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Isolation and Characterization of Biosurfactant-Producing Bacteria for Enhancing Oil Recovery

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Abstract: Biosurfactants produced by bacteria possess remarkable emulsification properties for crude oil, significantly enhancing oil mobility and recovery rates. This study aimed to isolate and screen biosurfactant-producing bacteria for oil enhancing recovery. A total of 93 bacterial strains were isolated from marine sediments, with three high-yield biosurfactant-producing strains identified: *Pseudomonas aeruginosa* N33, *Bacillus paralicheniformis* Nian2, and *Stenotrophomonas nematodicola* T10. The fermentation conditions, such as pH, carbon source, nitrogen source, and C/N ratio, were optimized to maximize the yield and activity of biosurfactants. Further evaluations were performed to assess the stability of the bio-surfactant activity and its emulsification properties. The results indicated that all three strains produced biosurfactants that retained their oil displacement activity in the presence of Na^+ and Mg^{2+}, but showed a significant reduction in their activities in the presence of Ca^{2+}. The biosurfactants maintained their original activity after treatment at 120 °C for 3 h. Additionally, the biosurfactants produced by all three strains demonstrated excellent oil emulsification capabilities. Static oil-washing and dynamic displacement experiments revealed static oil recovery rates of 28.1%, 23.4%, and 7.1%, respectively, for N33, Nian2, and T10, and dynamic oil displacement recovery rates of 95.0%, 74.1%, and 69.0%, respectively. This research provides valuable microbial resources for enhancing oil recovery via microorganisms and lays a foundation for practical application.

Keywords: microbial enhancing oil recovery (MEOR); bacterial biosurfactants; oil recovery rate; stability; emulsification

Citation: Jiang, M.; Wang, H.; Liu, J.; Hou, X.; Zhang, Y.; Liu, X.; Wei, S.; Cui, Q. Isolation and Characterization of Biosurfactant-Producing Bacteria for Enhancing Oil Recovery. *Processes* **2024**, *12*, 2575. https://doi.org/10.3390/pr12112575

Academic Editors: Tatiana Felix Ferreira and Priscilla Amaral

Received: 21 October 2024
Revised: 13 November 2024
Accepted: 14 November 2024
Published: 17 November 2024

1. Introduction

Petroleum resources have been pivotal in driving societal development. With rapid economic development, the demand for petroleum resources continues to grow. However, after primary and secondary recovery stages, numerous oil fields worldwide have now entered mid-to-late development stages, with many experiencing declining production rates and some nearing depletion. Consequently, the need for advanced tertiary recovery techniques has emerged as a critical challenge for the petroleum industry [1].

Currently, enhanced oil recovery (EOR) methods consist of four main approaches: thermal recovery, chemical flooding, gas miscible flooding, and microbial enhancing oil recovery (MEOR) [2,3]. Among these, chemical surfactant flooding has become widely used, drawing considerable attention within the EOR field [4,5]. Although chemical surfactants have significantly improved oil recovery rates, their limited biodegradability raises environmental concerns, and their high cost and restricted applicability have hindered

broader adoption [6]. In contrast, biosurfactants, recognized for their biodegradability, low toxicity, and environmental compatibility, have garnered increasing interest as a more sustainable alternative for enhancing oil recovery [7,8].

Biosurfactants are surface-active compounds produced by microorganisms that possess both hydrophilic and hydrophobic domains within their structure, enabling them to act at the interface between water and non-water phases, thereby reducing surface tension. Marchant and Banat discovered that microbial biosurfactants can lower the oil–water interfacial tension, disrupt the water film that traps residual oil in the rock pores, and release the oil as small free droplets dispersed in the aqueous phase [9]. Additionally, biosurfactants can emulsify the residual oil, forming microemulsions, which improves the flow properties of the oil–water phases and enhances oil recovery rates [10]. They also alter the wettability of rock surfaces, shifting the internal surface from oleophilic to hydrophilic. This alteration causes residual oil to detach from the surface as free droplets under capillary forces, allowing it to be carried out with water, ultimately increasing oil recovery [11].

Microbial biosurfactants can enhance oil recovery by reducing the interfacial tension between oil and water, emulsifying and dispersing residual oil, and altering rock wettability [12–14]. A wide range of microorganisms produce biosurfactants, which are broadly classified into three main categories: glycolipids, lipopeptides, and phospholipids [15]. Previous studies have shown that several species within the genera *Pseudomonas*, *Dietzia*, *Rhodococcus*, *Arthrobacter*, *Corynebacterium*, *Torulopsis*, and *Alcanivorax* produce glycolipid-based biosurfactants [16–21]. Meanwhile, lipopeptide-based biosurfactants are produced by species from the genera *Bacillus*, *Arthrobacter*, *Virgibacillus*, and *Acinetobacter* [22–27]. Additionally, species within the genera *Corynebacterium* and *Thiobacillus* are known to produce phospholipid-based biosurfactants [28,29]. These bacteria are capable of synthesizing significant quantities of effective and stable biosurfactants [30,31]. Khademolhosseini et al. investigated the rhamnolipids produced by *P. aeruginosa* [21], finding that these biosurfactants exhibited remarkable stability at temperatures ranging from 40 °C to 121 °C, pH values from 3 to 10, and in the presence of 10% NaCl. Notably, in microbial enhanced oil recovery (MEOR) experiments, these biosurfactants significantly increased oil recovery rates by 43% compared to the control. Zhao et al. examined surfactin production by *Bacillus subtilis* AnPL-1 [27], evaluating its ability to emulsify in situ and reduce crude oil viscosity under conditions simulating a reservoir environment. Their results demonstrated that surfactin produced by this strain effectively reduced oil viscosity by 40.6% through in situ emulsification, ultimately increasing oil recovery by nearly 10%. Liu et al. reported that surfactin from *Bacillus licheniformis* L20 maintained robust emulsification activity across a pH range of 4–11 [26], at temperatures up to 85 °C, and in the presence of up to 25 wt% NaCl, resulting in a 19.58% increase in oil recovery.

Microbial enhanced oil recovery primarily comprises two approaches: in situ and ex situ MEOR [32]. In situ MEOR involves injecting nutrients into the oil well to activate or enhance the activity of naturally occurring microorganisms within the reservoir, thereby achieving oil recovery. In contrast, ex situ MEOR introduces specific microbial strains directly into the reservoir to further improve oil recovery efficiency. However, the high salinity and elevated temperatures typical of oil reservoir environments can create substantial challenges for the survival of these introduced microbes and the stability of their metabolic products [33,34]. This study aimed to isolate and screen biosurfactant-producing bacteria from marine sediments. It further investigated the optimal conditions for biosurfactant production, as well as the salt tolerance, thermal stability, and emulsification properties of the produced biosurfactants. Static oil-washing and displacement experiments were conducted to evaluate their oil displacement efficiency. The primary objective was to identify highly efficient and stable biosurfactant-producing strains, providing a foundation for developing ex situ MEOR technologies and improving oil recovery rates.

2. Materials and Methods

2.1. Isolation and Screening of Biosurfactant-Producing Bacteria

Sediment samples were collected from the Qilihai Lagoon (119.27° E, 39.59° N) and Xinhe estuary (119.31° E, 39.50° N) (both located in Beidaihe, China), as well as from Hainan, China (109.59° E, 19.25° N). All samples were collected from the top 5–10 cm layers of sediment, immediately transferred into sterilized 50 mL centrifuge tubes, and then shipped to the laboratory for bacterial isolation. Each sample (5 g) was suspended in 45 mL of sterile water, creating a 10^{-1} suspension. This suspension was then serially diluted tenfold to 10^{-5}. Twenty microliters of each dilution was spread onto nutrient agar plates (containing beef extract 3 g/L, peptone 10 g/L, NaCl 5 g/L, agar 20) and incubated upside down at 30 °C for 2–3 days until single colonies appeared. Single colonies were then picked and inoculated into 5 mL of basal fermentation medium (composed of glucose 5 g/L, peptone 5.0 g/L, K_2HPO_4 5.0 g/L, KH_2PO_4 2.0 g/L, NaCl 0.1 g/L, $MgSO_4$ 0.2 g/L, and adjusted to pH 7.0) in test tubes. The tubes were incubated in a shaking incubator at 30 °C and 150 rpm for 4 days. This culture was then used for screening of surfactant-producing bacteria following the method described below. All chemicals were purchased from China National Pharmaceutical Group Co., Ltd. (Sinopharm, Beijing, China).

The oil displacement method was used for the screening of surfactant-producing bacteria [35]. A 10 cm diameter Petri dish was placed over a sheet of graph paper and filled with 30 mL of water. One hundred microliters of Sudan red-stained paraffin oil, heated to 60 °C, were then added to the water surface. Once the paraffin oil had spread evenly, forming a film, 20 µL of each bacterial culture was carefully dropped into the center of the oil film. A clear zone formed around the droplet, indicating surfactant-producing bacteria, and the diameter of this clear zone was recorded.

2.2. Identification of Surfactant-Producing Bacteria

The isolated surfactant-producing strains were incubated in the liquid nutrient culture. Genomic DNA was extracted from pure culture with the Fast Spin Kit (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. The 16S rRNA gene sequences were amplified by PCR with universal primers (27f and 1492r) targeting the bacterial 16S rRNA gene, following the method described by Wei et al. [36]. The PCR amplicons were visualized on an agarose gel, excised, purified, and sequenced. The obtained 16S rRNA gene sequences were then compared against sequences in the NCBI database using a BLAST search to determine their identity. The sequences were aligned with their relatives using Clustal W, and a phylogenetic tree was constructed by the neighbor-joining method using the maximum-parsimony algorithm in MEGA10 software with 1000 bootstrap replicates.

2.3. Optimization of Biosurfactant Production Fermentation Conditions

Inoculum Preparation: An inoculum was prepared by inoculating surfactant-producing bacteria into 5 mL of nutrient medium and incubated in a shaking incubator at 30 °C and 150 rpm for 4 days. The inoculum was then used for subsequent experiments.

Initial pH: To optimize the initial pH of the fermentation medium, the basal fermentation medium (100 mL) was adjusted to pH 6, 7, and 8, respectively.

Carbon and Nitrogen Sources: To optimize the carbon and nitrogen sources, three different carbon source media were prepared by replacing the glucose (5.0 g/L) in the basal fermentation medium with beef extract (10.0 g/L), glucose (10.0 g/L), and sucrose (10.0 g/L), respectively [37,38]. Similarly, three different nitrogen source media were prepared by replacing the peptone (3.0 g/L) with peptone (5.0 g/L), $NaNO_3$ (5.0 g/L), and NH_4Cl (5.0 g/L), respectively [37,39].

C/N Ratio: Following the optimization of pH, carbon source, and nitrogen source, a new fermentation medium was formulated using the optimal conditions. The nitrogen source was fixed at $NaNO_3$ (5 g/L), and glucose was added at varying concentrations (2.65, 3.52, 4.41, 5.29, 6.18, and 7.00 g/L) to achieve C:N ratios of 15:1, 20:1, 25:1, 30:1, 35:1, and 40:1, respectively. Six different C/N ratio media were thus prepared.

All of the media described above were sterilized by autoclaving, after which a 5% (*v*/*v*) inoculum was added to each medium. The inoculated media were then incubated in a shaking incubator at 30 °C and 150 rpm for 4 days. The diameter of the oil displacement zone in the fermentation broth was measured, with each experiment performed in triplicate.

2.4. Determination of Biosurfactant Stability

To evaluate the stability of biosurfactants in the presence of mineral ions and varying temperatures, two separate experiments were conducted.

The approach used to evaluate the influence of mineral ions on biosurfactant activity was performed by Amani et al. described with modifications [40]. The bacteria were inoculated into the optimized medium and incubated at 30 °C and 150 rpm for 4 days. Then, 50 mL of the culture was taken, and different amounts of NaCl, $MgCl_2$, and $CaCl_2$ were added to achieve salt concentrations (*w*/*v*) ranging from 2.5% to 50%, in increments of 2.5%. After thorough mixing, 10 µL was taken to measure the oil displacement diameter produced by the biosurfactant at each salt concentration. To evaluate the thermal stability of the biosurfactant, 50 mL of the culture was taken and heated at 20 °C, 40 °C, 60 °C, 80 °C, 100 °C, and 120 °C for 3 h, respectively. The diameter of the oil displacement zone was measured every 30 min to monitor changes in biosurfactant activity.

2.5. Emulsification Properties of Biosurfactants

To evaluate the emulsification properties of the biosurfactants, 5 mL of bacterial culture (volume denoted as V_1) was added to a 50 mL centrifuge tube. Ten milliliters of crude oil was then added, and the mixture was thoroughly mixed using a vortex mixer. The mixture was then quickly transferred to a glass test tube, and the volume of water separated at the bottom of the tube was recorded at 0 min, 30 min, 60 min, and 120 min, respectively. The volume of separated water was denoted as V_2. Control experiments were performed using water and culture medium alone. The emulsification rate for each time point was calculated using the following formula: Emulsification rate = $1 - V_2/V_1 \times 100\%$. Each experiment was performed in triplicate.

2.6. Static Oil Washing Experiment

The static oil washing experiment was adapted from the method described by Datta et al. [41]. Quartz sand and crude oil were mixed thoroughly at a ratio of 9:1 (*w*/*w*) and aged at room temperature for 24 h to allow the oil to adhere to the sand surface. One hundred and fifty grams of the oil–sand mixture was then placed into an Erlenmeyer flask, and its weight was recorded as m_1. One hundred and fifty milliliters of bacterial biosurfactant fermentation broth were added to the flask, and the mixture was allowed to stand at room temperature for 10 days. The liquid was then decanted, and the flask and oil–sand mixture were dried in an oven at 80 °C until a constant weight was reached. The final weight was recorded as m_2. The static oil recovery rate was calculated using the following formula: Static oil recovery rate = $(m_1 - m_2)/m_1 \times 100\%$. Each experiment was performed in triplicate.

2.7. Oil Displacement Experiment

Seven grams of the aged oil–sand mixture was weighed and packed into a syringe. The weight was recorded as G_1. Ten milliliters of bacterial biosurfactant was slowly injected from the bottom of the syringe. The syringe was allowed to stand at room temperature for approximately 1 h. The syringe was then inverted, the liquid was removed, and the syringe was dried in an oven at 80 °C until a constant weight was reached. The final weight was recorded as G_2. The oil displacement rate was calculated using the following formula: Oil displacement rate = $(G_1 - G_2)/G_1 \times 100\%$.

3. Results

3.1. Isolation, Screening, and Identification of Surfactant-Producing Bacteria

A total of 93 colonies were selected based on the bacterial morphologies observed on the plates, with 21, 22, and 50 single colonies picked from sediment samples collected from Xinhe, Qilihai, and Hainan, respectively. All 93 strains were inoculated into nutrient media and incubated with shaking at 30 °C for 4 days. Twenty microliters of each culture was then subjected to the oil displacement test, which revealed that eight bacterial strains produced clear oil displacement zones. These included one strain isolated from the Qilihai Lagoon sediments, designated as T10, and seven strains from the Hainan sediments, designated as N23, N28, N33, N43, N44, Nian1, and Nian2 (Figure 1). No surfactant-producing bacteria were found in the Xinhe estuary sediments. Among these strains, N33, Nian2, and T10 displayed the largest oil displacement zone diameters, measuring 5.9 ± 0.3 cm, 3.1 ± 0.2 cm, and 2.3 ± 0.3 cm, respectively, and were selected for further experiments.

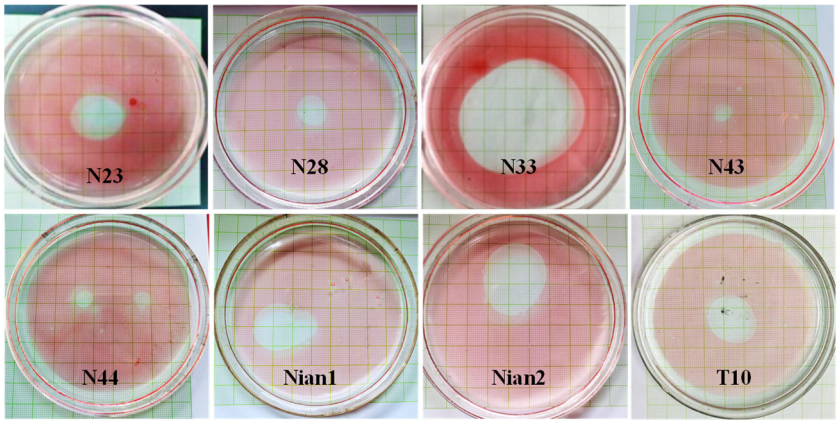

Figure 1. Screening of surfactant-producing bacteria by the oil displacement ring method. The paraffin oil was stained with Sudan red for easier observation.

The 16S rRNA gene sequences of the three strains, N33, Nian2, and T10, were compared to sequences in the GenBank database. Phylogenetic analysis revealed that the 16S rRNA gene sequences of N33, Nian2, and T10 exhibited 99.8% similarity to *Pseudomonas aeruginosa* DSM50071 (NR117678), *Bacillus paralicheniformis* LXJ3 (MN746205), and *Stenotrophomonas nematodicola* W5 (NR181111), respectively (Figure 2).

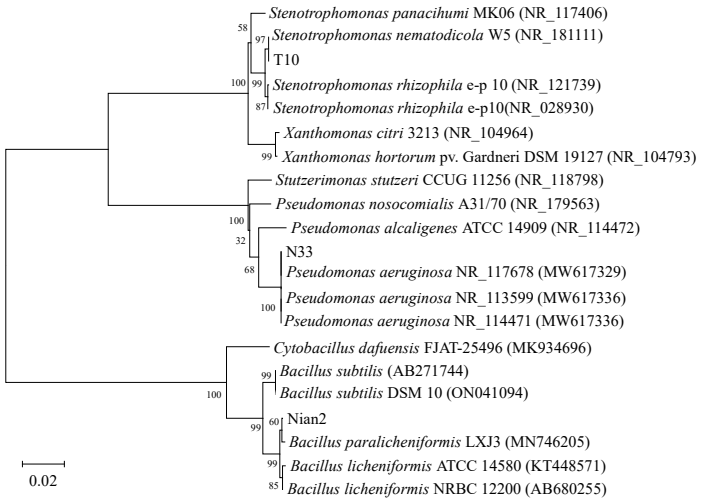

Figure 2. Phylogenetic tree of bacteria N33, Nian2, T10 with the related species of biosurfactant based on 16S rRNA sequence. The scale bar represents a 2% nucleotide sequence divergence.

3.2. Optimization of Biosurfactant Production Fermentation Conditions

The optimization of fermentation conditions was performed to enhance biosurfactant production and activity, with the results summarized in Figure 3.

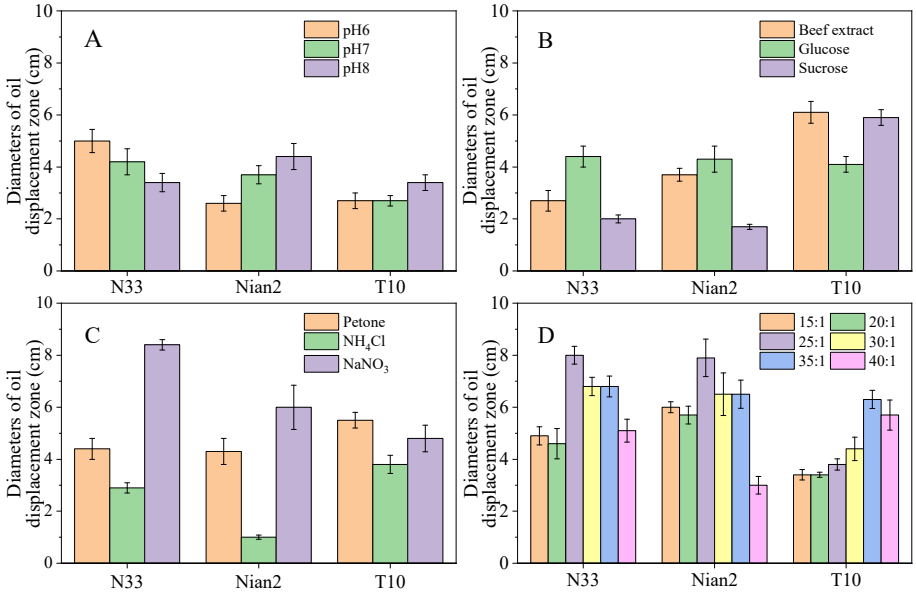

Figure 3. Optimization of fermentation conditions for bacterial surfactant production ((**A**), pH; (**B**), carbon source; (**C**), nitrogen source; (**D**), C/N ratio).

Initial pH: The initial pH of the media significantly impacted biosurfactant production. N33 exhibited the largest oil displacement zone diameter (5.0 cm) at pH 6. Nian2 and T10 displayed their largest oil displacement zone diameters (approximately 4.5 ± 0.5 cm and 3.3 ± 0.3 cm, respectively) at pH 8 (Figure 3A).

Carbon source: The carbon source also plays a crucial role in biosurfactant production. Glucose was found to be the optimal carbon source for N33 and Nian2, resulting in oil displacement zone diameters of 4.4 ± 0.4 cm and 4.3 ± 0.5 cm, respectively. For T10, beef extract was the optimal carbon source, producing an oil displacement zone diameter of 6 ± 0.3 cm (Figure 3B).

Nitrogen source: The nitrogen source had a significant impact on biosurfactant production. Sodium nitrate was found to be the optimal nitrogen source for N33 and Nian2, resulting in a significant increase in oil displacement capability, with oil displacement zone diameters of approximately 8.1 ± 0.2 cm and 6.0 ± 0.85 cm, respectively (Figure 3C). This suggests that nitrate plays a critical role in biosurfactant production by N33 and Nian2. For T10, peptone was the optimal nitrogen source, achieving an oil displacement zone diameter of 5.3 ± 0.3 cm.

C/N ratio: Following the optimization of pH, carbon source, and nitrogen source, the impact of the C/N ratio on biosurfactant production was further investigated (Figure 3D). The largest oil displacement zone diameters for N33 and Nian2 were observed at a C:N ratio of 25:1, measuring 8.0 ± 0.34 cm and 7.8 ± 0.72 cm, respectively. For T10, the largest oil displacement zone diameter 6.2 ± 0.35 cm occurred at a C:N ratio of 30:1.

The strains N33, Nian2, and T10 were re-fermented using the optimized media, which included adjustments to the initial pH, carbon source, nitrogen source, and C/N ratio. Ten microliters of each fermentation broth (half the volume used before optimization) was tested for oil displacement. The results revealed oil displacement zone diameters of 8.2 ± 0.5 cm for N33, 7.9 ± 0.3 cm for Nian2, and 5.0 ± 0.2 cm for T10. Compared to the oil displacement zone diameter produced by the bacterial surfactant before optimization, its oil displacement activity significantly improved.

3.3. Stability Tests of Biosurfactants

To investigate the impact of mineral ions and temperature on the stability of the biosurfactants produced by the bacteria, the stability of the biosurfactants was assessed in the presence of different concentrations of Na^+, Mg^{2+}, and Ca^{2+}, as well as the different temperatures (Figure 4).

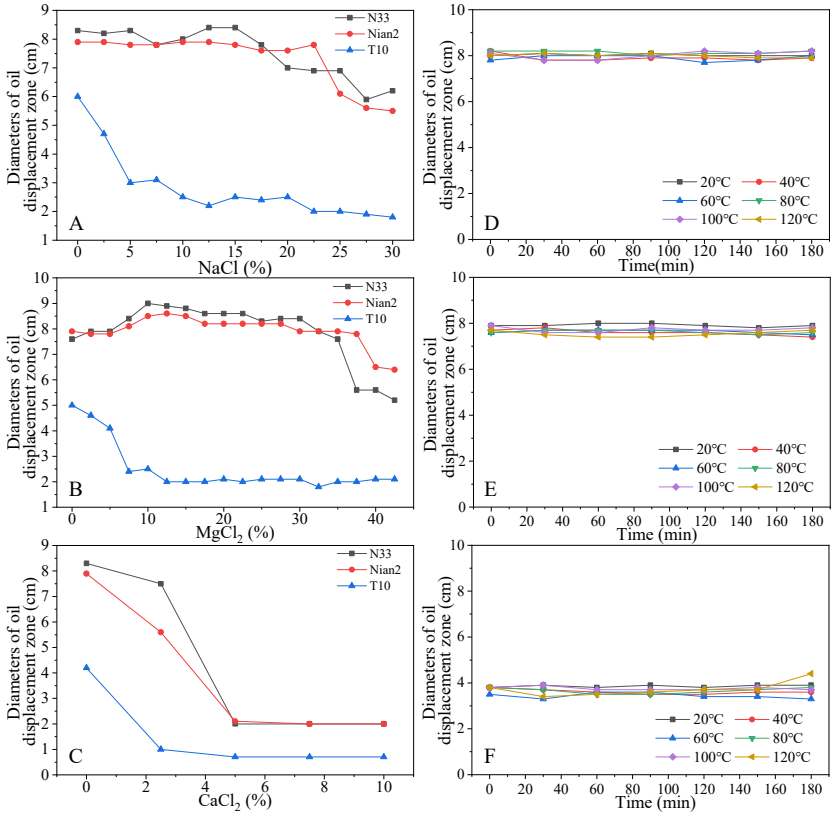

Figure 4. Effects of mineral ions (**A**–**C**) and temperature (**D**–**F**) on the stability of surfactants produced by N33, Nian2, T10 strains ((**A**), Na^+; (**B**), Mg^{2+}; (**C**), Ca^{2+}; (**D**), N33, (**E**), Nian2, (**F**), T10).

The stability of the biosurfactants produced by N33, Nian2, and T10 in the presence of different mineral ions was investigated, with the results displayed in Figure 4A–C.

Sodium ions: The effect of Na^+ on biosurfactant stability is shown in Figure 4A. Biosurfactants produced by N33 and Nian2 maintained good oil displacement activity at NaCl concentrations ranging from 0 to 25%. However, their oil displacement activity decreased when the NaCl concentration exceeded 25%. In contrast, the fermentation broth of T10 exhibited significant oil displacement activity only at NaCl concentrations below 7.5%, with higher Na^+ concentrations causing inactivation, indicating a lower tolerance to Na^+ compared to the other two strains.

Magnesium ions: The impact of Mg^{2+} on biosurfactant stability is shown in Figure 4B. Biosurfactants produced by N33 and Nian2 maintained good oil displacement activity at $MgCl_2$ concentrations ranging from 0 to 32.5% and 0 to 37.5%, respectively. Their oil displacement activity decreased as the Mg^{2+} concentration exceeded 37.5%. The biosurfactant from T10 demonstrated limited oil displacement activity at $MgCl_2$ concentrations below 5%, suggesting lower tolerance to Mg^{2+} compared to N33 and Nian2.

Calcium ions: The effect of Ca^{2+} on biosurfactant stability is shown in Figure 4C. All three biosurfactants were sensitive to Ca^{2+}, which led to the inactivation of the biosurfactants. At $CaCl_2$ concentrations ranging from 0 to 5%, the biosurfactant activity decreased significantly with increasing Ca^{2+} concentration.

The effect of temperature on biosurfactant stability was investigated, with the results shown in Figure 4D–F. The results indicate that the biosurfactants produced by N33

(Figure 4D), Nian2 (Figure 4E), and T10 (Figure 4F) maintained their oil displacement activity after continuous exposure at temperatures ranging from 0 to 120 °C for 3 h. The oil displacement zone diameters remained relatively constant at approximately 8.1 ± 0.6 cm, 7.9 ± 0.5 cm, and 3.8 ± 0.2 cm, respectively, for N33, Nian2, and T10. These results suggest that the biosurfactants produced by these three bacterial strains have a broad temperature tolerance and retain their original activity even after being treated at 120 °C for 3 h.

3.4. Emulsification of Crude Oil by Biosurfactants

Control experiments using culture medium and water mixed with crude oil showed that the oil and water layers separated within 10 s. Calculations revealed that the emulsification rates of controls with crude oil were close to 0%. In contrast, the biosurfactants produced by N33, Nian2, and T10 exhibited significant emulsification properties (Figure 5A). The rate of water separation at the bottom of the test tubes containing N33, Nian2, and T10 biosurfactants was slower. A distinct water layer was only observed after 5 to 30 min of settling, indicating that the biosurfactants produced by N33, Nian2, and T10 effectively reduced the interfacial tension between oil and water, leading to the formation of stable emulsions. Even after 120 min, the oil and water layers in the experimental groups had not fully separated, confirming the robust emulsification properties of the biosurfactants produced by N33, Nian2, and T10.

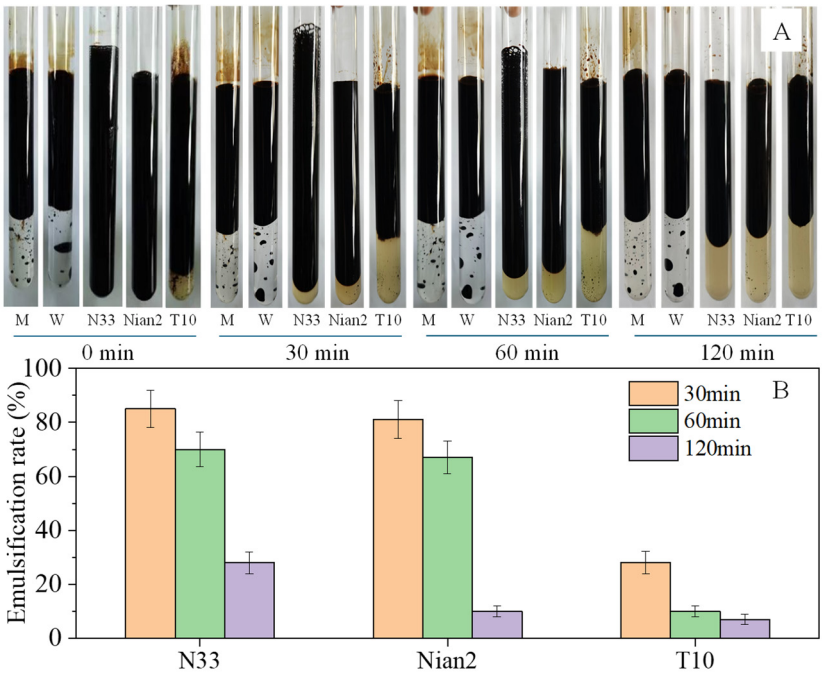

Figure 5. Emulsification of petroleum by bacterial surfactants (**A**) and emulsification rate (**B**). M: medium; W: water.

The emulsification rate at different time points was calculated based on the ratio of the volume of water separated at the bottom of the test tube to the initial volume of the biosurfactant solution (Figure 5B). The results show that the emulsification rates of the biosurfactants produced by N33, Nian2, and T10 all decreased over time. However, the emulsification rate decline was relatively slow for the biosurfactants produced by N33 and Nian2, suggesting that their emulsification properties were more stable than those of T10.

3.5. Static Oil-Washing Efficiency of Biosurfactants

The static oil-washing efficiency of the biosurfactants produced by N33, Nian2, and T10 was evaluated by treating oil–sand mixtures for 10 days (Figure 6A–D). The control group, treated only with medium (CK), showed negligible oil removal, with a recovery rate

of 3.8%. In contrast, significant oil removal was observed with the biosurfactants, with N33 and Nian2 showing the most promising results. Visual inspection revealed a whitening effect in the oil–sand mixture treated with N33 and Nian2, indicating oil extraction, while T10 showed minimal oil removal. Quantitatively, N33 and Nian2 achieved oil recovery rates of 28.1% and 23.4%, respectively, whereas T10 reached only 7.1%. These findings highlight the superior oil-washing potential of biosurfactants produced by N33 and Nian2 compared to T10.

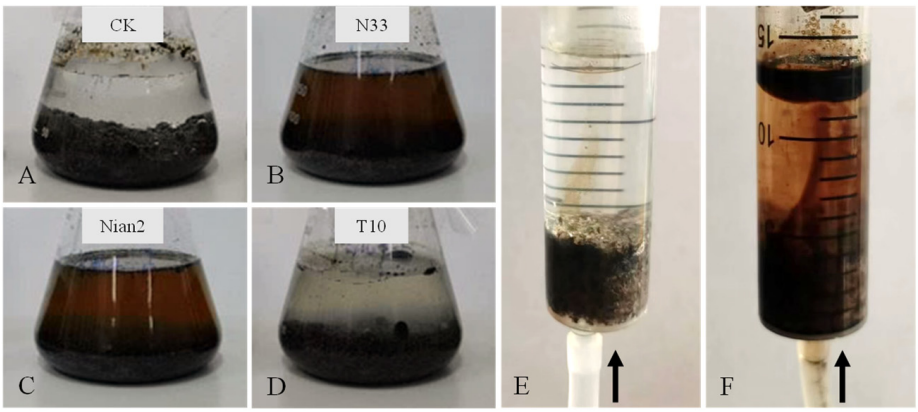

Figure 6. Static oil washing efficiency (**A**–**D**) and dynamic oil recovery performance (**E**,**F**) of biosurfactants produced by strains N33, Nian2, and T10. Arrows indicate injection water (**E**) and bacteria-produced surfactant (**F**).

3.6. Oil Displacement Efficiency of Biosurfactants

The dynamic oil displacement efficiency of the biosurfactants was assessed using a simulated water flooding method (Figure 6E,F). A control group (CK) involving only water injection showed no oil displacement, highlighting the necessity of biosurfactants for enhancing oil recovery. In contrast, all three bacterial strains (N33, Nian2, and T10) exhibited significant oil displacement when their respective biosurfactant solutions were injected. The biosurfactant produced by N33 demonstrated the highest oil displacement efficiency, achieving a rate of 95.0%, followed by Nian2 (74.1%) and T10 (69.0%). Visual observations of a gradual whitening of the oil–sand mixture further confirmed the successful displacement of oil by the biosurfactants. These results indicate that the biosurfactants produced by the three bacterial strains possess promising potential for enhancing oil recovery in a dynamic setting, particularly the biosurfactant produced by N33.

4. Discussion

4.1. Diversity of Biosurfactant-Producing Bacteria

Bacteria capable of producing biosurfactants are widely distributed across various natural ecosystems, including soil, oceans, oil-contaminated sites, industrial wastewater, and extreme environments [42–46]. The frequently identified bacterial genera include *Pseudomonas*, *Bacillus*, *Acinetobacter*, *Rhodococcus*, *Mycobacterium*, *Antarctobacter*, *Halomonas*, *Alcanivorax*, *Marinobacter*, etc. [40,47–50]. In this study, 8 surfactant-producing bacterial strains were identified from a total of 93 bacterial strains isolated from the sediments of Qilihai Lagoon and the intertidal zone of Hainan. In contrast, no surfactant-producing bacteria were found in the sediments of the Beidaihe estuary. This absence may be attributed to the estuary's unique environmental conditions. Domingues et al. conducted an investigation and isolated surfactant-producing bacteria from three different microenvironments: sea surface water, sediments, and rhizosphere within the estuary. They found that the proportion of surfactant-producing bacteria isolated from sediments was the lowest, and the reasons for this phenomenon require further investigation [51].

Among the eight surfactant-producing bacterial strains, three highly efficient biosurfactant producers, N33, Nian2, and T10, were identified as belonging to the genera

Pseudomonas, *Bacillus*, and *Stenotrophomonas*, respectively. Previous studies indicate that *Pseudomonas* sp. and *Bacillus* sp. are predominant biosurfactant-producing bacteria, commonly isolated from various environments and known for their remarkable ability to survive and adapt to a wide range of conditions [6]. The biosurfactants produced by these bacteria have important applications in enhancing oil recovery, as well as in agriculture and medicine [52]. The study of the diversity of surfactant-producing bacteria is beneficial for exploring their potential use as microbial resources in oil recovery technology.

4.2. Factors Affecting Bacterial Biosurfactant Production

Optimizing the carbon source, nitrogen source, C/N ratio, and pH is essential for enhancing bacterial biosurfactant production. This study determined that *P. aeruginosa* N33 and *B. paralicheniformis* Nian2 utilize glucose and sodium nitrate as their optimal carbon and nitrogen sources, respectively, with a C/N ratio of 25:1 resulting in the highest biosurfactant activity. In contrast, *S. nematodicola* T10 prefers beef extract and peptone as its optimal carbon and nitrogen sources, respectively, with a C/N ratio of 30:1. These findings differ from those of previous studies. Singh and Sachan examined the carbon and nitrogen sources for three biosurfactant-producing bacterial strains isolated from soil [53]. They discovered that *Bacillus* sp. PS1 utilizes glycerol and yeast extract as its optimal carbon and nitrogen sources, while *Bacillus* sp. DS2 prefers olive oil and peptone, and *Bacillus* sp. MS4 favors glycerol and peptone for biosurfactant synthesis. Bezza et al. observed that *B. subtilis* exhibits significantly enhanced biosurfactant production when utilizing sunflower oil as a carbon source compared to glycerol [54]. Notably, the addition of 5% (*w/v*) sunflower oil after glycerol depletion led to a more than 200% increase in biosurfactant yield. Deepika et al. demonstrated that *P. aeruginosa* KVD-HR42 achieves maximum biosurfactant production when using karanja oil and sodium nitrate as its carbon and nitrogen sources, respectively [55]. Furthermore, Ezebuiro et al. found that the optimal carbon and nitrogen sources for *Stenotrophomonas* sp. are glucose and a combination of yeast extract and NH_4NO_3 [56]. These studies indicate that different bacterial strains have varying requirements for carbon and nitrogen sources. Optimizing these nutrient sources is essential in industrial applications such as MEOR to enhance biosurfactant yield and cost efficiency [37–39,57,58].

Moreover, the initial pH of the medium significantly influences the yield and activity of bacterial biosurfactants. In this study, it was found that *P. aeruginosa* N33 required a lower initial pH of 6 to achieve higher biosurfactant yield and activity. In contrast, *B. paralicheniformis* Nian2 and *S. nematodicola* T10 reached the highest biosurfactant yield and activity at a higher initial pH of 8. These findings are somewhat aligned with those of Singh and Sachan [53] and Deepika et al. [55], who noted that *Bacillus* sp. PS1, DS2, and MS4 achieved their peak biosurfactant yield and emulsification activity at an initial pH of 9. In comparison, *P. aeruginosa* KVD-HR42 showed the highest biosurfactant yield at an initial pH of 7.8 [55].

4.3. Bacterial Surfactant Stability: Thermal and Ion Tolerance

Previous research has shown that bacterial surfactants exhibit good stability under high-temperature conditions. Ahmad et al. reported that monosaccharide lipid biosurfactants produced by *Klebsiella* sp. maintained good stability at 60 °C, achieving an emulsification index over 40% [59]. Amani et al. discovered that surfactants produced by *B. subtilis* NLIM 0110, *P. aeruginosa* NLIM 0112, and *B. cereus* NLIM 0111 remained stable even at 120 °C [60], which is consistent with our findings. Surfactants produced by *P. aeruginosa* N33 and *B. paralicheniformis* Nian2 maintained their oil displacement activity across a temperature range of 0–120 °C. This indicates that surfactants produced by *Bacillus* sp. and *Pseudomonas* sp. typically exhibit a broad tolerance to temperature variations [61,62]. Gargouri et al. isolated a strain of *S. rhizophila* from hydrocarbon-contaminated water [63], with its surfactant retaining emulsification activity within a temperature range of 4–55 °C. In contrast, the surfactant produced by *S. nematodicola* T10 in this study maintained its

oil displacement activity even at 120 °C. The discovery of these thermotolerant bacterial surfactants holds potential for application in microbial-enhancing oil recovery in high-temperature oil wells.

Oil wells used for petroleum production typically have a certain level of salinity [64], making it essential for bacterial surfactants to maintain stability in high-salinity environments to enhance oil recovery (MEOR). Khademolhosseini et al. [21] and Haque et al. [65] reported that surfactants produced by *P. aeruginosa* strain HAK01 and ENO14 showed relative stability at sodium chloride concentrations up to 12% (*w*/*v*), maintaining at least 90% of their activity compared to the control. In this study, however, it was found that the surfactants produced by *P. aeruginosa* N33 and *B. paralicheniformis* Nian2 retained their oil-displacement activity even at NaCl concentrations as high as 25%, indicating a greater tolerance to Na^+ than observed in previous studies.

Furthermore, the surfactants produced by *P. aeruginosa* N33 and *B. paralicheniformis* Nian2 also demonstrated a high tolerance to Mg^{2+}, maintaining effective oil-displacement activity at $MgCl_2$ concentrations ranging from 32.5% to 37.5%. However, the activity of these surfactants was significantly affected by Ca^{2+}, as a 5% $CaCl_2$ concentration caused a loss of up to 75% of their surface activity. In contrast, the surfactant produced by *S. nematodicola* T10 showed higher sensitivity to Na^+, Mg^{2+}, and Ca^{2+}, with its oil-displacement activity significantly diminished when the salt concentration exceeded 5%.

4.4. Emulsification Properties of Bacterial Surfactants

Biosurfactants, due to their amphiphilic properties, efficiently lower interfacial surface tension, leading to the formation of stable oil-in-water emulsions and improving emulsification stability. In this study, it was found that the surfactants produced by *P. aeruginosa* N33, *B. paralicheniformis* Nian2, and *S. nematodicola* T10 were all capable of forming stable emulsions with petroleum. The emulsification rates of the surfactants produced by *P. aeruginosa* N33 and *B. paralicheniformis* Nian2 with petroleum were as high as 81–85%. In contrast, the surfactant produced by *S. nematodicola* T10 exhibited a relatively low emulsification rate of only 28%. In comparison, the *P. aeruginosa* strain isolated by Câmara et al. from soil contaminated with crude oil produced a biosurfactant with a 69% emulsification rate for crude oil [66]. This indicates that the efficacy of bacterial biosurfactants in emulsification may differ based on the microbial source and the chemical structure of the surfactant they produce.

4.5. Bacterial Surfactants and Their Environmental and EOR Applications

Biosurfactants are known for their biodegradability and low toxicity, making them environmentally friendly and attracting widespread attention in fields such as oil spill remediation and enhancing oil recovery [67]. Previous studies have shown that surfactants produced by bacteria like *Bacillus* sp. and *Pseudomonas* sp. can effectively dissolve oil residues trapped in rock formations or contaminated soils. For example, Zhu et al. reported that biosurfactants produced by *B. subtilis* isolated from the Atlantic Ocean significantly improved the removal rate of crude oil from polluted soils, achieving a removal rate of 58–65% [44]. Similarly, Datta et al. conducted static oil-washing experiments in the laboratory using biosurfactants produced by *B. tequilensis* on oil-saturated sand, resulting in a static oil-washing rate of 80 ± 2% [41]. However, in this study, the biosurfactants produced by *P. aeruginosa* N33, *B. paralicheniformis* Nian2, and *S. nematodicola* T10 displayed lower static oil-washing rates for oil sand (9:1), with values of 28.1%, 23.4%, and 7.1%, respectively. These results may be influenced by several factors, including the type of bacteria, biosurfactant yield, oil-to-sand ratio, washing volume, and washing duration.

Dynamic oil displacement experiments were performed using the biosurfactants produced by *P. aeruginosa* N33, *B. paralicheniformis* Nian2, and *S. nematodicola* T10, yielding dynamic oil recovery rates of 95.0%, 74.1%, and 69.0%, respectively. These values were significantly higher than their corresponding static oil-washing rates (Figure 6). Notably, the *P. aeruginosa* N33 strain exhibited an oil recovery rate as high as 95%, which is consistent

with previous findings. For instance, Gogoi et al. demonstrated that the biosurfactant produced by *P. aeruginosa* could remove crude oil from packed sand columns with a removal rate of 70 ± 3.5% [68]. Raouf et al. isolated three surfactant-producing bacteria, *B. massiliigabonensis* A-LB, *P. nitritolerans* C-LB2, and *A. seohaensis* B-YM2, from Egyptian oil fields. They used core flooding micromodels to assess oil recovery, with results showing that the biosurfactant produced by *B. massiliigabonensis* significantly enhanced oil recovery, yielding an additional 69.96%. In comparison, *P. nitritolerans* and *A. seohaensis* resulted in additional oil recoveries of 68.11% and 63.34%, respectively [69]. Fulazzaky et al. isolated the bacterium *Geobacillus toebii* R-32639 and discovered that it reduces crude oil viscosity through biosurfactant production. Further, a core fooling simulation of microbial enhanced oil recovery revealed that it can degrade 7.4–28.8% fractions of (C_{12}–C_{34}) hydrocarbons in the crude oil, leading to a substantial increase in oil recovery. In this study, the *P. aeruginosa* N33, *B. paralicheniformis* Nian2, and *S. nematodicola* T10 strains have shown the potential to enhance oil recovery through biosurfactant production [70]. However, further research is needed to determine whether these strains also have oil-degrading capabilities that could reduce crude oil viscosity and improve its fluidity. Additionally, the laboratory simulation conditions may not fully replicate the environment of real oil reservoirs, and additional evaluation is required to assess its performance in actual EOR scenarios.

5. Conclusions

Enhanced oil recovery (EOR) remains a critical challenge in the oil and gas industry. This study successfully isolated and characterized three biosurfactant-producing bacterial strains from marine sediments, designated as N33, Nian2, and T10. These strains, identified as *P. aeruginosa*, *B. paralicheniformis*, and *S. nematodicola*, respectively, demonstrated significant biosurfactant production. Optimization of fermentation conditions, including pH, carbon source, nitrogen source, and C/N ratio, markedly improved biosurfactant yield and activity.

The three biosurfactants demonstrated excellent thermal stability, remaining effective at temperatures up to 120 °C and exhibiting broad temperature adaptability. However, their activities were affected by the presence of various ions. The biosurfactants produced by *P. aeruginosa* N33 and *B. paralicheniformis* Nian2 maintained their oil displacement activity in relatively high concentrations of Na^+ and Mg^{2+}, but experienced significant activity loss when exposed to Ca^{2+}. In contrast, the biosurfactants produced by *S. nematodicola* T10 were more vulnerable to activity loss in the presence of Na^+, Mg^{2+}, and Ca^{2+}. Moreover, all three biosurfactants exhibited strong emulsification properties with crude oil, with emulsification rates ranked as N33 > Nian2 > T10. In simulated oil displacement experiments, the oil displacement rates for N33, Nian2, and T10 were 95%, 74.1%, and 69%, respectively.

Although these results are promising, the laboratory conditions used in this study may not fully replicate the complexities of real oil reservoirs, which could impact the applicability of the findings. Additionally, while the strains showed effective biosurfactant production in controlled settings, their scalability for industrial applications and their performance in real-world EOR scenarios still need to be thoroughly evaluated.

Author Contributions: Conceptualization, S.W.; methodology, M.J., H.W. and Q.C.; software, M.J. and H.W.; validation, S.W., J.L., X.H. and Y.Z.; formal analysis, M.J., H.W. and X.L.; investigation, M.J., H.W. and X.L.; resources, M.J., H.W., J.L., X.H., Y.Z. and X.L.; data curation, M.J. and H.W.; writing—original draft preparation, M.J. and H.W.; writing—review and editing, Q.C. and S.W.; visualization, S.W., J.L., X.H. and Y.Z.; supervision, S.W.; project administration, M.J.; funding acquisition, S.W. and H.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Innovation and Entrepreneurship Training Program for College Students of China University of Geosciences (Beijing) (S202311415119), the National Natural Science Foundation of China (42272359), and the Chinese "111" project (B20011).

Data Availability Statement: Data are available upon request.

Conflicts of Interest: Author Xiaolin Liu and Qingfeng Cui was employed by the Research Institute of Petroleum Exploitation and Development, China National Petroleum Corporation. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Thomas, S. Enhanced oil recovery—An overview. *Oil Gas Sci. Technol.* **2008**, *63*, 9–19. [CrossRef]
2. Nikolova, C.; Gutierrez, T. Use of microorganisms in the recovery of oil from recalcitrant oil reservoirs: Current state of knowledge, technological advances, and future perspectives. *Front. Microbiol.* **2020**, *10*, 2996. [CrossRef] [PubMed]
3. Alvarado, V.; Manrique, E. Enhanced oil recovery: An update review. *Energies* **2010**, *3*, 1529–1575. [CrossRef]
4. Kamal, M.S.; Hussein, I.A.; Sultan, A.S. Review on surfactant flooding: Phase behavior, retention, IFT, and field applications. *Energy Fuels* **2017**, *31*, 7701–7720. [CrossRef]
5. Negin, C.; Ali, S.; Xie, Q. Application of nanotechnology for enhancing oil recovery—A review. *Petroleum* **2016**, *2*, 324–333. [CrossRef]
6. Banat, I.M.; Makkar, R.S.; Cameotra, S.S. Potential commercial applications of microbial surfactants. *Appl. Microbiol. Biotechnol.* **2000**, *53*, 495–508. [CrossRef]
7. Santos, D.; Rufino, R.; Luna, J.; Santos, V.A.; Sarubbo, L. Biosurfactants: Multifunctional biomolecules of the 21st century. *Int. J. Mol. Sci.* **2016**, *17*, 401. [CrossRef]
8. Jiang, J.; Zu, Y.; Li, X.; Meng, Q.; Long, X. Recent progress towards industrial rhamnolipids fermentation: Process optimization and foam control. *Bioresour. Technol.* **2020**, *298*, 122394. [CrossRef]
9. Marchant, R.; Banat, I.M. Microbial biosurfactants: Challenges and opportunities for future exploitation. *Trends Biotechnol.* **2012**, *30*, 558–565. [CrossRef]
10. Liu, Q.; Myers, R.; Niu, J.; Gonçalves, T.; Rosa, Y.; Liu, Y.; Li, L.; Lv, J. Optimization of lipopeptide biosurfactant production by *Bacillus licheniformis* L20 and performance evaluation of biosurfactant mixed system for enhanced oil recovery. *J. Pet. Sci. Eng.* **2022**, *208*, 109678. [CrossRef]
11. Alkan, S.; Sehitoglu, H. Investigation of spontaneous imbibition induced by wettability alteration as a recovery mechanism in microbial enhanced oil recovery. *J. Pet. Sci. Eng.* **2019**, *175*, 182–195. [CrossRef]
12. Niu, J.; Liu, Q.; Lv, J.; Peng, B. Review on microbial enhanced oil recovery: Mechanisms, modeling, and field trials. *J. Pet. Sci. Eng.* **2020**, *192*, 107350. [CrossRef]
13. Sen, R. Biotechnology in petroleum recovery: The microbial EOR. *Prog. Energy Combust. Sci.* **2008**, *34*, 714–724. [CrossRef]
14. Al-Sulaimani, H.; Joshi, S.; Al-Wahaibi, Y.; Al-Bahry, S.; Elshafie, A.; Al-Bemani, A. Microbial biotechnology for enhancing oil recovery: Current developments and future prospects. *Biotechnol. Bioinform. Bioeng. J.* **2011**, *1*, 147–158.
15. Ron, E.Z.; Rosenberg, E. Natural roles of biosurfactants. *Environ. Microbiol.* **2001**, *3*, 229–236. [CrossRef] [PubMed]
16. Hommel, R.K.; Stiiwer, O.; Stuber, W.; Haferburg, D.; Kleber, H.P. Production of water-soluble surface-active exolipids by *Torulopsis apicola*. *Appl. Microbiol. Biotechnol.* **1987**, *26*, 199–205. [CrossRef]
17. Hassanshahian, M.; Emtiazi, G.; Cappello, S. Isolation and characterization of crude-oil-degrading bacteria from the Persian Gulf and the Caspian Sea. *Mar. Pollut. Bull.* **2012**, *64*, 7–12. [CrossRef]
18. Wang, W.; Cai, B.; Shao, Z. Oil degradation and biosurfactant production by the deep sea bacterium *Dietzia maris* As-13-3. *Front. Microbiol.* **2014**, *5*, 119073. [CrossRef]
19. Kügler, J.H.; Le Roes-Hill, M.; Syldatk, C.; Hausmann, R. Surfactants tailored by the class *Actinobacteria*. *Front. Microbiol.* **2015**, *6*, 212.
20. Koretska, N.; Karpenko, O.; Baranov, V.; Lubenets, V.; Nogina, T. Biological properties of surface-active metabolites of *Rhodococcus erythropolis* Au-1 and their prospects for crop technology. *Innov. Biosyst. Bioeng.* **2019**, *3*, 77–85. [CrossRef]
21. Khademolhosseini, R.; Jafari, A.; Mousavi, S.; Hajfarajollah, H.; Noghabi, K.; Manteghian, M. Physicochemical characterization and optimization of glycolipid biosurfactant production by a native strain of *Pseudomonas aeruginosa* HAK01 and its performance evaluation for the MEOR process. *RSC Adv.* **2019**, *9*, 7932–7947. [CrossRef] [PubMed]
22. Morikawa, M.; Daido, H.; Takao, T.; Murata, S.; Shimonishi, Y.; Imanaka, T. A new lipopeptide biosurfactant produced by *Arthrobacter* sp. strain MIS38. *J. Bacteriol.* **1993**, *175*, 6459–6466. [CrossRef] [PubMed]
23. Elazzazy, A.M.; Abdelmoneim, T.S.; Almaghrabi, O.A. Isolation and characterization of biosurfactant production under extreme environmental conditions by alkali-halo-thermophilic bacteria from Saudi Arabia. *Saudi J. Biol. Sci.* **2015**, *22*, 466–475. [CrossRef] [PubMed]
24. Sarwar, A.; Brader, G.; Corretto, E.; Aleti, G.; Abaid-Ullah, M.; Sessitsch, A.; Hafeez, F.Y. Qualitative analysis of biosurfactants from *Bacillus* species exhibiting antifungal activity. *PLoS ONE* **2018**, *13*, e0198107.
25. Jiao, R.; Cai, Y.; He, P.; Munir, S.; Li, X.; Wu, Y.; Wang, J.; Xia, M.; He, P.; Wang, G.; et al. *Bacillus amyloliquefaciens* YN201732 produces lipopeptides with promising biocontrol activity against fungal pathogen *Erysiphe cichoracearum*. *Front. Cell. Infect. Microbiol.* **2021**, *9*, 598999. [CrossRef]
26. Liu, Q.; Niu, J.; Yu, Y.; Wang, C.; Lu, S.; Zhang, S.; Lv, J.; Peng, B. Production, characterization, and application of biosurfactant produced by *Bacillus licheniformis* L20 for microbial enhanced oil recovery. *J. Clean. Prod.* **2021**, *307*, 127193. [CrossRef]

27. Zhao, F.; Zhu, H.; Cui, Q.; Wang, B.; Su, H.; Zhang, Y. Anaerobic production of surfactin by a new *Bacillus subtilis* isolate and the in situ emulsification and viscosity reduction effect towards enhanced oil recovery applications. *J. Pet. Sci. Eng.* **2021**, *201*, 108508. [CrossRef]
28. Beebe, J.L.; Umbreit, W.W. Extracellular lipid of *Thiobacillus thiooxidans*. *J. Bacteriol.* **1971**, *108*, 612–614. [CrossRef]
29. Akit, J.; Cooper, D.J.; Mannien, K.I.; Zajic, J.K. Investigation of potential biosurfactant production among phytopathogenic corynebacteria and related soil microbes. *Curr. Microbiol.* **1981**, *6*, 145–150. [CrossRef]
30. Luo, P.; Tang, Y.; Lu, J.; Jiang, L.; Huang, Y.; Jiang, Q.; Chen, X.; Qin, T.; Shiels, H.A. Diesel degradation capability and environmental robustness of strain *Pseudomonas aeruginosa* WS02. *J. Environ. Manag.* **2023**, *351*, 119937. [CrossRef]
31. Gaur, S.; Gupta, S.; Jha, P.N.; Jain, A. Rhamnolipid production by *Pseudomonas aeruginosa* (SSL-4) on waste engine oil (WEO): Taguchi optimization, soil remediation, and phytotoxicity investigation. *Environ. Technol.* **2023**, *45*, 4536–4549. [CrossRef] [PubMed]
32. Geetha, S.J.; Banat, I.M.; Joshi, S.J. Biosurfactants: Production and potential applications in microbial enhanced oil recovery (MEOR). *Biocatal. Agric. Biotechnol.* **2018**, *14*, 23–32.
33. Wu, B.; Wang, J.; Shi, S.; Zhang, Y.; Nie, Z.; Li, Y. Scale modelling, prediction, and management strategy in high-temperature high-salinity reservoir in the Middle East. In Proceedings of the ASME 2023 42nd International Conference on Ocean, Offshore and Arctic Engineering, Melbourne, Australia, 11–16 June 2023.
34. Hadia, N.J.; Ng, Y.H.; Stubbs, L.P.; Torsæter, O. High salinity and high temperature stable colloidal silica nanoparticles with wettability alteration ability for EOR applications. *Nanomaterials* **2021**, *11*, 707. [CrossRef] [PubMed]
35. Hassanshahian, M. Isolation and characterization of biosurfactant-producing bacteria from Persian Gulf (Bushehr provenance). *Mar. Pollut. Bull.* **2014**, *86*, 361–366. [CrossRef] [PubMed]
36. Wei, S.; Cui, H.; Jiang, Z.; Liu, H.; He, H.; Fang, N. Biomineralization processes of calcite induced by bacteria isolated from marine sediments. *Braz. J. Microbiol.* **2015**, *46*, 455–464. [CrossRef]
37. Parthipan, P.; Preetham, E.; Machuca, L.L.; Rahman, P.K.; Murugan, K.; Rajasekar, A. Biosurfactant and degradative enzymes mediated crude oil degradation by bacterium *Bacillus subtilis* A1. *Front. Microbiol.* **2017**, *8*, 237675. [CrossRef]
38. Phulpoto, I.A.; Yu, Z.; Hu, B.; Wang, Y.; Ndayisenga, F.; Li, J.; Liang, H.; Qazi, M.A. Production and characterization of surfactin-like biosurfactant produced by novel strain *Bacillus nealsonii* S2MT and its potential for oil-contaminated soil remediation. *Microb. Cell Fact.* **2020**, *19*, 145. [CrossRef]
39. Santa Anna, L.M.; Sebastian, G.V.; Menezes, E.P.; Alves, T.L.M.; Santos, A.S.; Pereira, N., Jr.; Freire, D.M.G. Production of biosurfactants from *Pseudomonas aeruginosa* PA 1 isolated in oil environments. *Braz. J. Chem. Eng.* **2002**, *19*, 159–166. [CrossRef]
40. Amani, H.; Haghighi, M.; Keshtkar, M.J. Production and optimization of microbial surfactin by *Bacillus subtilis* for ex situ enhanced oil recovery. *Pet. Sci. Technol.* **2013**, *31*, 1249–1258. [CrossRef]
41. Datta, P.; Tiwari, P.; Pandey, L.M. Oil washing proficiency of biosurfactant produced by isolated *Bacillus tequilensis* MK 729017 from Assam reservoir soil. *J. Pet. Sci. Eng.* **2020**, *195*, 107612. [CrossRef]
42. Joshi, S.J.; Suthar, H.; Yadav, A.K.; Hingurao, K.; Nerurkar, A. Occurrence of biosurfactant-producing *Bacillus* spp. in diverse habitats. *ISRN Biotechnol.* **2012**, *2013*, 652340. [CrossRef] [PubMed]
43. Kaya, T.; Aslim, B.; Kariptaş, E. Production of biosurfactant by *Pseudomonas* spp. isolated from industrial waste in Turkey. *Turk. J. Biol.* **2014**, *38*, 307–317. [CrossRef]
44. Zhu, Z.; Zhang, B.; Chen, B.; Cai, Q.; Lin, W. Biosurfactant production by marine-originated bacteria *Bacillus subtilis* and its application for crude oil removal. *Water Air Soil Pollut.* **2016**, *227*, 328. [CrossRef]
45. Talukdar, P.; Sharma, C.; Doley, A.; Baruah, K.; Borah, A.; Agarwal, P.; Deori, P. Isolation and characterization of biosurfactant-producing microorganisms from petroleum-contaminated soil samples for EOR and bioremediation. *Pet. Sci. Technol.* **2017**, *35*, 2102–2108. [CrossRef]
46. Uyar, E.; Sağlam, Ö. Isolation, screening, and molecular characterization of biosurfactant-producing bacteria from soil samples of auto repair shops. *Arch. Microbiol.* **2021**, *203*, 4929–4939. [CrossRef]
47. Ivanova, A.E.; Sokolova, D.S.; Kanat'eva, A.Y. Hydrocarbon biodegradation and surfactant production by acidophilic mycobacteria. *Microbiology* **2016**, *85*, 317–324. [CrossRef]
48. Portal, A.; Lima de Albuquerque, T.; Maciel Melo, V.M.; Silveira Vieira, R.; Ponte Rocha, M.V. Biosurfactant production by *Acinetobacter venetianus* and its application in bioremediation. *Chem. Eng. Technol.* **2023**, *46*, 1106–1114. [CrossRef]
49. Sanmartin Negrete, P.; Ghilardi, C.; Rodriguez Pineda, L.; Pérez, E.; Herrera, M.L.; Borroni, V. Biosurfactant production by *Rhodococcus* ALDO1 isolated from olive mill wastes. *Biocatal. Agric. Biotechnol.* **2024**, *57*, 103106. [CrossRef]
50. Nikolova, C.; Gutierrez, T. Biosurfactants and their applications in the oil and gas industry: Current state of knowledge and future perspectives. *Front. Bioeng. Biotechnol.* **2021**, *9*, 626639. [CrossRef]
51. Domingues, P.M. Isolation of Estuarine Biosurfactant-Producing Bacteria. Master's Thesis, Universidade de Aveiro, Aveiro, Portugal, 2011.
52. Hadiya, P.; Ansari, R. The multifaceted role of microbial biosurfactants in various fields. *Int. J. Multidiscip. Res.* **2024**, *6*, 17112.
53. Singh, S.; Sachan, A. Evaluation of nutritional and environmental factors for biosurfactant production by soil bacteria. *Res. Sq.* 2024; *preprint*. [CrossRef]
54. Bezza, F.; Chirwa, E. Improvement of biosurfactant production by microbial strains through supplementation of hydrophobic substrates. *Chem. Eng. Trans.* **2020**, *79*, 91–96.

55. Deepika, K.V.; Kalam, S.; Sridhar, P.R.; Podile, A.R.; Bramhachari, P.V. Optimization of rhamnolipid biosurfactant production by mangrove sediment bacterium *Pseudomonas aeruginosa* KVD-HR42 using response surface methodology. *Biocatal. Agric. Biotechnol.* **2016**, *5*, 38–47. [CrossRef]
56. Ezebuiro, V.; Otaraku, I.; Oruwari, B.; Okpokwasili, G. Effects of nitrogen and carbon sources on biosurfactant production by hydrocarbon-utilizing *Stenotrophomonas* sp. *Microbiol. Res. J. Int.* **2019**, *29*, 1–10. [CrossRef]
57. Purwasena, I.A.; Amaniyah, M.; Astuti, D.I.; Firmansyah, Y.; Sugai, Y. Production, characterization, and application of *Pseudoxanthomonas taiwanensis* biosurfactant: A green chemical for microbial enhanced oil recovery (MEOR). *Sci. Rep.* **2024**, *14*, 10270. [CrossRef]
58. Gurjar, J.; Sengupta, B. Production of surfactin from rice mill polishing residue by submerged fermentation using *Bacillus subtilis* MTCC 2423. *Bioresour. Technol.* **2015**, *189*, 243–249. [CrossRef]
59. Ahmad, Z.; Zhang, X.; Imran, M.; Zhong, H.; Andleeb, S.; Zulekha, R.; Liu, G.; Ahmad, I.; Coulon, F. Production, functional stability, and effect of rhamnolipid biosurfactant from *Klebsiella* sp. on phenanthrene degradation in various medium systems. *Ecotoxicol. Environ. Saf.* **2021**, *207*, 111514. [CrossRef]
60. Amani, H.; Sarrafzadeh, M.H.; Haghighi, M.; Mehrnia, M.R. Comparative study of biosurfactant-producing bacteria in MEOR applications. *J. Pet. Sci. Eng.* **2010**, *75*, 209–214. [CrossRef]
61. Silva, S.N.R.L.; Farias, C.B.B.; Rufino, R.D.; Luna, J.M.; Sarubbo, L.A. Glycerol as substrate for the production of biosurfactant by *Pseudomonas aeruginosa* UCP0992. *Colloids Surf. B Biointerfaces* **2010**, *79*, 174–183. [CrossRef]
62. Vaz, D.A.; Gudiña, E.J.; Jurado Alameda, E.; Teixeira, J.A.; Rodrigues, L.R. Performance of a biosurfactant produced by a *Bacillus subtilis* strain isolated from crude oil samples as compared to commercial chemical surfactants. *Colloids Surf. B Biointerfaces* **2012**, *89*, 167–174. [CrossRef]
63. Gargouri, B.; Contreras, M.D.; Ammar, S.; Segura-Carretero, A.; Bouaziz, M. Biosurfactant production by the crude oil degrading *Stenotrophomonas* sp. B-2: Chemical characterization, biological activities, and environmental applications. *Environ. Sci. Pollut. Res.* **2017**, *24*, 3769–3779. [CrossRef] [PubMed]
64. Gowthaman, S.; Krishnya, S.; Nasvi, M. Effect of salinity on mechanical behavior of well cement: Application to carbon capture and storage wells. *Eng. J. Inst. Eng. Sri Lanka* **2016**, *49*, 21.
65. Haque, E.; Riyaz, M.; Shankar, S.; Hassan, S. Physiochemical and structural characterization of biosurfactant produced by halophilic *Pseudomonas aeruginosa* ENO14 isolated from seawater. *Int. J. Pharm. Investig.* **2020**, *10*, 437–444. [CrossRef]
66. Câmara, J.; Sousa, M.; Barros Neto, E.; Oliveira, M. Application of rhamnolipid biosurfactant produced by *Pseudomonas aeruginosa* in microbial-enhanced oil recovery (MEOR). *J. Pet. Explor. Prod. Technol.* **2019**, *9*, 2333–2341. [CrossRef]
67. Hu, X.; Subramanian, K.; Wang, H.; Roelants, S.L.K.W.; To, M.H.; Soetaert, W.; Kaur, G.; Lin, C.S.K.; Chopra, S.S. Guiding environmental sustainability of emerging bioconversion technology for waste-derived sophorolipid production by adopting a dynamic life cycle assessment (dLCA) approach. *Environ. Pollut.* **2021**, *269*, 116101. [CrossRef]
68. Gogoi, D.; Bhagowati, P.; Gogoi, P.; Bordoloi, N.K.; Rafay, A.; Dolui, S.K.; Mukherjee, A.K. Structural and physico-chemical characterization of a dirhamnolipid biosurfactant purified from *Pseudomonas aeruginosa*: Application of crude biosurfactant in enhanced oil recovery. *RSC Adv.* **2016**, *6*, 70669–70681. [CrossRef]
69. Raouf, S.; Ismail, Y.; Gamaleldin, N.; Aboelkhair, H.; Attia, A. Screening of sustainable biosurfactant produced by indigenous bacteria isolated from Egyptian oil fields for microbial enhanced oil recovery. *Geoenergy Sci. Eng.* **2024**, *239*, 212974. [CrossRef]
70. Fulazzaky, M.; Astuti, D.; Fulazzaky, M.A. Laboratory simulation of microbial enhanced oil recovery using *Geobacillus toebii* R-32639 isolated from the Handil reservoir. *RSC Adv.* **2015**, *5*, 3908. [CrossRef]