

SUSTAINABLE BIOSURFACTANT PRODUCTION FROM INDUSTRIAL WASTE USING *PSEUDOMONAS PUTIDA* ICCF 391: A CIRCULAR ECONOMY AND CONSUMER PROTECTION PERSPECTIVE

DOI: 10.26341/issn.2241-4010-2026-1c-8

Roxana Mădălina Stoica

National Institute for Chemical Pharmaceutical Research and Development-ICCF, Bucharest, Romania

roxym_stoica@yahoo.com

Nicoleta Stamate-Ene

National Institute for Chemical Pharmaceutical Research and Development-ICCF, Bucharest, Romania

ene.nicoleta27@yahoo.ro

Elena Simina Lakatos

Institute for Research in Circular Economy and Environment “Ernest Lupan”, Cluj-Napoca, Romania

simina.lakatos@ircem.ro

Elena Cristina Rada

Insubria University, Italy

elena.rada@uninsubria.it

Abstract

The transition toward circular and sustainable production systems has increased the need for biodegradable, non-toxic, and environmentally friendly compounds that can serve as alternatives to conventional synthetic chemicals. Microbial biosurfactants are a promising class of bio-based molecules that can facilitate this shift, particularly when produced using agro-food waste as a substrate.

*In this study, the biosurfactant produced by *Pseudomonas putida* ICCF 391 was synthesized using a cost-effective mixed substrate consisting of glycerol (3%) and waste cooking oil (2%), effectively incorporating waste valorization into the bioprocess. Its physico-chemical stability was assessed over a broad range of temperatures (30-115 °C) and pH values (2-12), while its antimicrobial activity was evaluated against *Pseudomonas aeruginosa* ATCC 9027. The biosurfactant exhibited thermal and pH stability, and demonstrated antibacterial activity, producing a 29 mm inhibition zone.*

Beyond its functional properties, the study frames the biosurfactant within the framework of the circular economy, highlighting its potential to reduce waste, promote sustainable industrial practices, and enhance consumer protection through safer, biodegradable, and renewable alternatives to chemical surfactants. These findings support integrating biosurfactants into sustainable industrial systems and highlight their relevance to EU policy objectives on waste reduction, chemical safety, and consumer protection.

Key words: *microbial surfactant, *Pseudomonas* strain, industrial waste, circular economy.*

Introduction

The transition toward a circular economy has emphasized the need to minimize waste, extend product lifecycles, and develop environmentally friendly alternatives to conventional chemicals. Within the European Union, these objectives are embedded in strategic frameworks such as the Circular Economy Action Plan (CEAP, 2020), which promotes waste valorization, the use of renewable resources, and the development of safer chemical products with reduced environmental impact (Gaur et al., 2022; Qamar & Pacifico, 2023). In this context, microbial biosurfactants have emerged as relevant bio-based compounds due to their functional versatility and compatibility with sustainability and consumer safety goals.

Biosurfactants are amphiphilic molecules synthesized by a wide range of microorganisms, including species belonging to the genera *Pseudomonas*, *Bacillus*, *Candida*, and *Rhodococcus*. In comparison with conventional synthetic surfactants, microbial biosurfactants generally exhibit enhanced biodegradability, reduced toxicity, and stability under a broad range of environmental conditions (Deshmukh & Kathwate, 2023). These characteristics support their applicability in diverse industrial sectors such as detergents, cosmetics, pharmaceuticals, agriculture, environmental remediation, and food-related processes (Mohanty et al., 2021).

The use of agro-industrial residues as substrates for biosurfactant production represents a key strategy within the circular bioeconomy, enabling the transformation of low-value waste into high-value functional products. Substrates such as waste cooking oil, whey, and crude glycerol are abundant, low-cost, and environmentally challenging materials that can be effectively valorized as feedstocks for microbial biosurfactant production (Praveesh et al., 2011; Soares da Silva et al., 2017). Their utilization into bioprocesses not only contributes to waste reduction, but also improves the economic feasibility and sustainability of biosurfactant production.

Therefore, in this study, the biosurfactant produced by *Pseudomonas putida* ICCF 391 using a low-cost mixed substrate composed of glycerol and waste cooking oil was evaluated in terms of its physico-chemical stability and antibacterial activity. In addition to assessing its functional properties, the work situates the production process within a circular economy framework, emphasizing its relevance for sustainable industrial applications and the development of safer, bio-based alternatives to synthetic surfactants.

Materials and methods

Biologic material

Pseudomonas putida ICCF 391 strain used in this study belongs to Collection of Microorganisms of Industrial Importance (CMII-ICCF-WFCC 232).

Culture medium and cultivation conditions

The strain was maintained on M44 agar slant, with the following composition (g/v): glycerol 5.0%, yeast extract 1.0%, bacto-peptone 1.0%, agar 2.0%. The culture medium was prepared with distilled water, adjusted to a pH of 6.5-7.0, and sterilized for 15 minutes at 121°C. The pre-inoculum consisted of bacterial strains incubated at 30°C for 48-72 hours, on M44 agar plates. The inoculum and biosynthesis media used for biosurfactant production contained % (v/v): glycerol 3.0, waste cooking oil 2.0, as carbon sources, 1.0 yeast extract, and 1.0 bacto-peptone as nitrogen sources. Also in this case, the culture media were prepared with distilled water, adjusted to a pH of 6.5-7.0, and sterilized at 121°C, for 15 minutes. For the submerged

fermentation, Erlenmeyer flasks of 500 mL capacity, with 100 mL medium, were used. Bioprocess conditions were performed at 30°C for 72 h on a rotary shaker at 220 rpm.

Stability and antibacterial activity of biosurfactant

In order to evaluate the production of biosurfactant by *Pseudomonas putida* ICCF 391, submerged bioprocesses in 500 mL flasks with 100 mL medium on a rotary shaker at 220 rpm were performed. Then, the procedure used by Foukia et al. (2016) to test the stability of the biosurfactant was followed.

To investigate the stability of biosurfactants at different temperature values, the supernatant obtained after centrifugation of the biosynthesis medium (9000 rpm for 20 minutes, 4°C), was kept at a constant temperature (30°C, 37°C, 50°C, 75°C, and 115°C) for 30 minutes, and then the emulsification index was determined. Similarly, the effect of pH was evaluated by adjusting the pH of the supernatant in the range of 2.0-12.0 using 2N HCl and 1N NaOH, and determining the emulsification index ($E_{24\%}$) (Alyousif et al., 2020).

In this case, 4 mL of supernatant was mixed with 6 mL of heptane, octane, and sunflower oil in separate tubes. Then, tubes were mixed vigorously for 2-5 minutes and kept for 24 hours (Pathak & Keharia, 2014). The emulsification index was determined by using the following formula:

$$E_{24\%} = (\text{height of the emulsified layer} / \text{total height of the liquid column}) \times 100$$

The experiments were performed in triplicate.

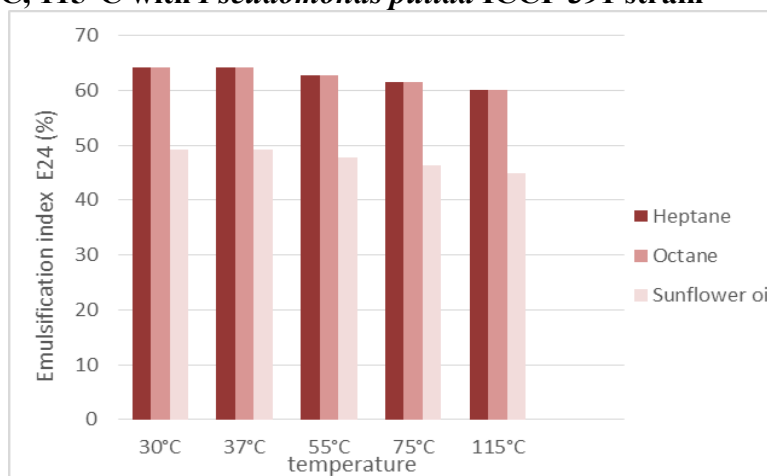
Regarding the evaluation of the antimicrobial activity, the biosurfactant produced by *Pseudomonas putida* ICCF 391 was isolated after 72 h of submerged fermentation through centrifugation, acid precipitation (pH 2.0, 2 N HCl, 4°C), and extraction with ethyl acetate. The organic phase was concentrated under reduced pressure to obtain a partially purified biosurfactant.

Its antibacterial activity was assessed against *Pseudomonas aeruginosa* ATCC 9027 using the agar diffusion (cylinder-plate) method adapted from Niamah, 2014. M44 agar medium was inoculated with a *Pseudomonas aeruginosa* ATCC 9027 suspension (10^8 CFU/mL), and 15 mL of the medium was poured into Petri plates and allowed to solidify. Two sterile stainless steel cylinders were placed at approximately 20 mm from the plate center, and 200 μ L of the partially purified biosurfactant was added to each cylinder. Plates were incubated at 30-35 °C for 18-24 h, and inhibition zones were measured. Experiments were conducted in triplicate, and results are reported as the mean of three independent assays.

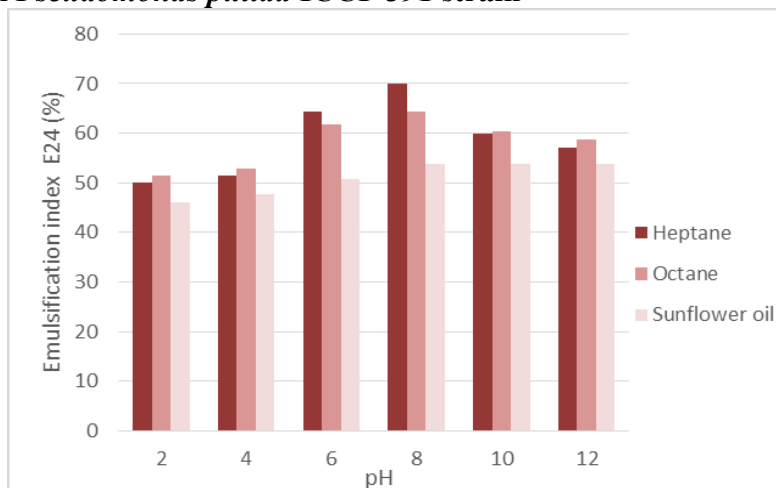
Results

Following 72 hours of submerged fermentation using a low-cost substrate composed of glycerol and waste cooking oil, the supernatant exhibited clear emulsification activity, indicating that the microorganism successfully utilized the mixed carbon sources to produce surface-active biocompounds. The results highlight that the biosurfactant produced by *Pseudomonas putida* ICCF 391 possesses physico-chemical and biological characteristics that support its application in sustainable and circular industrial systems.

As shown in Fig. 1, the $E_{24\%}$ results indicate that the biosurfactant maintains emulsification stability across a broad temperature range for heptane, octane, and sunflower oil. Even at higher temperatures, the emulsions remain stable, highlighting the thermal tolerance of the biosurfactant. The thermal stability broadens the potential applications of the biosurfactant, particularly in industrial processes subjected to demanding operating conditions.

Figure 1. Emulsification index for heptane, octane, and sunflower oil obtained at 30°C, 37°C, 55°C, 75°C, 115°C with *Pseudomonas putida* ICCF 391 strain

Also, the evaluation of pH tolerance provided similarly favorable results. The highest $E_{24\%}$ values were observed around neutral to slightly alkaline conditions (pH 6-8). Stability decreased slightly at acidic pH values below 6, while still maintaining good emulsification for heptane, octane, and sunflower oil. At strongly alkaline pH, $E_{24\%}$ values were lower than in the optimal range, but remained higher than those measured under strongly acidic conditions (Figure 2). Such broad pH tolerance confirms its versatility for industrial applications requiring stability under chemically demanding conditions, such as cleaning formulations, environmental remediation, and variable-pH processing environments.

Figure 2. Emulsification index for heptane, octane and sunflower oil obtained at pH 2, 4, 6, 8, 10, 12 with *Pseudomonas putida* ICCF 391 strain

Regarding the antimicrobial activity of the partially purified biosurfactant, obtained after post-biosynthesis processing, it was analyzed against the microbial strain *Pseudomonas aeruginosa* ATCC 9027. As we can see in Figure 3, biosurfactant showed antimicrobial activity against the test bacterial strain, indicated by an inhibition zone of 29 mm. This bioactivity enhances the biosurfactant's potential applicability in the formulation of natural disinfectants, preservative systems, cosmetic products with antimicrobial attributes, and food industry sanitation technologies. The dual functionality-surfactant behavior combined with antimicrobial activity-further strengthens its commercial and environmental value.

Figure 3. Antibacterial activity of biosurfactant against *Pseudomonas aeruginosa* ATCC 9027 strain



Therefore, the results are consistent with recent studies demonstrating that biosurfactants maintain stability at elevated temperatures and across a broad pH range (2-12) (Adebajo et al., 2019).

Additionally, the biosurfactant produced by *Pseudomonas putida* ICCF 391 exhibited antibacterial activity against *Pseudomonas aeruginosa*, indicating its potential application as an antimicrobial agent in pharmaceutical, food, and related industries. These functional properties highlight the biosurfactant as a competitive and sustainable alternative to conventional synthetic surfactants in industrial and consumer applications.

Discussion

The results of this study demonstrate that the biosurfactant produced by *Pseudomonas putida* ICCF 391 using a mixed substrate of glycerol and waste cooking oil exhibits functional properties compatible with circular economy-oriented bioprocesses. The ability to convert waste-derived substrates into a stable and bioactive compound highlights the potential of microbial biotechnology to support waste valorization strategies and to reduce the environmental impacts associated with the uncontrolled disposal of lipid-rich residues, such as waste cooking oil.

By integrating low-value by-products into the biosurfactant production process, this approach contributes to improved resource efficiency while decreasing reliance on conventional, non-renewable raw materials. Such waste-to-resource pathways are consistent with circular bioeconomy principles, as they promote material reuse and enhance the sustainability of industrial supply chains. Furthermore, the utilization of renewable substrates supports diversification away from petrochemical-based surfactant production, which remains associated with environmental persistence and increased ecological risk.

Beyond the sustainability of the production process, the properties of the biosurfactant itself are relevant for applications where environmental compatibility and product performance are essential. The observed stability across a wide range of temperatures and pH values supports its potential use in industrial formulations exposed to variable processing conditions. In addition, the demonstrated antibacterial activity against *Pseudomonas aeruginosa* indicates that this biosurfactant may provide functional benefits in formulations

requiring antimicrobial performance, including cleaning agents, cosmetic products, and sanitation-related applications.

The biodegradability and reduced toxicity commonly associated with microbial biosurfactants further enhance their suitability for applications involving direct or indirect human exposure. By limiting the persistence of surfactant residues in aquatic and terrestrial environments, biosurfactants can mitigate ecological risks while supporting the development of safer consumer products. Accordingly, the biosurfactant evaluated in the present study is consistent with European policy objectives that promote the use of safer chemical alternatives, improved environmental performance, and responsible resource management.

The waste-based production, and antibacterial activity of the biosurfactant from *Pseudomonas putida* ICCF 391 make it a promising candidate for sustainable industrial applications. Its integration into circular economy-oriented production systems may help reduce environmental impact while facilitating the shift toward bio-based, consumer-focused markets (Kumari et al., 2023; Ali et al., 2024).

Conclusions

This study demonstrates that the biosurfactant produced by *Pseudomonas putida* ICCF 391 using waste cooking oil and glycerol as substrates exhibits physico-chemical stability and notable antibacterial activity. These characteristics make it a viable and sustainable substitute for traditional synthetic surfactants. Its production pathway aligns with circular economy principles by converting waste materials into high-value bioproducts, mitigating environmental pollution, and promoting resource efficiency. Furthermore, the biodegradability, low toxicity, and antimicrobial activity of the biosurfactant contribute to the development of safer formulations and reinforce consumer protection. Overall, these findings underscore the biosurfactant's potential for application in green industrial processes and its relevance for policy initiatives promoting sustainable and consumer-oriented markets.

References

- Adebajo, S.O., Akintokun, A.K., Ojo, A.E., Akinremi, C.A. (2019). Stability of biosurfactant produced by *Pseudomonas taenensis*. Journal of Natural Sciences, Engineering & Technology, vol. 18, 155-165. <https://doi.org/10.51406/jnset.v18i1.2039>.
- Ali, A., Aziz, A.R.A., Ismael, A.M, Alqaed, S. (2024). Biosurfactants as an alternative eco-friendly solution for water-in-diesel emulsions - A review paper. Heliyon, vol. 10, issue 17. doi: 10.1016/j.heliyon.2024.e37485.
- Alyousif, N.A.; Al-Tamimi, W.H.; Al-Luaibi, Y.Y.Y. (2020). Screening, enhance production and characterization of biosurfactant produced by *Pseudomonas aeruginosa* isolated from hydrocarbon contaminated soil. Eurasia Journal of Bioscience, vol. 14, 4377-4391.
- Deshmukh, N., Kathwate, G. (2023). Biosurfactant production by *Pseudomonas aeruginosa*. Biointerface Research in Applied Chemistry, vol. 13, 1-13.
- Foukia, E.M., Mostafa, M.A., Maysa, E.M. (2016). Optimization of biosurfactant production by *Bacillus brevis*. Biotechnology Reports, vol. 9, 31-37.
- Gaur, V.K., Sharma, P., Sirohi, R., Varjani, S., Taherzadeh, M.J., Chang, J.S., Ng, H.Y., Wong, J.W.C., Kim, S.H. (2022). Production of biosurfactants from agro-industrial waste and waste cooking oil in a circular bioeconomy: An overview, Bioresource Technology, vol. 343, <https://doi.org/10.1016/j.biortech.2021.126059>.

- Kumari, R., Singha, L.P., Shukla, P., (2023). Biotechnological potential of microbial biosurfactants, their significance, and diverse applications, FEMS Microbes, vol. 4, <https://doi.org/10.1093/femsmc/xtad01>.
- Mohanty, S.S., Koul, Y., Varjani, S., Pandey, A., Ngo, H.H., Chang, J.S., Wong, J.W.C., Bui, X.T. (2021). Review on sustainable substrates for biosurfactant production. Microbial Cell Factories, vol. 20, 1-13.
- Niamah, A. (2014). Determination, identification of bioactive compounds extracts from yellow banana peels and used in vitro as antimicrobial. International Journal of Phytomedicine, vol. 6, 625-632.
- Pathak, K.V., Keharia, H. (2014). Application of extracellular lipopeptide biosurfactant produced by endophytic *Bacillus subtilis* K1 isolated from aerial roots of banyan (*Ficus benghalensis*) in microbially enhanced oil recovery (MEOR). 3 Biotech, vol. 4, 41-48.
- Praveesh, B.V., Soniyambi, A.R., Mariappan, C., Kavithakumari, P., Palaniswamy, M., Sundaram, L. (2011). Biosurfactant production from soil microorganisms using whey. New York Science Journal, vol. 4, 99-103.
- Qamar, S.A., Pacifico, S. (2023). Cleaner production of biosurfactants via bio-waste valorization: A comprehensive review of characteristics, challenges, and opportunities in bio-sector applications, Journal of Environmental Chemical Engineering, vol. 11, issue 6, <https://doi.org/10.1016/j.jece.2023.111555>.
- Soares da Silva, R.C.F., Almeida, D.G., Meira, H.M., Silva, E.J., Farias, C.B.B., Rufino, R.D. (2017). Biosurfactant production from *Pseudomonas cepacia* using low-cost substrates. Biocatalysis and Agricultural Biotechnology, vol. 12, 206-215.