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Using Crude Biosurfactant from *Pseudomonas stutzeri* strain ASWISA6 for Cleaning Oil-Contaminated Containers

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Abstract

This study investigated the potential of biosurfactants for various applications. The research focused on *Pseudomonas stutzeri* strain ASWISA6, demonstrating its capability to produce biosurfactants with notable properties. The strain exhibited strong emulsification capabilities, significant surface tension reduction, and a satisfactory yield, indicating its potential for applications such as oil spill remediation, enhanced oil recovery, and industrial cleaning processes. Furthermore, the efficacy of these biosurfactants in removing oil from contaminated surfaces across different vessel types was confirmed. The environmental compatibility and high performance of the biosurfactants make them suitable for oil tank cleaning and related applications, with potential for further optimization and scale-up in industrial cleaning protocols.

Keywords: Biosurfactant, *Pseudomonas stutzeri*, oil contamination.

Introduction

The global oil industry continues to face major technical and environmental challenges, particularly in managing the buildup of heavy hydrocarbon residues inside storage tanks and transport vessels. These deposits reduce storage efficiency and complicate maintenance, while conventional cleaning methods—such as the use of high-pressure water, steam, or chemical solvents—often generate hazardous waste, incur high costs, and pose environmental risks (1).

In response to these limitations, the use of biosurfactants, surface-active molecules pr

oduced by specific microorganisms, has gained momentum as a more sustainable and efficient solution. These naturally derived compounds, which include glycolipids (e.g., rhamnolipids), lipopeptides (e.g., surfactin), and others, possess both hydrophilic and hydrophobic domains. This structure allows them to lower interfacial tension, disperse hydrocarbons, and form emulsions even under challenging environmental conditions such as high salinity, temperature, and pressure (2).

One of the most promising applications of biosurfactants in the petroleum sector is in the cleaning of crude oil storage tanks. Microbial biosurfactants can loosen and emulsify the thick sludge that accumulates at the tank bottom, improving oil recovery and reducing the need for harsh chemicals. For instance, in a field application, a biosurfactant-rich culture derived from Pseudomonas was used to treat tank sludge, recovering over 90% of residual hydrocarbons in less than a week (3). Laboratory studies further confirm that biosurfactants can rapidly emulsify crude oil and separate it from tank surfaces within minutes of application (4).

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These biological surfactants are not only effective but also environmentally advantageous. Unlike synthetic surfactants, biosurfactants are biodegradable, non-toxic, and often produced from low-cost or waste substrates, such as agricultural residues or used cooking oil (5). This aligns with global goals for cleaner production and circular economy practices in industrial operations (1).

Additionally, biosurfactants play a role in enhancing the bioavailability of hydrocarbons during microbial degradation. Recent findings from studies conducted in marine environments, such as the North Sea, indicate that biosurfactants outperform chemical dispersants in stimulating the breakdown of oil by native microorganisms (6). Beyond cleaning and bioremediation, they are being explored for other petroleum-related uses, including emulsification in drilling fluids and preventing pipeline corrosion (7).

Emerging microbial strains, such as *Rhodococcus indonesiensis* SARSHI1, have shown promise for industrial use due to their high biosurfactant yields and strong hydrocarbon-degrading capabilities, achieving over 85% degradation efficiency in oil-contaminated media (8). Coupled with technological advances in microbial engineering, fermentation techniques, and formulation science, these innovations are helping to overcome cost and scalability issues that previously limited the widespread industrial use of biosurfactants (9).

In summary, biosurfactants offer a multifunctional and eco-friendly alternative for various oil industry applications, particularly in tank cleaning operations where they improve safety, reduce waste, and recover valuable hydrocarbons. As production methods become more efficient and research continues to uncover novel strains and formulations, biosurfactants are positioned to become central components of sustainable practices in petroleum management.

Materials & Methods

Microorganism

A previously isolated and characterized bacterial strain (10), *Pseudomonas stutzeri* ASWISA6 (GenBank accession number MN309833), isolated from oil-contaminated soil near the Al-Rafdi oil well in Basra, was used for the experiments.

Enrichment of biosurfactant-producing bacteria

The selected strains were inoculated for 48 hours in mineral salt media (MSM) for screening of biosurfactant production (11).

Enhancement of Biosurfactant Bacteria

Cultivation:

Pseudomonas aeruginosa is inoculated into a suitable production medium, such as Mineral Salt Medium (MSM) or Bushnell Haas broth, supplemented with a carbon source like glucose, sunflower oil, or corn oil. The culture is incubated at 30–37°C for 5–7 days on a rotary shaker at 150–200 rpm.

Biosurfactant extraction

- Cell Removal
- After incubation, the culture is centrifuged at 10,000 rpm for 15 minutes to separate the bacterial cells. The clear supernatant is collected for biosurfactant extraction.
- Acid Precipitation:
  The pH of the supernatant is adjusted to pH 2.0 using 1N HCl, then incubated at 4°C for 24 hours to allow the biosurfactant to precipitate.
- Collection and Purification:

The precipitate is harvested by centrifugation, washed with distilled water, and dissolved in a solvent mixture such as chloroform: methanol (2:1). The solvent is evaporated using a rotary evaporator to obtain the crude biosurfactant.

Screening Tests of Biosurfactant-Producing Bacteria

Screening bacterial isolates for their ability to produce biosurfactants is a fundamental step in identifying strains suitable for industrial and environmental applications. In this study, a combination of qualitative and semi-quantitative tests was applied to evaluate the surface activity of bacterial supernatants. These assays helped prioritize isolates with high biosurfactant-producing potential, including: interfacial reduction, foaming activity, hemolytic activity, CTAB agar plate, drop collapse assay, oil displacement test, and emulsification index E24% (10).

Calculating the weight of the extracted oil-

Various clean glass containers were weighed (150 ml bottles, 125 ml separating funnels, and test tubes), All were immersed in crude oil for 24 hours, then removed from the oil and placed on blotting paper for an hour .They were weighed again while saturated with crude oil .50 ml of the test solutions (tap water, crude biosurfactant (free cell culture supernatants), and 5% SDS) were placed in each of the separating funnels and bottles, and 10 ml in the test tubes. The mixture was shaken by hand ten times (12).

-The resulting solution was kept for oil extraction and weighing.

-The containers were weighed after cleaning.

-The weight difference was calculated for each of the four treatments.

-Weight of the oil sticking to the beaker = Weight after immersion - Weight before immersion.

- Weight of the extracted oil = Weight of the container saturated with oil - Weight of the container after cleaning.

Results and Discussion

- **Characterization and identification of BS-producing isolate:**

Morphological characteristics were studied by performing Gram staining, and cultural characteristics were recorded from a Nutrient agar plate. and molecular identification is shown in Table 1.

Table 1: Characterization and identification of BS-producing isolate

Isolate number	Gram stain	Colony characteristics	Molecular identification
17	Gram-negative rod-shaped, non-spore-forming	Rigid colonies are usually brown.	*Pseudomonas stutzeri strain ASWISA6*

Table 2: Screening of biosurfactant production.

Isolate	Haemolytic Activity	Emulsification index Ei%	Oil spreading Assay (cm)	Drop collapse	Surface tension m N/m	Foam formation	Dry weight of biosurfactant g/l
Pseudomonas stutzeri strain ASWISA6	Clear zone Beta hemolytic	45.3	6	+	32	+	2

Screening of biosurfactant production

The isolate Pseudomonas stutzeri strain ASWISA6 exhibited several promising indicators of biosurfactant production based on multiple qualitative and quantitative assays Table 2.

Hemolytic Activity

The isolate demonstrated a clear beta-hemolytic zone on blood agar plates, which is a commonly used preliminary screening method for biosurfactant production. Beta hemolysis indicates the ability of biosurfactant compounds to disrupt erythrocyte membranes, which suggests surface activity. This aligns with findings by (13), who reported that hemolytic activity is strongly correlated with biosurfactant production in ***Pseudomonas spp***.

Emulsification Index (E24%)

The emulsification index (E24) of 45.3% indicates a strong emulsifying capacity, signifying that the produced biosurfactant can effectively stabilize oil-water mixtures. According to (14), an E24% value exceeding 40% is considered high and reflects robust biosurfactant activity. This is especially important for bioremediation and oil recovery applications Figure 1.

Oil Spreading Assay

A 6 cm oil displacement diameter reflects the biosurfactant's high surface activity and its potential to reduce interfacial tension between oil and water. (15) highlighted that oil displacement diameters greater than 5 cm are typical of potent biosurfactants, confirming the effectiveness of the isolate's extracellular metabolites Figure 2.

Drop Collapse Test

A positive result in the drop collapse test further supports the presence of surface-active agents in the culture supernatant. As described by (16). The test is a simple yet reliable method to detect biosurfactants based on their ability to destabilize liquid droplets on hydrophobic surfaces.

Surface Tension Reduction

The isolate was able to reduce surface tension to 32 mN/m, a significant drop from the standard water surface tension of approximately 72 mN/m. (17). Note that an effective biosurfactant typically reduces surface tension below 40 mN/m, and values near 30 mN/m are characteristic of rhamnolipids and similar glycolipids produced by Pseudomonas species.

Foam Formation

The ability to generate stable foam is an indicator of amphiphilic compound production. Foam formation is desirable in several industrial applications and correlates with the surface activity of the biosurfactant (18). observed that the presence of foam in culture supernatants is a hallmark of biosurfactant-producing bacteria Figure 3.

Dry Weight of Biosurfactant

The isolate produced 2 g/L of crude biosurfactant, which is within the range considered industrially relevant. According to (19). Pseudomonas spp. generally produce between 0.5–4 g/L of biosurfactant under optimized conditions, suggesting that strain ASWISA6 has a good production potential figure (4a,4b & 4c).

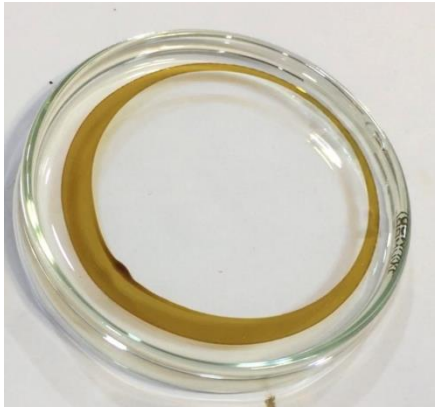

Figure 1: Oil Spreading

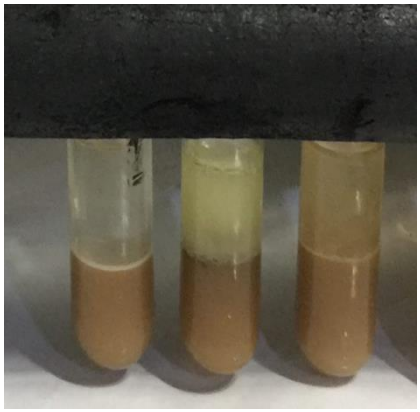

Figure 2: Emulsification Index (E24%)

Figure 3: Foam formation

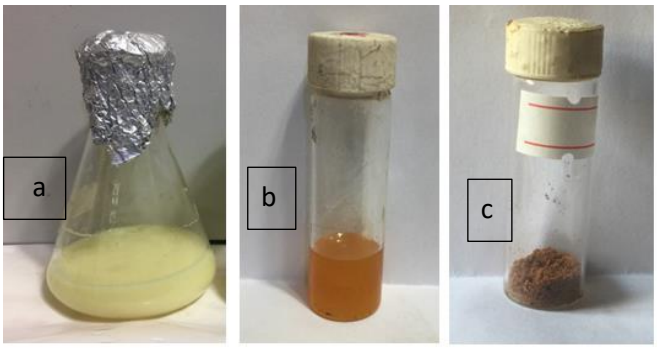

Figure 4: a-biosurfactant production b- biosurfactant extraction c- Dry biosurfactant

Calculating the weight of the extracted oil

The efficiency of oil extraction using three different cleaning agents—tap water, sodium dodecyl sulfate (SDS), and biosurfactant—was assessed using three types of vessels: test tubes, separation funnels, and bottles. The primary parameters measured included weight without oil (W.wo.o), weight with oil (W.w.o), weight after cleaning (W.afc), extracted oil weight (W.ex), and the percentage of extracted oil (W.ex.%. Table 3.

Oil Removal Efficiency in Test Tubes

The results showed that the biosurfactant achieved the highest oil removal efficiency in test tubes, with a W.ex% of 79%, compared to 75% for SDS and only 18% for tap water. The corresponding extracted oil weights were 0.51 g for the biosurfactant, 0.45 g for SDS, and 0.11 g for tap water. These findings clearly indicate the superior emulsification and oil solubilization capacity of the biosurfactant over both the synthetic surfactant (SDS) and tap water Figures 5 &6.

Oil Removal Efficiency in Separation Funnels

In separation funnels, the trend remained consistent, with the biosurfactant showing the highest oil removal efficiency (W.ex% = 59%), followed by SDS (47%) and tap water (19%). The amount of oil extracted was 1.33 g using the biosurfactant, 1.12 g with SDS, and only 0.45 g with tap water. This improvement in extraction efficiency with larger vessels might be attributed to better mixing and surface contact, enhancing the action of the surfactants Figures 5 & 6.

Oil Removal Efficiency in Bottles

The bottle setup demonstrated the most efficient oil removal using the biosurfactant, which reached an extraction percentage of 79%, similar to its performance in test tubes. SDS also showed high efficiency at 76%, whereas tap water remained the

least effective at 29%. Extracted oil weights were 1.06 g, 1.12 g, and 0.42 g for biosurfactant, SDS, and tap water, respectively Figures 5 &6.

Table 3: Removing oil from containers using different liquids

Vessels \ Liquid		Tap water	SDS	Biosurfactant
Test tube	(W.wo.o) (g)	13.34	13.37	13.24
	(W.w.o) (g)	13.93	13.97	13.88
	(W.o) (g)	0.59	0.60	0.64
	(W.afc) (g)	13.82	13.34	13.37
	(W.ex. o) (g)	0.11	0.45	0.51
	(W.eX oil .%)	18%	75%	79%
Separation funnel	(W.wo.o) (g)	110.03	111.02	110.31
	(W.w.o) (g)	112.29	113.4	112.66
	(W.o) (g)	2.26	2.38	2.35
	(W.afc) (g)	111.84	112.28	111.33
	(W.ex. o) (g)	0.45	1.12	1.33
	(W.eX oil .%)	19%	47%	56%
Bottle	(W.wo.o) (g)	85.50	85.58	85.46
	(W.w.o) (g)	86.84	86.93	86.79
	(W.o) (g)	1.34	1.35	1.33
	(W.afc) (g)	**86.42**	**85.90**	**85.73**
	(W.ex. o) (g)	**0.42**	**1.03**	**1.06**
	(W.eX oil .%)	**29%**	**76%**	**79%**

weight without oil (W.wo.o), weight with oil (W.w.o) . oil weight (W.o), weight after cleaning (W.afc), weight of extracted oil (W.ex), Percentage of weight extracted oil (W.eX%).

Figure 5: Vessels before and after cleaning with various liquids

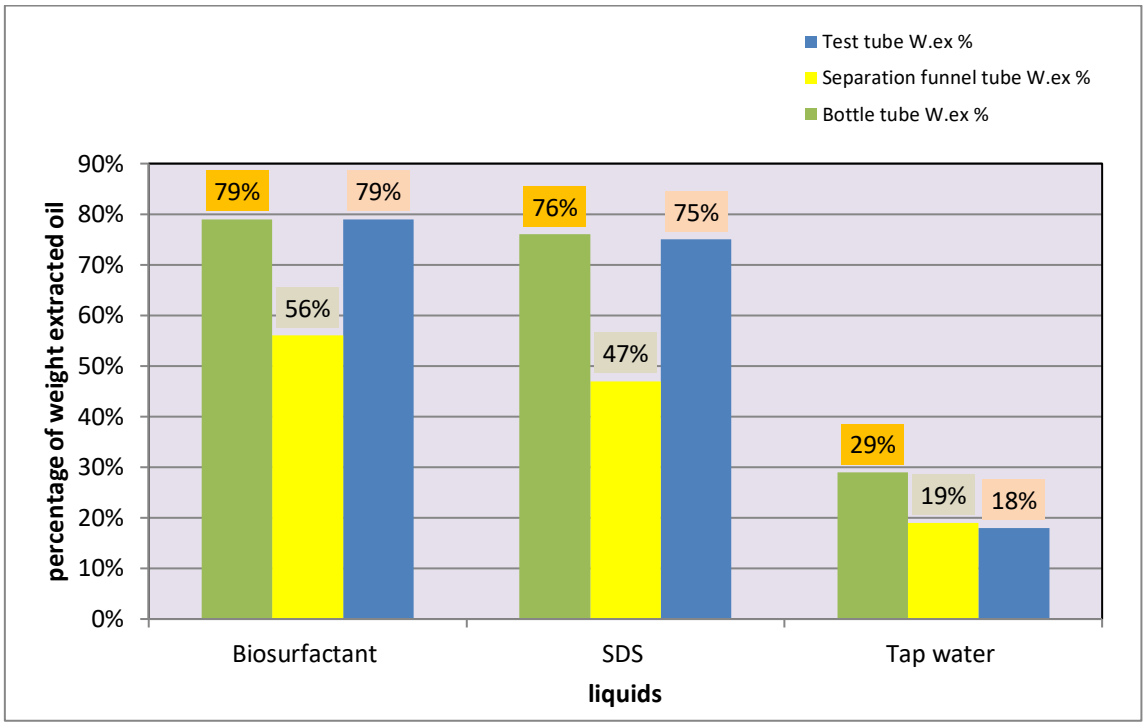

Figure 6: Percentage of weight extracted oil of the vessels

The biosurfactant consistently outperformed SDS and tap water across all vessel types, confirming its potential as a green and effective alternative to synthetic surfactants. The high oil removal efficiency observed with the biosurfactant is likely due to its ability to reduce surface and interfacial tension more effectively and form stable emulsions, facilitating the detachment and solubilization of oil residues.

The performance of SDS, while also significantly better than tap water, was slightly lower than that of the biosurfactant, highlighting that microbial biosurfactants can be equally or more effective than conventional surfactants in industrial cleaning processes. Tap water showed minimal oil removal efficiency, underscoring the necessity of using surface-active agents for such applications.

These findings align with previous studies that have reported the superior oil-displacement and emulsification properties of biosurfactants derived from Pseudomonas aeruginosa, Bacillus subtilis, and other microbial species (20).

Conflict of interest: NIL
Funding: NIL

References

1. Chrysalidis, A., & Kyzas, G. Z. (2020). Applied cleaning methods of oil residues from industrial tanks. Processes, 8(5), 569.

2. Santos, D. K. F., et al. (2023). “Industrial applications of biosurfactants: New trends and future outlook.” Current Opinion in Biotechnology, 81, 102-112.

3. Elazzazy, A. M., et al. (2023). “Microbial cleaning of oil tanks using rhamnolipid-rich cultures.” Journal of Industrial Microbiology & Biotechnology, 50(1), 45-56.

4. Perfumo, A., Rancich, I., & Banat, I. M. (2010). Possibilities and challenges for biosurfactants use in petroleum industry. Biosurfactants, 135-145.

5. Ali, M. A., et al. (2024). “Biosurfactants in petroleum industry: Eco-friendly alternatives for oil spill and tank cleaning.” Environmental Biotechnology Reports, 18(2), 101-115.

6. Zhao, Y., et al. (2023). “Comparative study of rhamnolipids and chemical dispersants in oil bioremediation.” Marine Pollution Bulletin, 191, 114867.

7. Gertsen, M. M., Arlyapov, V. A., Perelomov, L. V., Kharkova, A. S., Golysheva, A. N., Atroshchenko, Y. M., ... & Reverberi, A. P. (2024). Environmental Implications of Energy Sources: A Review on Technologies for Cleaning Oil-Contaminated Ecosystems. Energies (19961073), 17(14).

8. Farooq, M., et al. (2025). “High-efficiency oil degradation by biosurfactant-producing Rhodococcus strains.” Microbial Biotechnology Advances, 12(1), 77-89.

9. Singh, P., Patil, Y., & Rale, V. (2019). Biosurfactant production: emerging trends and promising strategies. Journal of applied microbiology, 126(1), 2-13.

10. Hamzah, A. F., Al-Mossawy, M. I., Al-Tamimi, W. H., Al-Najm, F. M., & Hameed, Z. M. (2020). Enhancing the spontaneous imbibition process using biosurfactants produced from bacteria isolated from Al-Rafidiya oil field for improved oil recovery. Journal of Petroleum Exploration and Production Technology, 10(8), 3767-3777.

11. Tabatabaee, A., Assadi, M. M., Noohi, A. A., & Sajadian, V. A. (2005). Isolation of biosurfactant producing bacteria from oil reservoirs. Journal of Environmental Health Science & Engineering, 2(1), 6-12.

12. Hamzah, A. F., & Al-Tamimi, W. H. (2021). Enhanced oil recovery by sand packed column supplemented with biosurfactants produced by local oil fields bacteria. Marsh Bulletin, 16(2).

13. Carrillo, P. G., Mardaraz, C., Pitta-Alvarez, S. I., & Giulietti, A. M. (1996). Isolation and selection of biosurfactant-producing bacteria. World Journal of Microbiology and Biotechnology, 12(1), 82–84. https://doi.org/10.1007/BF00327707.

14. Pacwa-Płociniczak, M., Płaza, G. A., Piotrowska-Seget, Z., & Cameotra, S. S. (2011). Environmental applications of biosurfactants: recent advances. International journal of molecular sciences, 12(1), 633-654

15. Morikawa, M., Hirata, Y., & Imanaka, T. (2000). A study on the structure–function relationship of lipopeptide biosurfactants. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1488(3), 211-218.

16. Jain, D. K., Collins-Thompson, D. L., Lee, H., & Trevors, J. T. (1991). A drop-collapsing test for screening surfactant-producing microorganisms. Journal of Microbiological Methods, 13(4), 271-279.

17. Ron, E. Z., & Rosenberg, E. (2001). Natural roles of biosurfactants: Minireview. Environmental microbiology, 3(4), 229-236.

18. Desai, J. D., & Banat, I. M. (1997). Microbial production of surfactants and their commercial potential. Microbiology and Molecular Biology Reviews, 61(1), 47-64.

19. Mukherjee, S., Das, P., & Sen, R. (2006). Towards commercial production of microbial surfactants. TRENDS in Biotechnology, 24(11), 509-515.

20. Degang, L., Auricchio, G., de Oliveira Schmidt, V. K., Della-Flora, I. K., & Andrade, C. J. D. (2023). Oil displacement properties of surfactin: a comparative study. Tenside Surfactants Detergents, 60(5), 414-418.