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Tilapia viscera wastewater: an innovative substrate for sustainable biosurfactant production by *Penicillium citrinum* UCP 1183

Água residual de vísceras de tilápia: um substrato inovador para a produção sustentável de biossurfactante por *Penicillium citrinum* UCP 1183

Everton Ricardo Carneiro Costa[1], *Dayana Montero Rodríguez*[1], *Adriana Ferreira de Souza*[1], *Galba Maria de Campos-Takaki*[1], *Rosileide Fontenele da Silva Andrade*[1]

ABSTRACT

Sustainable fish waste management is a critical issue linked to the United Nations Sustainable Development Goals, particularly SDG 12 (Sustainable Consumption and Production). Improper disposal of fish processing residues, including viscera, causes significant environmental problems by worsening pollution and wasting valuable biotechnological resources. In order to contribute to the solution of this economic and environmental challenge, this study sought to use wastewater from the processing of Nile tilapia (*Oreochromis niloticus*) viscera as a raw material for biosurfactant production by *Penicillium citrinum* UCP 1183. This strain was cultivated in alternative media composed of tilapia viscera wastewater and post-frying soybean oil, based on the concentrations established by a 2^2 full-factorial design. Biosurfactant production was verified in condition 4 of the full-factorial design, obtaining a surface tension of 36 mN/m. The biosurfactant showed an anionic and lipopeptide nature, moderate zeta potential, and excellent stability and emulsifying capacity. Hence, tilapia viscera wastewater proved to be an excellent substrate for sustainable biosurfactant production, minimizing the environmental impact of fish processing waste and promoting the circular economy.

Keywords: fish processing waste; fungal surfactant; emulsifying properties.

RESUMO

A gestão sustentável dos resíduos de pescado é uma questão crítica vinculada aos Objetivos de Desenvolvimento Sustentável das Nações Unidas, especialmente ao ODS 12 (Consumo e Produção Sustentáveis). O descarte inadequado dos resíduos do processamento de pescado, incluindo as vísceras, causa problemas ambientais significativos ao contribuir para a poluição e o desperdício de recursos biotecnológicos valiosos. Com o intuito de colaborar para a solução desse desafio econômico e ambiental, este estudo buscou utilizar a água residual do processamento de vísceras da tilápia-do-nilo (*Oreochromis niloticus*) como matéria-prima para a produção de biossurfactante por *Penicillium citrinum* UCP 1183. Esta cepa foi cultivada em meios alternativos compostos por água residual de vísceras de tilápia e óleo de soja pós-fritura, conforme as concentrações estabelecidas por um planejamento fatorial completo 2^2. A produção de biossurfactante foi verificada na condição 4 do planejamento fatorial completo, obtendo-se uma tensão superficial de 36 mN/m. O biossurfactante apresentou natureza aniônica e lipopeptídica, potencial zeta moderado e excelente estabilidade e capacidade emulsificante. Assim, a água residual de vísceras de tilápia demonstrou ser um excelente substrato para a produção sustentável de biossurfactante, minimizando o impacto ambiental dos resíduos do processamento de pescado e promovendo a economia circular.

Palavras-chave: resíduos do processamento de pescado; surfactante fúngico; propriedades emulsificantes.

[1]Universidade Católica de Pernambuco – Recife (PE), Brazil.

Corresponding author: Rosileide Fontenele da Silva Andrade – Catholic University of Pernambuco (UNICAP) – Rua do Príncipe, 526, Boa Vista – CEP: 50050-900 – Recife (PE), Brazil. E-mail: rosileide.andrade@unicap.br

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Introduction

Oreochromis niloticus or Nile tilapia is one of the most consumed freshwater fish in the world, and 60–70% of its body is composed of head, skin, bones, scales, fins, and viscera (Arias et al., 2022; Rajabimashhadi et al., 2023). Annually, fish processing industries discard millions of tons of fish waste, which contains large amounts of water and compounds that make it susceptible to auto-oxidation (Lee et al., 2022; Vaishnav et al., 2025).

Nile tilapia wastes can be biologically hazardous due to their disposal methods, which make them potential pathogenic contaminants. They are typically discarded in landfills or buried in soil, resulting in environmental, social, and human health impacts (Mozumder et al., 2022; Santana et al., 2023; Rasmiya Begum et al., 2024). Therefore, one of the targets of the Sustainable Development Goals (SDG 12 – Sustainable Consumption and Production) promotes the sustainable reuse of these wastes (Sanches et al., 2024).

Fish wastes are generally rich in bioactive peptides, lipids, proteins, essential amino acids, proteases, fatty acids, collagen, vitamins, and minerals of great value for the industrial sector (Borges et al., 2023; Jasrotia et al., 2024). Thus, when properly reused, they become byproducts with the potential to generate high-value-added products such as fish meal, hydrolysates, biodiesel, and glue, among others, increasing the full utilization of fish (Kuley et al., 2020; Jimenez-Champi et al., 2024).

Accordingly, tilapia wastes can serve as an excellent renewable raw material for the production of biosurfactants due to their rich nutritional composition (Maksimenko et al., 2024). For example, the wastewater generated from tilapia processing, especially from washing viscera, is rich in organic matter, including proteins, lipids, and other essential nutrients for microbial metabolism. These compounds potentially serve as natural sources of carbon and nitrogen, promoting microbial growth and the biosynthesis of secondary metabolites, such as biosurfactants (Ponsano et al., 2019; Sar et al., 2021; Rifna et al., 2024). They are amphipathic compounds capable of reducing surface and interfacial tension, often exhibiting emulsifying activity and finding applications in various sectors due to their multifunctionality, biodegradability, biocompatibility, low toxicity, and stability under extreme environmental conditions (Sharma et al., 2022; Gautam et al., 2023; Sankhyan et al., 2024). Therefore, it is estimated that the demand for biosurfactants will grow at a rate of 35% annually, driving the biosurfactant market to reach $52.4 billion (Dini et al., 2024; Chauhan et al., 2025). However, the economic viability of biosurfactant production remains a challenge, with the cost of substrates being one of the main limiting factors. Thus, alternative substrates, such as tilapia viscera wastewater (TVWW), emerge as a promising strategy to reduce biosurfactant production costs while promoting the reuse of commonly discarded by-products, aligning with the principles of circular economy and sustainable biotechnology.

Various microorganisms, including bacteria, yeasts, and filamentous fungi produce biosurfactants. However, filamentous fungi represent a promising yet underexplored group with significant potential for biosurfactant production even under adverse environmental conditions, and limited nutrients (Luft et al., 2020; Silva et al., 2021; Sankhyan et al., 2024). Among them, *Penicillium citrinum* can produce tensioactive compounds, such as biosurfactants and bioemulsifiers (Camargo-de-Morais et al., 2003; Kumar et al., 2023; Olivia et al., 2023). Our previous study highlighted the ability of *P. citrinum* UCP 1183 to produce a bioemulsifier using agro-industrial substrates, revealing its potential to also produce biosurfactants, as indicated by the reduction in surface tension (Costa et al., 2023).

In this context, the present study aimed to investigate the suitability of wastewater generated from the laboratory processing of Nile tilapia viscera as an alternative substrate for producing biosurfactant by *P. citrinum* UCP 1183.

Materials and Methods

Microorganism and growth conditions

P. citrinum UCP 1183 was isolated from mangrove sediment contaminated with crude oil in the municipality of Rio Formoso, state of Pernambuco, Brazil. This strain is maintained in the Culture Collection of the Multiuser Center for Analysis and Characterization of Biomolecules and Material Surfaces (CEMACBIOS), from the Catholic University of Pernambuco (Recife-PE), and registered at the World Federation for Culture Collections (WFCC). *P. citrinum* was cultivated on Petri plates containing Sabouraud medium (40 g/L glucose, 10 g/L peptone, and 18 g/L agar) and incubated at 28°C until mycelial mat growth.

Wastes

Post-frying soybean oil (PFSO) was kindly supplied by a local food trade in the city of Recife-PE, Brazil.

Nile tilapia (*Oreochromis niloticus*) viscera were kindly donated by Noronha Pescados Ltd., a local fish processing plant in Pernambuco, and kept frozen until use. In the laboratory, 1 kg of viscera was thawed at room temperature and then washed with 1 L of distilled water before processing. This "wastewater" was sieved through a household sieve to separate the remaining solids, filtered through gauze, and squeezed manually. The obtained TVWW was subjected to Fourier transform infrared (FTIR) spectroscopy to investigate its composition and used as an alternative substrate for biosurfactant production.

Biosurfactant production

For the production of biosurfactant by *P. citrinum* UCP 1183, low-cost media were formulated using TVWW and PFSO, according to concentrations established by the 2^2 full-factorial design (FFD). Twenty mycelium discs (8 mm in diameter) of the fungus grown in Sabouraud medium were used to inoculate each production medium (100 mL). Erlenmeyer flasks were incubated in a shaker at 150 rpm and

28°C for 96 h. Afterward, the cultures were filtered and centrifuged to obtain cell-free metabolic liquids, which were used to carry out the analyses described below.

Full-factorial design

A 2^2 FFD was performed in order to investigate the effects of the independent variables, concentrations of TVWW and PFSO, on the production of biosurfactant, verified by the dependent variable, surface tension, considering that only surface tension was analyzed as a response or dependent variable in the full factorial design. Seven experimental assays were performed in a randomized order, with three replicates at the central point, evaluating the independent variables at three levels: minimum (-1), central (0), and maximum (+1) (Table 1). The choice of factor levels was based on preliminary tests and literature data. The concentrations tested for TVWW and PFSO were selected to represent ranges that would provide sufficient nutrients for fungal metabolism and biosurfactant production, while allowing the evaluation of synergistic effects between the substrates. The data obtained from the experiments were subjected to statistical analysis by Statistica® software, version 12.0 (StatSoft Inc., USA), and the significance of the results was tested at $p<0.05$ level.

Determination of surface tension

The occurrence of biosurfactant production was confirmed by measuring the surface tension on the cell-free metabolic liquids by the Du Noüy ring method using a tensiometer model Sigma 70 (KSV Instruments Ltd., Finland). The ring was submerged in the liquid and slowly pulled through the liquid-air interface, which was correlated with the force required to lift the platinum ring from the liquid. The values obtained were expressed in mN/m (Kuyukina et al., 2005).

Determination of emulsification index

The emulsification index was determined using cell-free metabolic liquid and hydrophobic substrates (engine oil and burnt engine oil). The samples were transferred to test tubes in a 1:1 (*v/v*) ratio and vortexed for 2 min, following the methodology of Cooper and Goldenberg (1987). After 24 h, the emulsions were measured using a ruler, and the results were calculated according to Equation 1 and expressed in percentage:

$$EI_{24}\ (\%) = emulsion\ height\ /\ total\ height\ x\ 100 \quad (1)$$

Table 1 – Variables and levels used in the full-factorial design applied to investigate biosurfactant production by *Penicillium citrinum* UCP 1183.

Variables	Levels		
	-1	0	+1
Tilapia viscera wastewater (%)	0	1.5	3.0
Post-frying soybean oil (%)	0	3.0	6.0

Biosurfactant stability

The stability of the biosurfactant was evaluated by measuring the surface tension in the cell-free metabolic liquid after 24 h of exposure to different temperatures (0, 5, 50, 70, 100, and 120˚C), pH (2, 4, 6, 8, 10, and 12), and sodium chloride (NaCl) concentrations (2, 4, 6, 8, 10, and 12%) (Purwasena et al., 2024).

Biosurfactant isolation

The produced biosurfactant was isolated from the cell-free metabolic liquid by the addition of acetone (1:1, *v/v*) and subsequent acidification to pH 2.0 using 2 normal solution of hydrochloric acidA (2 N HCl). The acidified mixture was left overnight at 4°C for complete precipitation of the biosurfactant and subsequently centrifuged for 15 min at 5,000 *g* using a HERMLE Z 513K centrifuge (HERMLE Labortechnik, Wehingen, Germany). The supernatant was discarded, and the precipitate was resuspended in distilled water and centrifuged again to remove the residual acetone. This step was repeated three times, and the biosurfactant obtained was frozen, lyophilized (Advantage Plus EL-85 lyophilizer, SP Scientific, USA), and kept in a desiccator until a constant weight. Dry weight was determined by gravimetry, and yield was expressed in g/L.

Analysis of the zeta potential and ionic charge of biosurfactant

The determination of the ionic charge of the biosurfactant was carried out in the Zeta-Meter 4.0 (model ZM3-DG, direct video Zeta Meter, Inc., USA) at 100 volts. The isolated biosurfactant was solubilized in 20 mL of distilled water and transferred to the viewing chamber. Next, electrodes were connected, and the electric field was activated. The direction in which the biosurfactant particles moved in the chamber defined the biosurfactant's charge, which was recorded through video visualization (Lima et al., 2017).

Fourier transform infrared spectroscopy

The composition of the isolated biosurfactant and TVWW was investigated based on the identification of functional groups determined by FTIR spectroscopy. Spectroscopic analysis was carried out on Shimadzu equipment (IR-TRACER 100, Shimadzu, Japan), using an attenuated total reflection accessory consisting of a mixed "diamond/ZnSe" crystal. Infrared spectra were recorded within the wavelength range of 500–4,000 cm^{-1}.

Results and Discussion

Composition of tilapia viscera wastewater

The composition of TVWW can vary due to factors such as fish diet, age, and processing method. According to data obtained by the FTIR spectroscopy (Figure 1), the spectrum showed a strong absorption band at 2,915 cm^{-1}, characteristic of C–H groups present in alkanes, suggesting the presence of unsaturated organic compounds in TVWW.

Another absorption peak at 2,850 cm^{-1} indicates the presence of carbon-hydrogen (C-H) bonds in lipid chains. In addition, the band identified at 1,710 cm^{-1} corresponds to the predominance of α or β secondary structures of proteins. The absorption band at 1,180 cm^{-1} refers to anhydrides (compounds derived from carboxylic acids) such as lactones or other cyclic compounds with carbonyl (C=O) groups. The presence of a peak in this region may indicate dehydration reactions of the lipid compounds contained in the wastewater (Naumann et al., 2000). Thus, the spectrum indicates the predominant concentration of lipids, followed by proteins, in the TVWW, which agrees with the composition previously reported for tilapia viscera (Table 2). Although FTIR confirmed the presence of lipids, a detailed lipid profile of TVWW was not determined in this study. Further investigations will be necessary to identify the specific lipid constituents and evaluate their influence on microbial biosurfactant production.

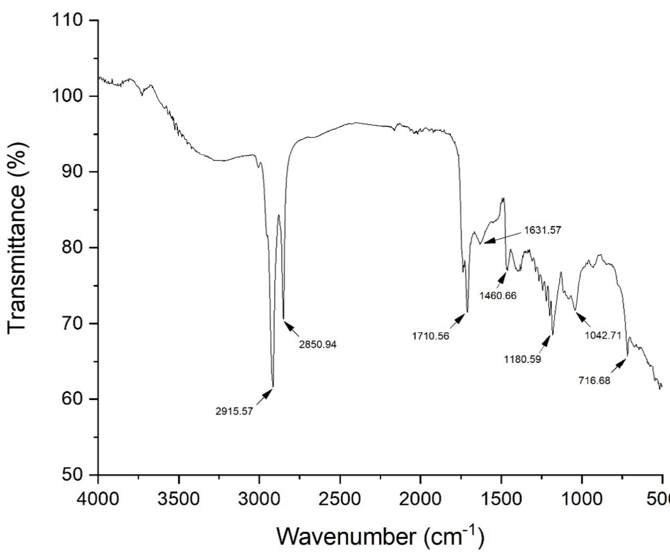


Figure 1 – Fourier transform infrared spectroscopy analysis of Nile tilapia viscera wastewater.

Table 2 – Composition of the physicochemical properties of tilapia viscera based on existing literature.

Moisture (%)	Proteins (%)	Lipids (%)	Reference
60.44	14.60	10.7	Villamil et al. (2017)
72.50	6.90	14.9	Suseno et al. (2021)
62.69	4.50	33.0	Arias et al. (2022)
62.00	7.80	26.8	Montoya et al. (2022)
63.71	11.20	23.3	Alexandre et al. (2022)
66.84	14.70	19.1	Cavenaghi-Altemio et al. (2022)
77.46	21.80	2.30	Zhang et al. (2023)
71.10	15.70	9.20	Wang et al. (2022)
76.20	19.70	2.10	Klahan et al. (2023)

Biosurfactant production by *Penicillium citrinum* UCP 1183 using tilapia viscera wastewater

Most researchers stated that biosurfactants can reduce surface tension to values below 40 mN/m (Kazemzadeh et al., 2022; Othman et al., 2022). In this context, the results obtained here evidenced that *P. citrinum* UCP 1183 could produce biosurfactant in alternative media containing alternative substrates, since the effective reduction of surface tension to ≤40 mN/m was verified in conditions 2–7 of FFD (Table 3). Among them, condition 4 stood out with a reduction in surface tension to 36 mN/m, in the medium with the highest concentrations of TVWW and PFSO, indicating the positive effect of both substrates. Indeed, the Pareto chart illustrated in Figure 2 confirmed that both substrates showed a significant and negative effect, from a statistical point of view, on the surface tension.

Table 3 – Results of the full-factorial design applied to investigate biosurfactant production by *Penicillium citrinum* UCP 1183 using alternative substrates.

Conditions	TVWW	PFSO	Surface tension (mN/m)
1	-1	-1	72.0
2	+1	-1	36.6
3	-1	+1	40.0
4	+1	+1	36.0
5	0	0	36.9
6	0	0	38.3
7	0	0	38.6

TVWW: tilapia viscera wastewater; PFSO: post-frying soybean oil.

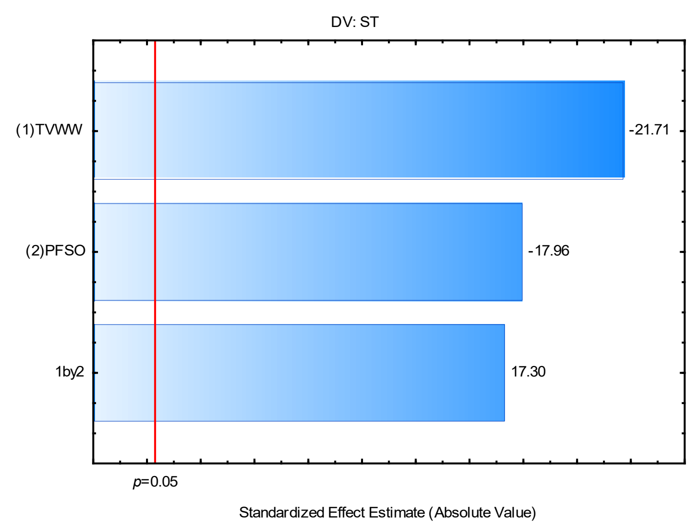


Figure 2 – Pareto chart of the standardized effects of alternative substrates on biosurfactant production by *Penicillium citrinum* UCP 1183. Effects of tilapia viscera wastewater and post-frying soybean oil concentrations on surface tension.

DV: dependent variable; ST: surface tension; TVWW: tilapia viscera wastewater; PFSO: post-frying soybean oil.

In other words, with the increase in the concentrations of each substrate, there was a reduction in the surface tension of the media, indicating the biosurfactant production.

From a phenomenological perspective, the significant effects of TVWW and PFSO on surface tension reduction (Figure 2) can be attributed to their nutritional composition and roles as carbon and energy sources, supplying the metabolic demands of *P. citrinum* during biosurfactant synthesis. TVWW contains high levels of organic matter, particularly proteins and lipids, which provide nitrogen and carbon elements essential for fungal metabolism and the biosynthesis of secondary metabolites, such as biosurfactants. PFSO, on the other hand, is predominantly composed of free fatty acids and triglycerides, which may be directly incorporated into the hydrophobic moiety of the biosurfactant or act as metabolic inducers for lipid-associated pathways (Valenzuela-Ávila et al., 2020; Liepins et al., 2021). The enhanced biosurfactant production in condition 4, where both substrates were at their highest concentrations, suggests a synergistic effect between the protein/lipid content of TVWW and the hydrophobic carbon supply from PFSO. This finding is consistent with the known metabolic pathways for microbial biosurfactant synthesis, which are often stimulated under nutrient-rich conditions, particularly when lipid carbon sources are present (Pathania et al., 2021; Chabhadiya et al., 2024).

Preliminary characterization of biosurfactant

The composition of the biosurfactant produced by *P. citrinum* UCP 1183 was investigated by analyzing the spectrum obtained by the FTIR spectroscopy (Figure 3). The presence of absorbance bands at 2,924.18 and 2,853.81 cm^{-1} was detected, corresponding to methyl and methylene groups (CH_2 and CH_3) containing fatty acids and the side chain of some amino acids.

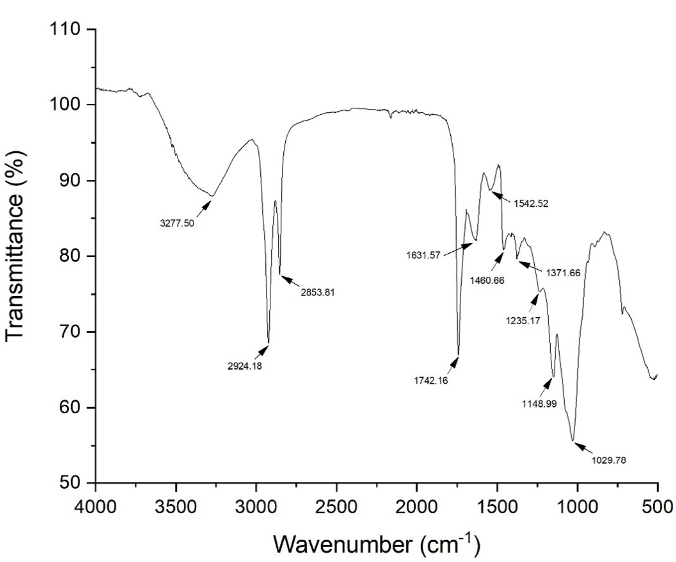


Figure 3 – Fourier transform infrared spectroscopy analysis of the isolated biosurfactant produced by *Penicillium citrinum* UCP 1183.

The strong absorption peak observed at 1,742.16 cm^{-1} is characteristic of amide I and II, being more intense with C=O stretching vibrations of the ester functional group in lipids. The spectrum suggests that the biosurfactant produced by *P. citrinum* belongs to the lipopeptide group. Similarly, Gautam et al. (2014) and Landa-Faz et al. (2022) reported the production of lipopeptide biosurfactants by *P. chrysogenum* SNP5 and *P. crustosum*, respectively. Lipopeptide biosurfactants have low molecular weights and a wide variation in amino acid and lipid composition (Gayathiri et al., 2022), and have biological activities such as antimicrobial, pharmaceutical, and biotechnological properties (Bjerk et al., 2021).

Zeta potential and ionic charge of biosurfactant

Zeta potential allows for the identification of the ionic charge present on the surface of the biosurfactant suspended in water, as well as the determination of the stability of this system, since the higher the absolute value of the zeta potential, the greater the stability. If the zeta potential is low, the particles tend to aggregate, resulting in instability (Barbosa et al., 2022; He et al., 2023). In this study, the solution containing the biosurfactant produced by *P. citrinum* UCP 1183 presented a zeta potential of -25.2 mV, indicating that the particles suspended in water have negative electrical charges on the surface, being considered an anionic biosurfactant. In addition, the zeta potential value of the biosurfactant shows moderate stability of the colloidal system since zeta potential between ±15–30 mV indicates moderate stability (Tadros et al., 2006). Other conditions, such as viscosity and temperature, can also influence the stability of the zeta potential.

Biosurfactant stability

The stability of a biosurfactant needs to be investigated, as this is a crucial parameter for its application in various industrial products (Dabaghi et al., 2023; Purwasena et al., 2024). In this context, the biosurfactant produced by *P. citrinum* showed stability of surfactant properties (reduction of surface tension) after being subjected to different temperatures, pH values, and NaCl concentrations (Figure 4).

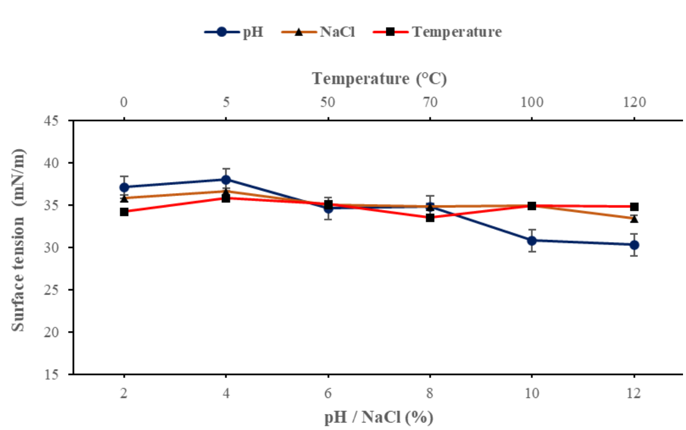


Figure 4 – Stability of the biosurfactant produced by *Penicillium citrinum* UCP 1183 at different temperatures, pH, and NaCl concentrations.
pH: potential hydrogen; NaCl: sodium chloride.

The high tolerance to extreme environmental conditions makes biosurfactants an excellent alternative to chemical surfactants (Lima et al., 2024).

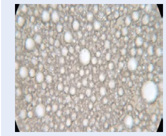
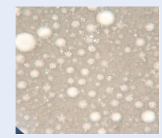
Biosurfactant as a forming and stabilizing agent of water-in-oil emulsions

The biosurfactant produced by *P. citrinum* UCP 1183 could form and stabilize emulsions with engine oil and burnt engine oil, showing emulsification index of 90.4% for both hydrophobic substrates after 48 h (Table 4). The ability of some biosurfactants to emulsify two immiscible liquids, such as water and oil, and form stable emulsions allows them to have wide industrial applicability in the formulation of detergents, cosmetics, and food products (Yang et al., 2023; Castor et al., 2024; D'Almeida et al., 2024). Some lipopeptide compounds with bioemulsifying potential have a tendency for biphasic interfacial adsorption (Soliman et al., 2023), as well as stability to withstand physicochemical changes, maintaining 50% of the original volume after 24 h of their formation (Kupikowska-Stobba et al., 2024).

Conclusion

This study demonstrated the potential of *P. citrinum* UCP 1183 for the sustainable production of biosurfactant from the reuse of TVWW. The produced biosurfactant showed anionic character and lipopeptide composition, in addition to stability under varied conditions and excellent emulsifying properties. Such multifunctional characteristics give it varied applications, making it competitive for future trends in the global surfactant market.

Table 4 – Characterization of emulsions formed by the biosurfactant produced by *Penicillium citrinum* UCP 1183.

Phases	Emulsification index (%)	Macroscopic analysis	Microscopic analysis
Oil-water burnt engine oil	90.4		
Oil-water engine oil	90.4		

Acknowledgments

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Authors' Contributions

Costa, E.R.C.: conceptualization, investigation, methodology, formal analysis, writing – original draft, writing – review & editing. **Montero-Rodríguez**, D.: investigation, software, writing – original draft, writing – review & editing. **Souza**, A.F.: investigation, data curation, visualization. **Campos-Takaki**, G.M.: resources, validation, visualization. **Andrade**, R.F.S.: conceptualization, funding, project administration, resources, supervision, writing – original draft, writing – review & editing.

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