in a growth chamber at 28 °C; seed germination was recorded after 3 days of incubation, while the shoot and root length was recorded after 5 days of incubation.[43] The seed germination % age as well as germination index (GI) was calculated by using the following formula

$$\text{Seed germination}(\%) = (\text{Total numberof seeds germinated} \div \text{Total number of seeds}) \times 100$$

$$\text{germination index (GI)}\% = (\%\ \text{of seed germination}) \times (\%\ \text{of root growth}) \div 100$$

Statistical Analysis. All experiments were performed in triplicate, and the significant difference between group means in engine oil degradation was evaluated with one-way analysis of variance (ANOVA) using IBM SPSS ver.18 software. Groups sharing different letters (a, b, c) indicate significant differences between two treatments by the one-way ANOVA test ($P < 0.05$). Bars carrying the same alphabets indicate no significant difference between treatments. The standard errors for mean (SEM), at the 95% confidence level, were calculated.

RESULTS AND DISCUSSION

Isolation and Screening of Used Engine Oil-Degrading Strains with Biosurfactant Producing Ability. Initially, nine strains were isolated that have the capability to degrade used engine oil when they were inoculated in the minimal medium containing only the engine oil as the sole source of carbon. All of these strains can utilize engine oil as the sole source of carbon. Further screening is done for their ability to produce biosurfactants, and out of these, NMA produces the maximum biosurfactant, and thus, this strain was selected for further bioremediation experiments. In a previous study, the bacterium *Bacillus licheniformis* LRK1 was isolated from the marine environment that produces biosurfactants and utilizes engine oil as a sole source of carbon.[44]

Selection of the Best Degrading Strain. Various assays were performed to confirm the ability of the bacterial strains to produce biosurfactants; among all, the best biosurfactant producer was NMA. The emulsification index is considered to be the main approach to estimate the production of biosurfactants, and the bacterial strain NMA has the high emulsification index with a value of 72%. This bacterial strain also showed the best degradation capability while using the used engine oil as the sole source of energy. Several studies have reported that the biosurfactant-producing strains have high ability to degrade petroleum products.[45] Biosurfactant producers have high substrate specificity; their hydrophilic and hydrophobic properties and rapid and controlled inactivation make them the best candidates for bioremediation of hydrocarbons.[46] Biosurfactants help lower the surface tension, and their high emulsification index promotes the bioavailability of hydrophobic compounds for bacterial cells that ultimately increase the degradation of crude oil.[13,47]

Identification of Bacterial Isolates. The most efficient bacterial strain was identified on the basis of its biochemical and 16S rRNA technique. The strain NMA has 99% similarity with *Pseudomonas oleovorans* and thus was designated as the *P. oleovorans* strain NMA. The sequence of the bacterial strain has been submitted in the NCBI database with accession numbers OQ740159. The phylogenetic relation was established for the bacterial isolate, and the phylogenetic tree for NMA is shown in Figure 1. Numerous bacterial strains have been reported that have the capability to degrade petrohydrocarbons including

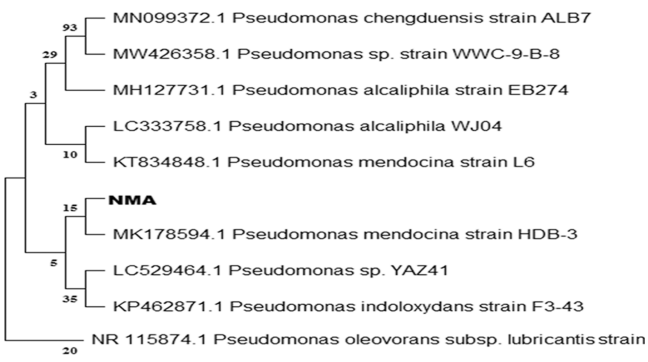

Figure 1. Neighbor-joining phylogenetic tree from 16S rRNA gene sequence data showing the positions of NMA (*P. oleovorans*) and related organism.

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ACS Omega 2025, 10, 541−549

diesel oil, crude oil, used engine oil, etc. as their sole source of energy. *P. oleovorans* is one of the most reported strains involved in the degradation of both aromatic and aliphatic hydrocarbons;[48] recently, a strain of *P. oleovorans* was reported that is able to degrade polycyclic hydrocarbons.[49] Various other *Pseudomonas* have also been reported that are biosurfactant producers and involved in the degradation of organic pollutants.[5] Recently, a strain of *Enterobacter hormaechei* was isolated from the oil-based mud of the petroleum drilling site of Egypt that can degrade oil using it as the sole source of carbon.[50]

Biodegradation Experiment. The biodegradation ability of free and immobilized cells of the *P. oleovorans* strain NMA was determined separately in minimal media carrying various concentrations (1, 3, 5, and 7%) of used engine oil as the sole source of carbon. Before degradation, the optimum temperature and pH for the growth of strains have been determined; the temperature is 32 °C. Compared to the biodegradation efficiency of free cells versus immobilized bacterial cells, NMA showed the best biodegradation result for immobilized cells, with 98−99% removal efficiency in 7 days of incubation irrespective of all oil concentrations, as shown in Figure 2a. This might be due to their high stability and greater mechanical strength as compared to their free cells. At low concentrations (1 and 3%), the immobilized bacterial cells of the *P. oleovorans* strain NMA efficiently degraded engine oil up to 99% in 5 days of incubation, and as the concentration increases (5 and 7%), the 98.6−99% degradation was observed in 7 days of incubation. An increased engine oil concentration (5 and 7%) has no effect on the degradation ability of the isolated bacterium, but it takes more time to degrade them, Figure 2b. The free cells of NMA showed the highest degradation rate of 85% of the used engine oil with the concentration of 5% (Figure 2a). During early days, the slow growth rate of free bacterial cells might be due to adaption in the new environment;[51] as time passes, the rate of biodegradation increased as shown in Figure 2b. As the concentration of the used engine oil increases (7%), the degradation rate is decreased. In a previous finding, the immobilized bacterial cells efficiently remove the crude oil. According to Sakdapetsiri et al., the immobilized *Exiguobacterium* sp. AO-11 removed 90% of crude oil (0.25%) in 9 days of incubation.[24] Zhang et al. reported the bacterial strain *P. aeruginosa* DQ8 that has the ability to degrade 79.9% of TPH in 10 days of treatment.[52] Similarly, *P. aeruginosa* degraded oil up to 77.8% at 22 °C in 28 days,[53] while *Pseudomonas* sp. BP10 and *Rhodococcus* sp. NJ2 degraded 60.6 and 49.5% of TPH, respectively, in 30 d at optimum conditions in MSM containing 2% of crude oil.[47] Varuvel et al. reported that RSM and ANN models can be used to optimize the engine parameters.[54]

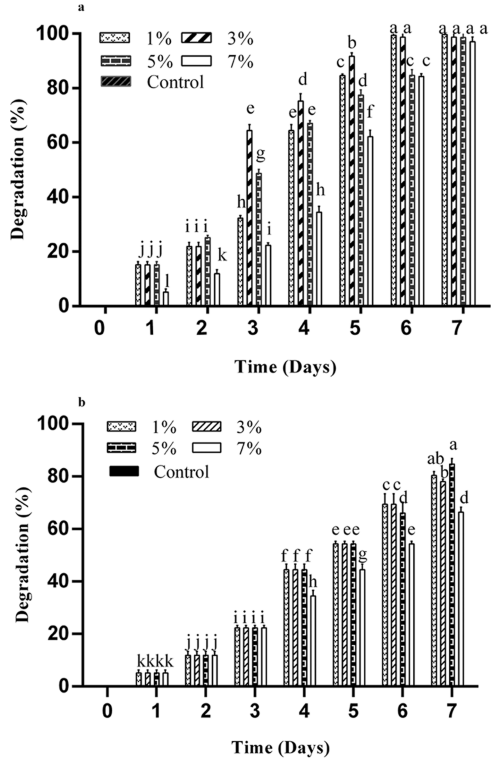

Figure 2. (a) Percentage degradation of used engine oil with various initial concentrations of oil (1, 3, 5, and 7%) by immobilized cells of *P. oleovorans* NMA. Groups sharing different letters (a, b, c) indicate significant differences between two treatments by the one-way ANOVA test ($P < 0.05$). Bars carrying the same alphabets indicate no significant difference between treatments. (b) Percentage degradation of used engine oil with various initial concentrations of oil (1, 3, 5, and 7%) by free cells of *P. oleovorans* NMA.

Another study reported that the bacterial consortium containing *P. aeruginosa* PP3 and PP4 is used to degrade the TPH up to 67% in contaminated soil.[8] The *P. aeruginosa* PP4 is also used to degrade 67% anthracene along with synthesized iron nanoparticles.[9] In comparison with other reported studies,[24,55,56] the NMA immobilized cells exhibit greater removal efficiency of used engine oil. In addition, the NMA immobilized cells could tolerate high substrate concentrations such as used engine oil up to 7%. This might be due to the protection of the carrier matrix.

The production of biosurfactants has also been maximum, and it was observed that the samples treated with the strain NMA have reduced surface tension (ST), i.e., from 59.8 to 28.6 mN m^{-1}. This reduction in surface tension is the major cause of maximum degradation after 7 days of incubation. In previous studies, the same was found where biosurfactants increase the biodegradation of petroleum hydrocarbons and oil.[57] In a previous study, the bacterium *P. aeruginosa* PG1 was isolated that produces a biosurfactant characterized as rhamnolipid, responsible for the enhanced degradation of crude oil.[45]

Gas chromatography−mass spectrometry (GC−MS) analysis reveals that the strains degrade engine oil very efficiently; the presence of cycloalkanes and aromatics in untreated samples but not in treated samples clearly demonstrates that the bacterial strain degrades these components in oil. The samples treated with the strain NMA degraded the pyrene and phytane after 5 days of incubation but unable to degrade 2,6-tetramethyl-7-pentadecane as shown in Figure 3 and Table 1. In most cases, a single bacterium is unable to degrade all of the components of crude oil due to its complex structure. Similar to present findings, *Rhodococcus* sp. P14 R and *Erythropolis* degraded PAHs and C32 *n*-alkanes in diesel oil and crude oil, respectively, but did not degrade all of its components.[58]

Phytotoxic Effects. The toxic effects of crude oil on plants are well documented.[59] The results revealed that the untreated engine oil restricts the germination of seeds and affects the shoot and root length of seedlings of *Solanum lycopersicum*. The treated oil also contains biosurfactants that have no toxic

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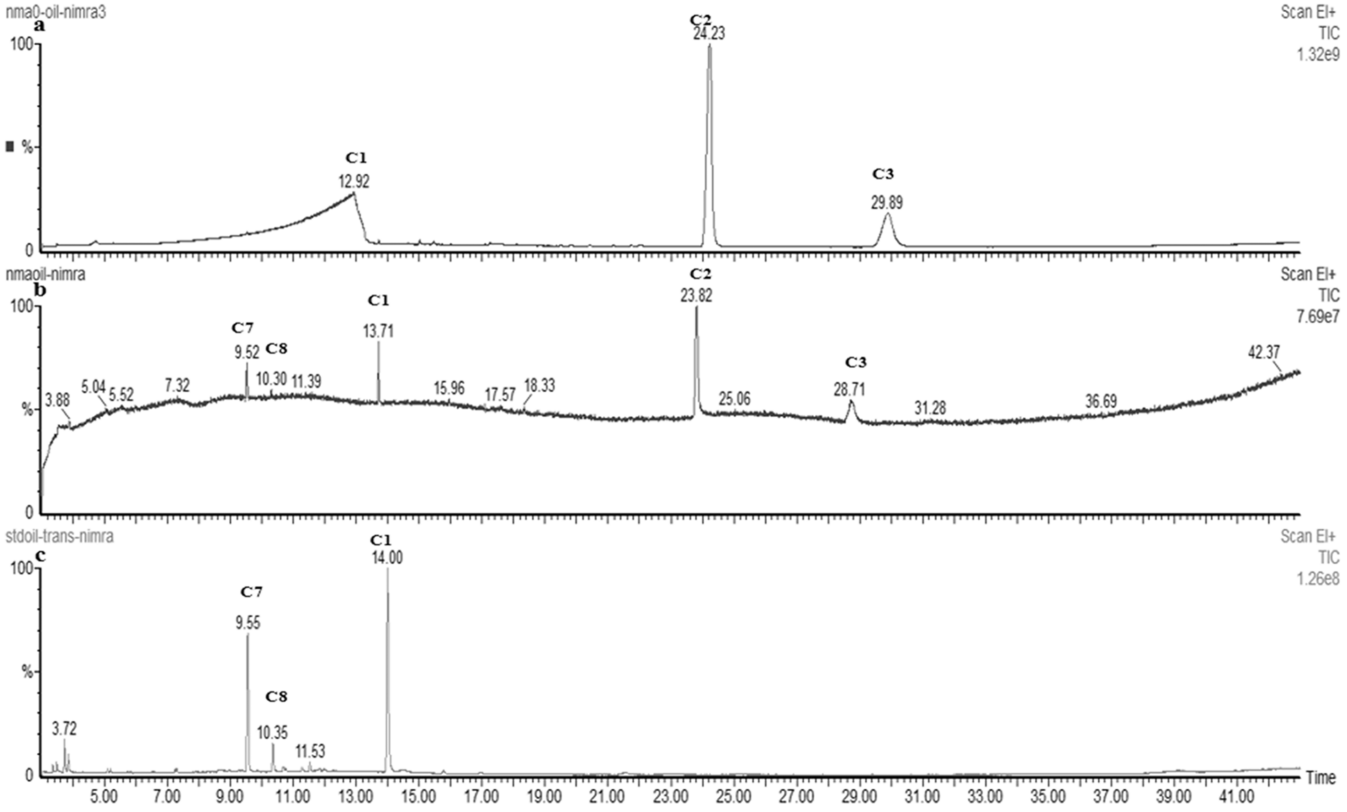

Figure 3. Gas chromatograms indicating the variations in the components of used engine oil before and after degradation by *P. oleovorans* NMA in MSM medium at 32 °C for 7 days. (a) Oil control; (b) crude oil degradation of NMA at 0 days; and (c) oil degradation by NMA after 7 days.

Table 1. Hydrocarbons in Engine oil before and after Incubation with the Bacterial Isolate *Pseudomonas oleovorans* NMA

oil fractions	compoundand formula	crude oil (control)	crude oil at 0 day NMA	crude oil at 5 day NMA
C1	2,6-tetramethyl-7-pentadecane ($C_{25}H_{48}$)	present	present	present
C2	pyrene ($C_{16}H_{10}$)	present	present	absent
C3	phytane ($C_{20}H_{42}$)	present	present	absent
C7	dodecane methyl ($C_{13}H_{28}$)	absent	present	present
C8	dimethyl (C_2H_6O)	absent	present	present

effect on the germination as well as the length of roots and shoot. In the NMA-treated oil, 85.5% of the seeds were germinated, with a germination index of 73.2, as shown in Table 2. Similarly, the shoot and root lengths were nearly the same in the control as in treated samples as compared to the untreated engine oil (Figure 4). The previous findings revealed the same type of results, where treated oil with *Bacillus pumilus* has no toxic effect on the growth parameters of *Sinapis alba*.[60] Biosurfactant producers in the rhizosphere enhanced the bioavailability of hydrophobic compounds by adsorbing them on soil particles and thus promoted plant growth directly or indirectly.[61]

Table 2. Phytotoxic Potential of Treated and Untreated Engine Oil (Mean ± SD, n = 3)

	Pseudomonas oleovorans NMA		
parameters	control (water)	pretreated	post-treated
root length (cm)	2.1 ± 0.02	0.3 ± 0.05	2 ± 0.07
shoot length (cm)	0.9 ± 0.02	0.4 ± 0.01	0.7 ± 0.02
seed germination (%)	91.5 ± 0.03	15.5 ± 0.03	85.5 ± 0.03
root growth (%)	85.7 ± 0.06	42.8 ± 0.02	85.7 ± 0.01
germination index (%)	78.42 ± 0.05	6.62 ± 0.05	73.2 ± 0.06

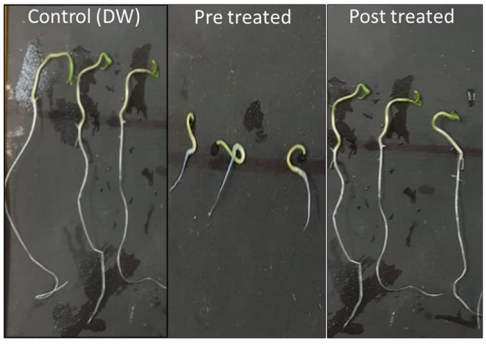

Figure 4. Germination of the *Solanum lycopersicum* (Riogrande) seeds before (pretreated) and after oil degradation (post-treated) by *P. oleovorans* NMA.

Growth Kinetics. The kinetic models and assessment of the parameters play a significant role in the understanding of the biodegradation process. Batch study was conducted to evaluate the growth kinetic parameters of isolated strains using crude oil as the sole carbon source. The Monod equation is the

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ACS Omega 2025, 10, 541−549

best fitted kinetic model for the single substrate utilization. The estimated value of the parameters was μ_{max} (h^{-1}) = 0.013 for the strain NMA (*P. oleovorans*) grown on crude oil with K_s = 10.6, which was lower than the μ_{max} (h^{-1}) = 0.112 and K_s = 16.7 as shown in Table 3. The link established between μ_{max} and KS can be difficult for microbial growth kinetic modeling utilizing a single substrate. Therefore, instead of analyzing separate kinetic parameters (μ_{max} and K_s), the best way to explain the utilization of low substrate concentration by bacterial cells is the ratio of μ_{max}/K_s. This ratio best explains the substrate utilization with respect to microbial growth. The highest value of μ_{max}/K_s (0.013 and 0.016 mg/L) was obtained when bacterial isolates (NMA) used oil as a sole carbon source as shown in Table 3. In the case of the control (devoid of carbon source), it remained zero. Previously, growth kinetics of *P. aeruginosa*, *P. fluorescens*, and *P. putida* were also studied. These kinetic values estimate the growth rate of isolated strains on various substrates.[5]

Table 3. Growth Kinetic Parameters of *Pseudomonas oleovorans* NMA Using the Monod Equation

monod kinetic parameters	*Pseudomonas oleovorans* NMA	control (NMA)
μ_{max} (h^{-1})	0.139	0.000
K_s (mg/L)	10.6	0.000
μ_{max}/K_s	0.013	0.000

CONCLUSIONS

In this study, the biodegradation efficiency of used engine oil was studied using the free and immobilized cells of the *P. oleovorans* strain NMA. Upon comparing free and immobilized cells, the *P. oleovorans* strain NMA showed excellent ability to utilize used engine oil as the sole source of carbon with profound ability to produce biosurfactants. Especially when the immobilized cells of the *P. oleovorans* strain NMA are inoculated in batch culture, the major components of engine oil are completely degraded. Furthermore, the results of the phytotoxic and bacteriotoxic assays confirmed that the metabolites derived after NMA treatment are nontoxic. Therefore, the *P. oleovorans* strain NMA can be a potential candidate to remediate oil-contaminated soil.

ASSOCIATED CONTENT

Data Availability Statement

The authors declare that all data supporting the findings of the present study are available within the article.

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REFERENCES

(1) Krishnan, G. M.; Rajkumar, S.; Devarajan, Y.; Rajiv, A. A comprehensive review on advancement and challenges of renewable biofueled reactivity controlled compression ignition (RCCI) engine. *J. Energy Inst.* **2024**, *113*, No. 101540.

(2) Ghaleb, A. A. S.; Kutty, S. R. M.; Salih, G. H. A.; Jagaba, A. H.; Noor, A.; Kumar, V.; Almahbashi, N. M. Y.; Saeed, A. A. H.; Saleh Al-dhawi, B. N. Sugarcane Bagasse as a Co-Substrate with Oil-Refinery Biological Sludge for Biogas Production Using Batch Mesophilic Anaerobic Co-Digestion Technology: Effect of Carbon/Nitrogen Ratio. *Water* **2021**, *13* (5), 590.

(3) Suganthi, S. H.; Murshid, S.; Sriram, S.; Ramani, K. Enhanced biodegradation of hydrocarbons in petroleum tank bottom oil sludge and characterization of biocatalysts and biosurfactants. *J. Environ. Manage.* **2018**, *220*, 87−95.

(4) Bao, M. T.; Wang, L. N.; Sun, P. Y.; Cao, L. X.; Zou, J.; Li, Y. M. Biodegradation of crude oil using an efficient microbial consortium in a simulated marine environment. *Mar. Pollut. Bull.* **2012**, *64*, 1177−1185.

(5) Ray, M.; Kumar, V.; Banerjee, C.; Gupta, P.; Singh, A.; Singh, S. Investigation of biosurfactants produced by three indigenous bacterial strains, their growth kinetics and their anthracene and fluorene tolerance. *Ecotoxicol. Environ. Saf.* **2021**, *208*, No. 111621.

(6) Albuquerque, C. F.; Finkler, C. L.; Rufino, R. D.; Luna, J. M.; Menezes, C. T. B.; Santos, V. A. Evaluation of biosurfactants for removal of heavy metal ions from aqueous effluent using flotation techniques. *Int. Rev. Chem. Eng.* **2012**, *4*, 1−6.

(7) Chen, J.; Huang, P. T.; Zhang, K. Y.; Ding, F. R. Isolation of biosurfactant producers, optimization and properties of biosurfactant

produced by *Acinetobacter sp*. from petroleum-contaminated soil. *J. Appl. Microbiol* **2012**, *112* (4), 660−671.
(8) Muthukumar, B.; Surya, S.; Sivakumar, K.; AlSalhi, S. M.; Rao, N. T.; Devanesan, S.; Arunkumar, P.; Rajasekar, A. Influence of bioaugmentation in crude oil contaminated soil by *Pseudomonas* species on the removal of total petroleum hydrocarbon. *Chemosphere* **2023**, *310*, No. 136826.
(9) Muthukumar, B.; Satheeshkumar, A.; Parthipan, P.; Laishram, B.; Duraimurugan, R.; Devanesan, S.; AlSalhi, S. M.; Rajamohan, R.; Rajasekar, A. Integrated approach of nano assisted biodegradation of anthracene by *Pseudomonas aeruginosa* and iron oxide nanoparticles. *Environ. Res.* **2024**, *244*, No. 117911.
(10) Sánchez, C. A review of the role of biosurfactants in the biodegradation of hydrophobic organopollutants: production, mode of action, biosynthesis and applications. *World J. Microbiol. Biotechnol.* **2022**, *38*, 216.
(11) Kaczorek, E.; Pacholak, A.; Zdarta, A.; Smułek, W. The Impact of Biosurfactants on Microbial Cell Properties Leading to Hydrocarbon Bioavailability Increase. *Colloids Interfaces* **2018**, *2* (3), 35.
(12) Das, K.; Mukherjee, A. K. Crude petroleum-oil biodegradation efficiency of *Bacillus subtilis* and *Pseudomonas aeruginosa* strains isolated from a petroleum-oil contaminated Soil from North-East India. *Bioresour. Technol.* **2007**, *98*, 1339−1345.
(13) Liu, C. W.; Liu, H. S. *Rhodococcus erythropolis* strain NTU-1 efficiently degrades and Traps diesel and crude oil in batch and fed-batch bioreactors. *Process Biochem.* **2011**, *46*, 202−209.
(14) Thavasi, R.; Jayalakshmi, S.; Banat, I. M. Effect of biosurfactant and fertilizer on biodegradation of crude oil by marine isolates of *Bacillus megaterium*, Cornynebacterium kutscheri and *Pseudomonas aeruginosa*. *Bioresour. Technol.* **2011**, *102*, 772−778.
(15) Wang, X. B.; Chi, C. Q.; Nie, Y.; Tang, Y. Q.; Tan, Y.; Wu, G.; Wu, X. L. Degradation of petroleum hydrocarbons (C6−C40) and crude oil by novel *Dietzia* strain. *Bioresour. Technol.* **2011**, *102*, 7755−7761.
(16) Zhao, Y.; Dong, Y.; Chen, X.; Wang, Z.; Cui, Z.; Ni, Q. S. Using sulfide as nitrite oxidizing bacteria inhibitor for the successful coupling of partial nitrification-anammox and sulfur autotrophic denitrification in one reactor. *Chem. Eng. J.* **2023**, *475*, No. 146286.
(17) Beilig, S.; Pannekens, M.; Voskuhl, L.; Meckenstock, R. U. Assessing anaerobic microbial degradation rates of crude light oil with reverse stable isotope labelling and community analysis. *Front. Microbiomes* **2024**, *3*, No. 1324967.
(18) Muthukumar, B.; Al Salhi, M. S.; Narenkumar, J.; Devanesan, S.; Rao, T. N.; Kim, W.; Rajasekar, A. Characterization of two novel strains of *Pseudomonas aeruginosa* on biodegradation of crude oil and its enzyme activities. *Environ. Pollut.* **2022**, *304*, No. 119223.
(19) Eldos, H. I.; Zouari, N.; Saeed, S.; Ashfaq, M. Y. M.; Al-Ghouti, A. M. Isolation, identification, and characterization of potential biosurfactant producing bacteria from processing wastewater for the development of eco-friendly green technology. *Bioresour. Technol. Rep.* **2024**, *25*, No. 101763.
(20) Singh, P.; Patil, Y.; Rale, V. Biosurfactant production: emerging trends and promising strategies. *J. Appl. Microbiol.* **2019**, *126*, 2−13.
(21) Rezvani, F.; Ardestani, F.; Najafpour, G. Growth kinetic models of five species of *Lactobacilli* and lactose consumption in batch submerged culture. *Braz. J. Microbiol.* **2017**, *48* (2), 251−258.
(22) Üçok, G.; Sert, D. Growth kinetics and biomass characteristics of *Lactobacillus plantarum* L14 isolated from sourdough: effect of fermentation time on dough machinability. *LWT: Food Sci. Technol.* **2020**, *129*, No. 109516.
(23) El-Mohdy, H. L. A. Radiation-induced degradation of sodium alginate and its plant growth promotion effect. *Arab. J. Chem.* **2017**, *10* (1), S431−S438.
(24) Sakdapetsiri, C.; Kaokhum, N.; Pinyakong, O. Biodegradation of crude oil by immobilized Exiguobacterium sp. AO-11 and shelf life evaluation. *Sci. Rep.* **2021**, *11* (1), No. 12990.
(25) Bayat, Z.; Hassanshahian, M.; Cappello, S. Immobilization of microbes for bioremediation of crude oil polluted environments: A mini review. *Open Microbiol. J.* **2015**, *9*, 48−54.
(26) Xue, J.; Wu, Y.; Fu, X.; Li, N.; Sun, J.; Qiao, Y. Study on degradation characteristics and bacterial community structure changes of immobilized cells in straw-alginate beads in marine environment. *Bioresour. Technol. Rep.* **2020**, *10*, No. 100402.
(27) Karamba, K. I.; Ahmad, S. A.; Zulkharnain, A.; et al. Optimisation of biodegradation conditions for cyanide removal by *Serratia marcescens* strain AQ07 using one-factor-at-a-time technique and response surface methodology. *Rend. Fis. Acc. Lincei* **2016**, *27*, 533−545.
(28) Ishaya, S.; Usman, S.; Nweke, D. O.; Adams, H. N.; Umar, R.; Ilyasu, S. N.; Jagaba, H. A.; Atangwho, J. I.; Yakasai, M. H. Degradation of used engine oil by *alcaligenes* sp. strain isolated from oil contaminated site: Isolation, identification, and optimization of the growth parameters. *Case Stud. Chem. Environ. Eng.* **2023**, *8*, No. 8100516.
(29) Sawadogo, A.; Harmonie, C. O.; Sawadogo, B. J.; Kaboré, A.; Traoré, S. A.; Dianou, D. 2014 Isolation and Characterization of Hydrocarbon-Degrading Bacteria from Wastewaters in Ouagadougou, Burkina Faso. *J. Environ. Prot.* **2014**, *5*, 1183−1196.
(30) Yu, T.; Liu, X.; Ai, J.; Wang, J.; Guo, Y.; Liu, X.; He, X.; Deng, Z.; Jiang, Y. Microbial community succession during crude oil-degrading bacterial enrichment cultivation and construction of a degrading consortium. *Front. Microbiol.* **2022**, *13*, No. 1044448.
(31) Narendra Kumar, P.; Swapna, T. H.; Khan, M. Y.; Reddy, G.; Hameeda, B. Statistical optimization of antifungal iturin A production from *Bacillus amyloliquefaciens* RHNK22 using agro-industrial wastes. *Saudi J. Biol. Sci.* **2017**, *24* (7), 1722−1740.
(32) Satpute, S. K.; Mone, N. S.; Das, P.; Banpurkar, A. G.; Banat, I. M. Lactobacillus acidophilus derived biosurfactant as a biofilm inhibitor: a promising investigation using microfluidic approach. *Appl. Sci.* **2018**, *8* (9), 1555.
(33) Morais, I. M. C.; AL; Teixeira, G. S.; Domingues, V. S.; Nardi, R. M. D.; Monteiro, A. S.; Santos, V. L.; et al. Biological and physicochemical Cordeiro properties of biosurfactants produced by *Lactobacillus jensenii* P 6A and *Lactobacillus gasseri* P 65. *Microb. Cell Fact.* **2017**, *16* (1), 1−15.
(34) Roy, A. A Review on the Biosurfactants: Properties. Types and its Applications. *J. Fundam. Renewable Energy Appl.* **2017**, *8*, 1.
(35) Dusane, D. H.; Pawar, V. S.; Nancharaiah, Y. V.; Venugopalan, V. P.; Kumar, A. R.; Zinjarde, S. S. Anti-biofilm potential of a glycolipid surfactant produced by a tropical marine strain of *Serratia marcescens*. *Biofouling* **2011**, *27* (6), 645−654.
(36) Campos, J. M.; Stamford, T. L. M.; Rufino, R. D.; Luna, J. M.; Stamford, T. C. M.; Sarubbo, L. A. Formulation of mayonnaise with the addition of a bioemulsifier isolated from *Candida utilis*. *Toxicol. Rep.* **2015**, *2*, 1164−1170.
(37) Zaffar, H.; Ahmad, R.; Pervez, A.; Naqvi, T. A. A newly isolated Pseudomonas sp. can degrade endosulfan via hydrolytic pathway. *Pestic. Biochem. Physiol.* **2018**, *152*, 69−75.
(38) Fareed, A.; Zaffar, H.; Bilal, M.; Hussain, J.; Jackson, C.; Naqvi, T. A. Decolorization of azo dyes by a novel aerobic bacterial strain *Bacillus cereus* strain ROC. *PLoS One* **2022**, *17* (6), No. e0269559.
(39) Ravi, A.; Ravuri, M.; Krishnan, R.; Narenkumar, J.; Anu, K.; Alsalhi, S. M.; Devanesan, S.; Kannan, K. S.; Rajasekar, A. Characterization of petroleum degrading bacteria and its optimization conditions on effective utilization of petroleum hydrocarbons. *Microbiol. Res.* **2022**, *265*, No. 127184.
(40) Meena, K. R.; Dhiman, R.; Singh, K.; Kumar, S.; Sharma, A.; Kanwar, S. S.; Mondal, R.; Das, S.; Franco, O. L.; Mandal, A. K. Purification and identification of a surfactin biosurfactant and engine oil degradation by *Bacillus velezensis* KLP2016. *Microb. Cell Fact.* **2021**, *20* (1), 26.
(41) Fu, X.; Wang, H.; Bai, Y.; Xue, J.; Gao, T. G. Y.; Hu, S.; Sun, J.; Wu, T.; Hu, S. Systematic degradation mechanism and pathways analysis of the immobilized bacteria: Permeability and biodegradation, kinetic and molecular simulation. *Environ. Sci. Ecotechnol.* **2020**, *2*, No. 100028.
(42) Chittepu, O. R. Isolation and characterization of biosurfactant producing bacteria from groundnut oil cake dumping site for the

https://doi.org/10.1021/acsomega.4c06964
ACS Omega 2025, 10, 541−549

control of foodborne pathogens. *Grain Oil Sci. Technol.* **2019**, *2*, 15−20.
(43) Ullah, N.; Haq, I. U.; Mirza, B. Phytotoxicity evaluation and photochemical analysis of three medicinally important plants from Pakistan. *Toxicol. Ind. Health* **2015**, *31*, 389−395.
(44) Nayak, N. S.; Purohit, M. S.; Tipre, D. R.; Dave, S. R. Biosurfactant production and engine oil degradation by marine halotolerant *Bacillus licheniformis* LRK1. *Biocatal. Agric. Biotechnol.* **2020**, *29*, No. 101808.
(45) Patowary, K.; Patowary, R.; Kalita, M. C.; Deka, S. Characterization of biosurfactant produced during degradation of hydrocarbons using crude oil as sole source of carbon. *Front. Microbiol.* **2017**, *8*, 279.
(46) Ambaye, T. G.; Chebbi, A.; Formicola, F.; Prasad, S.; Gomez, H. F.; Franzetti, A.; Vaccari, M. Remediation of soil polluted with petroleum hydrocarbons and its reuse for agriculture: Recent progress, challenges, and perspectives. *Chemosphere* **2022**, *293*, No. 133572.
(47) Kumari, B.; Singh, S. N.; Singh, D. P. Characterization of two biosurfactant producing strains in crude oil degradation. *Process Biochem.* **2012**, *47* (12), 2463−2471.
(48) Van Beilen, J. B.; Wubbolts, M. G.; Witholt, B. Geneticsof alkane oxidation by *Pseudomonas oleovorans*. *Biodegradation* **1994**, *5*, 161−174.
(49) Bhatawadekar, V. C.; Damare; Samir, R.; Anita, G. Biodegradation of mixed polycyclic aromatic hydrocarbons by Pseudomonas sp. isolated from estuarine sediment. *Biorem. J.* **2023**, *27* (2), 116−125.
(50) El-Liethy, M. A.; El-Noubi, M. M.; Abia, A. L. K.; et al. Eco-friendly bioremediation approach for crude oilpolluted soils using a novel and biostimulated *Enterobacter hormaechei* ODB H32 strain. *Int. J. Environ. Sci. Technol.* **2022**, *19*, 10577−10588.
(51) Ferreira, C. A.; Inga, J.; Buendia, H.; Raul, V.; Osir, R.; Danessa, G.; Walter, A.; Benji, M.; Madjory, M.; Utos, S.; Thonny, C.; Echeverri, V.; Andrés; Filho, M. Identification of 20 species from the Peruvian Amazon tropical forest by the wood macroscopic features. *Cerne* **2023**, *29*, 1.
(52) Zhang, Z.; Hou, Z.; Yang, C.; Ma, C.; Tao, F.; Xu, P. 2011 Degradation of n-alkanes and poly- Cyclic aromatic hydrocarbons in petroleum by a newly isolated *Pseudomonas aeruginosa* DQ8. *Bioresour. Technol.* **2011**, *102*, 4111−4116.
(53) Al-Wasify, R. S.; Hamed, S. R. Bacterial biodegradation of crude oil using local isolates. *Int. J. Bacteriol.* **2014**, *2014*, No. 863272.
(54) Varuvel, E. G.; Seetharaman, S.; Bai, S.; Devarajan, Y.; Balasubramanian, D. Development of artificial neural network and response surface methodology model to optimize the engine parameters of rubber seed oil Hydrogen on PCCI operation. *Energy* **2023**, *283*, No. 129110.
(55) Chen, Y.; Yu, B.; Lin, J.; Naidu, R.; Chen, Z. Simultaneous adsorption and biodegradation (SAB) of diesel oil using immobilized *Acinetobacter venetianus* on porous material. *Chem. Eng. J.* **2016**, *289*, 463−470.
(56) Mao, N.; Xue, S.; Zhang, G.; Xia, S.; Chen, X.; You, W.; Sun, Y.; Shen, L.; Wang, S. Crude oil degradation in oilfield produced water by immobilized microbes and oil recovery improvement after its reinjection. *Int. Biodeterior. Biodegrad.* **2022**, *173*, No. 105452.
(57) Lu, L.; Rughöft, S.; Straub, D.; Joye, B. S.; Kappler, A.; Kleindienst, S. Rhamnolipid biosurfactants enhance microbial oil biodegradation in surface seawater from the North Sea. *ACS ES&T Water* **2023**, *3* (8), 2255−2266.
(58) Song, X.; Xu, Y.; Li, G.; Zhang, Y.; Huang, T.; Hu, Z. Isolation, characterization of Rhodococcus sp. P14 capable of degrading high molecular weight polycyclic aromatic hydrocarbons and aliphatic hydrocarbons. *Mar. Pollut. Bull.* **2011**, *62* (10), 2122−2128.
(59) da Silva Correa, H.; Blum, C. T.; Galvão, F.; Maranho, L. T. Effects of oil contamination on plant growth and development: a review. *Environ. Sci. Pollut. Res. Int.* **2022**, *29* (29), 43501−43515.
(60) Marchut-Mikolajczyk, O.; Drozdzynski; Pietrzyk, D.; Antczak, T. Biosurfactant production and hydrocarbon degradation activity of endophytic bacteria isolated from *Chelidonium majus* L. *Microb. Cell Fact.* **2018**, *17*, 171.
(61) Khan, H. U.; Khan, R. A.; Ahmad, M.; Mushtaq, N.; Rashid, M.; Nowshad, M. Phytotoxic potential of methanolic extract of *Cardia obaliqua*. *Altern. Integ. Med.* **2014**, *3*, 150.

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