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Valorization of tilapia processing waste for sustainable production of fungal lipopeptide biosurfactant

Valorização de resíduos do processamento de tilápia para a produção sustentável de biossurfactante lipopeptídico fúngico

Dayana Montero Rodríguez[1], *Adriana Ferreira de Souza*[1], *Rafael de Souza Mendonça*[1], *Rosileide Fontenele da Silva Andrade*[1], *Josimar Gurgel Fernandes*[2], *Galba Maria de Campos-Takaki*[1]

ABSTRACT

The aquaculture industry generates a significant amount of organic waste, particularly from tilapia (*Oreochromis niloticus*) processing. Among these residues, tilapia viscera stand out for their high protein and lipid content, representing a promising but still underexplored resource for biotechnological applications. In this context, this study investigated the use of tilapia viscera broth (TVB) as a low-cost, renewable substrate for biosurfactant production by *Penicillium rubens* UCP 1213, aiming to contribute to the fish waste valorization. A 2^2 full-factorial design was applied to evaluate the effects of TVB and post-frying soybean oil (PFSO) as a component of the culture medium for biosurfactant production. The best results were achieved with 9% TVB and 3% PFSO, reducing surface and interfacial tensions to 35.68 and 6.4 mN/m, respectively. The biosurfactant was recovered by acid precipitation (1.36 g/L); its anionic profile was confirmed by zeta potential measurement, while preliminary compositional and spectroscopic analyses indicated a predominantly lipopeptide nature. Phytotoxicity assays showed no evidence of toxic effects on onion seed germination, indicating preliminary environmental safety. Overall, this study demonstrates the feasibility of using tilapia viscera as a renewable substrate for the sustainable production of fungal biosurfactant, reinforcing circular economy practices and contributing to waste mitigation in aquaculture. The results highlight the potential of this waste-derived lipopeptide and support further process optimization and broader toxicological evaluation for industrial and agricultural applications.

Keywords: tilapia viscera; waste valorization; lipopeptide biosurfactant; *Penicillium rubens*.

RESUMO

A indústria aquícola gera quantidades significativas de resíduos orgânicos, particularmente provenientes do processamento de tilápia (*Oreochromis niloticus*). Entre esses resíduos, as vísceras de tilápia se destacam por seu elevado teor de proteínas e lipídios, representando um recurso promissor, porém ainda pouco explorado, para aplicações biotecnológicas. Nesse contexto, este estudo investigou o uso do caldo de vísceras de tilápia (CVT) como substrato renovável e de baixo custo para a produção de biossurfactante por *Penicillium rubens* UCP 1213, visando contribuir para a valorização de resíduos de pescado. Um planejamento fatorial completo 2^2 foi aplicado para avaliar os efeitos do CVT e do óleo de soja pós-fritura (OSPF) como constituintes do meio na produção de biossurfactante. Os melhores resultados foram obtidos com 9% de TVB e 3% de OSPF, reduzindo as tensões superficial e interfacial para 35,68 e 6,4 mN/m, respectivamente. O biossurfactante foi recuperado por precipitação ácida (1,36 g/L); seu perfil aniônico foi confirmado por medição de potencial zeta, enquanto análises preliminares de composição e espectroscópicas indicaram uma natureza predominantemente lipopeptídica. Ensaios de fitotoxicidade não mostraram efeitos tóxicos sobre a germinação de sementes de cebola, indicando segurança ambiental preliminar. De modo geral, este estudo demonstra a viabilidade do uso de vísceras de tilápia como substrato renovável para a produção sustentável de biossurfactante fúngico, reforçando práticas de economia circular e contribuindo para a mitigação de resíduos na aquicultura. Os resultados destacam o potencial desse lipopeptídeo derivado de resíduos e apoiam a necessidade de maior otimização do processo e de avaliações toxicológicas mais abrangentes para aplicações industriais e agrícolas.

Palavras-chave: vísceras de tilápia; valorização de resíduos; biossurfactante lipopeptídico; *Penicillium rubens*.

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

[2]Instituto Agronômico de Pernambuco – Recife (PE), Brazil

Corresponding author: Galba Maria de Campos-Takaki. Multi-User Center for Analysis and Characterization of Biomolecules and Material Surfaces, Catholic University of Pernambuco, Rua Nunes Machado, 42, Bloco J, Térreo – Boa Vista – CEP: 50050-590 – Recife (PE), Brazil.
E-mail: galba.takaki@unicap.br

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Introduction

Global aquaculture has grown significantly in recent decades, driven by the rising demand for fish and the decline of wild stocks due to overfishing and sustainability concerns (FAO, 2024; Rodríguez-Hernández et al., 2025). Among farmed species, Nile tilapia (*Oreochromis niloticus*) ranks third in worldwide production, due to its adaptability, disease resistance, rapid growth, and high market acceptance, accounting for approximately 9% of global aquaculture production (Meurer et al., 2025). In Brazil, tilapia farming has expanded rapidly and currently represents the main aquaculture activity in the country, with production reaching over 850,000 tons in 2023 and contributing substantially to economic development, employment opportunities, and regional growth (França et al., 2024; Nunes et al., 2024).

However, the expansion of tilapia production also generates large volumes of organic waste, including scales, heads, bones, and viscera. Among these residues, viscera constitute a significant fraction and are particularly rich in proteins, lipids, and other nutrients (Mota et al., 2019; Kuepethkaew et al., 2024). When inadequately managed, this material may cause environmental contamination and promote the proliferation of pathogenic microorganisms, highlighting the urgent need for sustainable reuse strategies. Although part of this waste is currently converted into fish meal and animal feed, its biotechnological potential remains largely underexplored (Lee et al., 2022; Igansi et al., 2024).

In recent years, several fish-derived residues, such as heads, skin, bones, and scales, have been investigated as substrates for the microbial production of value-added biomolecules (Khiari, 2024; Kumari et al., 2024). However, tilapia viscera offer specific advantages that distinguish them from other processing wastes, including a high lipid content, a balanced protein profile, and readily assimilable nitrogen and fatty acid fractions, which are particularly favorable for the biosynthesis of amphiphilic compounds (He et al., 2021; Arias et al., 2022; Costa et al., 2025). Despite these characteristics, the use of tilapia viscera as a substrate for biosurfactant production remains underexplored, representing a significant knowledge gap.

Biosurfactants are surface-active secondary metabolites produced by various microorganisms that efficiently reduce surface and interfacial tensions while exhibiting low toxicity, high biodegradability, and stability across a wide range of environmental conditions (Araújo et al., 2019; Santos et al., 2025). These properties make them suitable for applications in agriculture, cosmetics, pharmaceuticals, and petrochemical industries. Consequently, the global biosurfactant market has shown sustained growth and is projected to reach USD 7.62 billion by 2029, with a compound annual growth rate of 9.8%, driven by a rising demand for environmentally friendly alternatives to synthetic surfactants (Markets and Markets, 2024; Pandit and Meena, 2025). Nevertheless, large-scale production remains economically constrained, mainly due to the high cost of conventional substrates, reinforcing the importance of low-cost renewable feedstocks (Mgbechidinma et al., 2022; Karmakar et al., 2025).

Filamentous fungi of the genus *Penicillium* have emerged as promising biosurfactant producers due to their metabolic versatility, ability to use complex substrates, and capacity to synthesize structurally diverse lipopeptides and glycolipids (Sanches et al., 2021; Kathiravan et al., 2024; Costa et al., 2025). In particular, *Penicillium rubens* UCP 1213 showed promising biosurfactant production performance in our preliminary study when cultivated on agro-industrial substrates (Montero-Rodríguez et al., 2025). However, its potential to convert fish-processing waste, especially tilapia viscera, into biosurfactants has not yet been systematically investigated. In this context, the present study aims to evaluate the feasibility of using tilapia viscera broth (TVB) as a renewable and low-cost substrate for biosurfactant production by *P. rubens* UCP 1213. In addition, a preliminary physicochemical characterization and a phytotoxicity assessment of the biosurfactant produced were conducted to evaluate its potential for agricultural and industrial applications. This work seeks to contribute to the valorization of aquaculture residues and to advance sustainable strategies aligned with circular economy principles.

Materials and Methods

Microorganism

Penicillium rubens UCP 1213, a filamentous fungus isolated from mangrove sediment in Rio Formoso-PE, was kindly provided by the Culture Collection of the Catholic University of Pernambuco- UCP (Recife-PE, Brazil). This strain was maintained on Sabouraud Agar medium (40 g/L glucose, 10 g/L peptone, and 20 g/L agar) at 5 °C.

Inoculum preparation

P. rubens was grown in Petri dishes containing Sabouraud Agar at 28°C for 5 days. Then, discs (6 mm in diameter) of young mycelium were removed and used to inoculate the production media.

Agro-industrial waste

Post-frying soybean oil (PFSO): PFSO was kindly supplied by a local food trade in the city of Recife-PE, Brazil (Santos et al., 2022).

Tilapia viscera: viscera from farmed Nile tilapia (*Oreochromis niloticus*) were kindly donated by Noronha Pescados Ltd., a local fish processing plant in Pernambuco State, Brazil, and kept frozen until further use. To process the viscera, 1 kg was thawed at room temperature and then boiled in 1 L of distilled water for 1 h. The resulting "broth" was left to stand at room temperature until it cooled, then filtered through a household sieve to separate the remaining solids. It was subsequently filtered through gauze and manually squeezed. The filtered tilapia viscera broth (TVB) obtained was frozen until further use.

Preliminary characterization of tilapia viscera broth

The pH of TVB was measured using a benchtop pH meter (Orion model 310). Its composition was determined by quantifying total proteins, carbohydrates, and lipids using the Labtest kit (Labtest Diagnostica S.A., Minas Gerais, Brazil), the phenol-sulfuric acid method (Dubois et al., 1956), and chloroform-methanol extraction (Manocha et al., 1980), respectively. In addition, the main functional groups of the TBV were identified by Fourier transform infrared (FTIR) spectroscopic analysis using an attenuated total reflection (ATR) accessory consisting of a mixed 'diamond/ZnSe' crystal. The infrared spectrum was recorded using the Shimadzu equipment IR-TRACER 100, within the wavelength range of 4,000–500 cm^{-1} (Costa et al., 2025).

Biosurfactant production

Biosurfactant production was carried out in 250 mL Erlenmeyer flasks containing 100 mL of production media formulated with the desired concentrations of alternative substrates (PFSO and TVB), according to the full-factorial design (FFD) described later. The pH of the media was adjusted to 6, and the flasks were sealed with cotton plugs and aluminum foil. All media were sterilized by autoclaving at 121°C for 15 min prior to inoculation. After cooling, the media were handled under aseptic conditions in a laminar flow cabinet to prevent contamination. Fungal inoculation was performed by adding 20 mycelial discs of *P. rubens* to each flask, followed by incubation for 96 h under controlled temperature and agitation conditions (28°C and 150 rpm), according to our previous studies (Oliveira et al., 2020; Costa et al., 2025). Throughout the fermentation process, the flasks were visually inspected for contamination, and only uncontaminated cultures were used for subsequent analyses.

Although environmental variables such as dissolved oxygen variation and real-time metabolic pH shifts were not monitored during fermentation, methodological robustness was maintained by strictly controlling the initial incubation parameters, including standardized agitation (150 rpm), temperature (28°C), and an initial pH adjustment to 6.0.

Full-factorial design

A 2^2 FFD was applied to investigate the influence of the concentration of each low-cost substrate (TVB and PFSO) and their interaction on surface tension and biomass yield, as response variables. Seven experimental assays were conducted randomly, with three replicates at the central point, and the independent variables evaluated at three levels: minimum (-1), central (0), and maximum (+1) (Table 1). The experimental levels were defined based on preliminary assays and prior studies (Montero-Rodríguez et al., 2025). The concentrations tested for TVB and PFSO were selected to represent ranges that would provide sufficient nutrients for fungal metabolism and biosurfactant production, while allowing for the evaluation of synergistic effects between the substrates. The data obtained from the experiments were subjected to statistical analysis using the Statistica® software, version 12.0 (StatSoft Inc., USA), and the significance of the results was assessed at the $p<0.05$ level. It is worth clarifying that the FFD was applied as an exploratory screening approach to identify significant culture conditions affecting biosurfactant production within the studied range, rather than as a definitive optimization strategy.

Table 1 – Levels of the variables studied in the 2^2 full-factorial design applied to investigate the production of biosurfactant and biomass by *P. rubens* UCP 1213 using alternative substrates.

Variables	Factors levels		
	Low (-1)	Central (0)	High (+1)
TVB (%, *v/v*)	3	6	9
PFSO (%, *v/v*)	3	6	9

TVB: tilapia viscera broth; PFSO: post-frying soybean oil.

Determination of the surface tension

The surface tension of cell-free metabolic liquids was determined at room temperature (±25°C) in an automatic tensiometer model called Sigma 701 (Biolin Scientific, São Paulo, Brazil), using the Du Noüy ring method (Kuyukina et al., 2001). The surface tension value was obtained as an average of ten measurements, and distilled water was used as a control (surface tension=72 mN/m).

Biomass yield

The biomass obtained by filtration and centrifugation was washed three times with distilled water to remove residues from the culture medium and centrifuged yet again. Then, it proceeded to be frozen, lyophilized (Advantage Plus EL-85 lyophilizer, SP Scientific, USA) and kept in a desiccator until a constant weight was reached. Dry weight was determined by gravimetry and yield expressed in g/L.

Determination of the tensoactive properties of the biosurfactant

After analyzing the factorial design results, the condition with the greatest reduction in surface tension was selected for the analyses described below.

Determination of interfacial tension: The interfacial tension of the cell-free metabolic liquid was determined at room temperature (±25°C) in an automatic tensiometer model Sigma 701 (Biolin Scientific, São Paulo, Brazil), using the Du Noüy ring method and n-hexadecane (Santos et al., 2022).

Determination of emulsification index (EI_{24}): The emulsification index (EI_{24}) of the cell-free metabolic liquid was determined in triplicate, following the methodology of Cooper and Goldenberg (1987). Two milliliters of the cell-free metabolic liquid were added to two milliliters of hydrophobic compounds (soybean oil, PFSO, motor oil, and burnt motor oil) in test tubes and vigorously vortexed for 2 min at room temperature (25°C). The mixtures were left to stand for 24 h, after which the EI_{24} was calculated according to Equation 1:

$$EI_{24}\,(\%) = He/Ht \times 100 \quad (1)$$

Where:
He: height of the emulsion;
Ht: total height of the mixture.

Parafilm M test: Parafilm M test was carried out by placing the cell-free metabolic liquid (25 µL) on the hydrophobic surface of the parafilm M strip. Distilled water and 2% sodium dodecyl sulfate (SDS) were used as negative and positive controls, respectively. The shape of the drops on the surface was examined after 1 min, and their diameters were measured using a caliper. The spreading of drops on the hydrophobic surface was considered an indicator of the presence of the biosurfactant (Pele et al., 2018).

Isolation of the biosurfactant

Different methodologies were tested for the isolation of the produced biosurfactant, using 50 ml of cell-free metabolic liquid. The methods of acid precipitation (adjustment of pH to 2) and extraction with solvents (acetone, ethanol 95% and ethanol 70%), were used separately and in combination, as described in Table 2. The samples were incubated overnight at 5°C and then centrifuged at 5,000 *g* for 15 min. After centrifugation, the supernatants were discarded, and the precipitates obtained were washed by centrifuging with distilled water and subjected to lyophilization using an Advantage Plus EL-85 lyophilizer (SP Scientific, USA). Then, the samples were kept in a desiccator up until they reached a constant weight, as determined by gravimetry, and the yield was expressed in grams per liter (g/L). Based on the results, the method for isolation of the biosurfactant produced by *P. rubens* was selected.

Preliminary characterization of the biosurfactant

The ionic charge of the biomolecule was determined using the Zeta potentiometer model ZM3-D-G, Zeta Meter System 3.0+, with direct images to the video of the Zeta Meter, San Francisco, CA, U.S.A. (Lima et al., 2017; Santos et al., 2022).

Table 2 – Methods used for the recovery of the biosurfactant produced by *P. rubens* UCP 1213.

Recovery method	Reference
Acid precipitation	Santos et al. (2022)
Extraction with acetone (1:1, *v/v*)	Pele et al. (2019)
Extraction with ethanol 95% (1:1, *v/v*)	Bueno et al. (2010)
Extraction with ethanol 70% (1:1, *v/v*)	Nogueira et al. (2020)
Extraction with ethanol 70% (2:1, *v/v*)	Bueno et al. (2010)
Extraction with acetone (1:1, *v/v*) + acid precipitation	This study
Extraction with ethanol 95% (1:1, *v/v*) + acid precipitation	This study
Extraction with ethanol 70% (1:1, *v/v*) + acid precipitation	This study
Extraction with ethanol 70% (2:1, *v/v*) + acid precipitation	This study

The compositional analyses and Fourier-transform infrared (FTIR) spectroscopy of the recovered biosurfactant were carried out using the same procedures and instrumental conditions previously described in the subsection "Preliminary characterization of tilapia viscera broth" (Costa et al., 2025).

Phytotoxicity of biosurfactant

Seeds collection and disinfection: Onion seeds of the "Vale Ouro IPA-11" cultivar, not previously exposed to pesticides, were kindly provided by the Seed Analysis and Pathology Laboratory of the Agronomic Institute of Pernambuco – IPA (Recife-PE). They were rinsed thoroughly with sterile distilled water (SDW), then immersed in 1% sodium hypochlorite solution for 1 min, and thereafter washed five times with SDW to remove any residual effect of the disinfectant (Faisal, 2024). The seeds were then dried at room temperature for 2 h.

Phytotoxicity test: Phytotoxicity of the biosurfactant was determined in a static test involving the seed germination and root growth of onion (*Allium cepa* L.) plants, according to the methodology of Tiquia et al. (1996). Test solutions of the biosurfactant at different concentrations (0.5 and 1%) were prepared in SDW. The test was performed in triplicate in sterile Petri plates (9 cm in diameter) containing Whatman No. 1 filter paper discs. Ten previously disinfected seeds were symmetrically placed in each plate, followed by inoculation with 5 mL of the test solution at 28 °C. SDW was used as the control. Seed germination, root growth (≥5 mm), and the germination index (GI) were calculated after five days of incubation using the Equation 2, 3 and 4:

$$\text{Relative seed germination (\%)} = \frac{(\text{number of seeds germinated in extract} \times 100}{\text{number of seeds germinated in control})} \quad (2)$$

$$\text{Relative root length (\%)} = \frac{(\text{average root length in extract} \times 100}{\text{average root length in control})} \quad (3)$$

$$\text{Germination index (\%)} = [(\text{\% seed germination}) \times (\text{\% root growth})]/100\% \quad (4)$$

The results of relative seed germination, relative root elongation, and germination index were expressed as mean±standard error (n=3). Data were subjected to Student's t-test to compare the effects of biosurfactant concentrations (0.5 and 1.0%) using the Statistica® software (version 12.0, StatSoft Inc., Tulsa, OK, USA). Significant differences between means were considered at p<0.05.

Results and Discussion

Preliminary characterization of tilapia viscera broth

The Nile tilapia processing industry in Brazil has experienced significant growth; however, this sector generates substantial waste, particularly viscera, which is often accumulated and discarded, resulting in environmental contamination. The methods currently used for waste reutilization are insufficient or inadequate, and new approaches are needed to enable a more effective use beyond the production of fertilizers and animal feed (Kuepethkaew et al., 2024; Ogava et al., 2024; Campos et al., 2025).

Interestingly, fish by-products provide an excellent source of nutrients for microbial growth (particularly nitrogen and minerals) that can be exploited to produce valuable metabolites, such as biosurfactants (Martins and Martins, 2018; Zhu et al., 2020). In this sense, the formulation of microbial growth media for biosurfactant production using TVB can reduce bioprocess costs and mitigate environmental problems associated with fish waste, thereby proposing an environmentally friendly alternative for disposal (Hu et al., 2021; Alvarado-Ramírez et al., 2023). The current study aimed to investigate the suitability of TVB as an innovative substrate for the low-cost production of biosurfactant.

The pH of TVB was 6.20, and its chemical composition revealed high contents of total proteins (64.1%) and lipids (25%), in agreement with previous findings on tilapia processing residues (Sales and Oliveira, 2015; Boscolo et al., 2018). Sellami et al. (2016) used the supernatant from boiled tuna processing by-product as a low-cost substrate for biosurfactant production by *Aneurinibacillus migulanus*. They characterized this raw material and reported findings similar to those reported here, in both high protein and lipid content.

The composition of TVB was confirmed by analyzing the FTIR spectrum, shown in Figure 1.

The absorbance peak at approximately 2,922 cm^{-1} was attributed to the typical vibrational modes of lipid fatty acids. In addition, the broad absorbance band at 3,200–3,600 cm^{-1} can be used to monitor the formation of hydroperoxide, a primary lipid oxidation product (Lozano et al., 2017; Choopan et al., 2021). The absorbance peak at 2,853 cm^{-1} was assigned to C-N stretching. Peaks in the 1,000–1,600 cm^{-1} region were also observed and attributed to the amide bands characteristic of proteins (Chaijan and Panpipat, 2021). The detection of a secondary protein structure was also possible, based on the amide I region, which consists of C=O stretching vibrations in the 1,610–1,690 cm^{-1} region (Ellepola et al., 2005; Choopan et al., 2021).

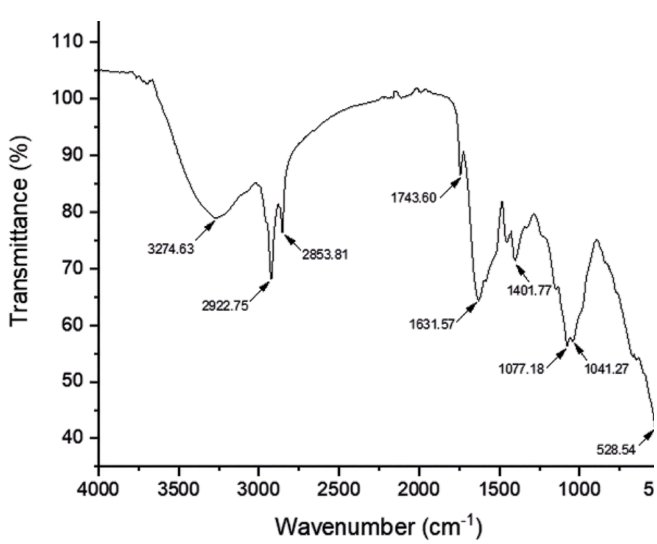


Figure 1 – Infrared spectrum of tilapia viscera broth (TVB) used in this study.

Several researchers stated that fish processing by-products contain growth-promoting factors, and are therefore promising substrates for the formulation of a microbial culture media. In this sense, various pretreatment methods (including thermal, chemical, and enzymatic treatments) have been applied to fish waste before its utilization in growth media, to enhance the soluble protein fraction and ash content (Vázquez et al., 2020; Petrova et al., 2021). As reported by Rebah and Miled (2013), heat treatment of fish by-products offers several advantages for microbial applications, including process simplification, reduced energy demand, and, consequently, lower production costs.

Production of biosurfactant by *P. rubens* UCP 1213

Several low-cost substrates have been used to produce microbial surfactants, including post-frying oils and fishery-processing waste (Martins and Martins, 2018; Santos et al., 2022). In this context, this study applied the FFD methodology to investigate biosurfactant production by *P. rubens* UCP 1213 using TVB and PFSO as alternative substrates. The results in Table 3 verify the effective reduction in surface tension under all evaluated conditions, with particular emphasis on assay 2 (medium containing 9% TVB and 3% PFSO), where the greatest reduction to 35.68 mN/m, was observed.

This result confirms the potential of *P. rubens* UCP 1213 as a biosurfactant-producing microorganism, as reported in our previous study (Montero-Rodríguez et al., 2025). Other researchers have verified the production of biosurfactants by fungi of the genus *Penicillium*; however, the use of conventional substrates such as glucose,

Table 3 – Results of the 2^2 full-factorial design applied to investigate the production of biosurfactant and biomass by *P. rubens* UCP 1213 using tilapia viscera broth (TVB) and post-frying soybean oil (PFSO) as alternative substrates.

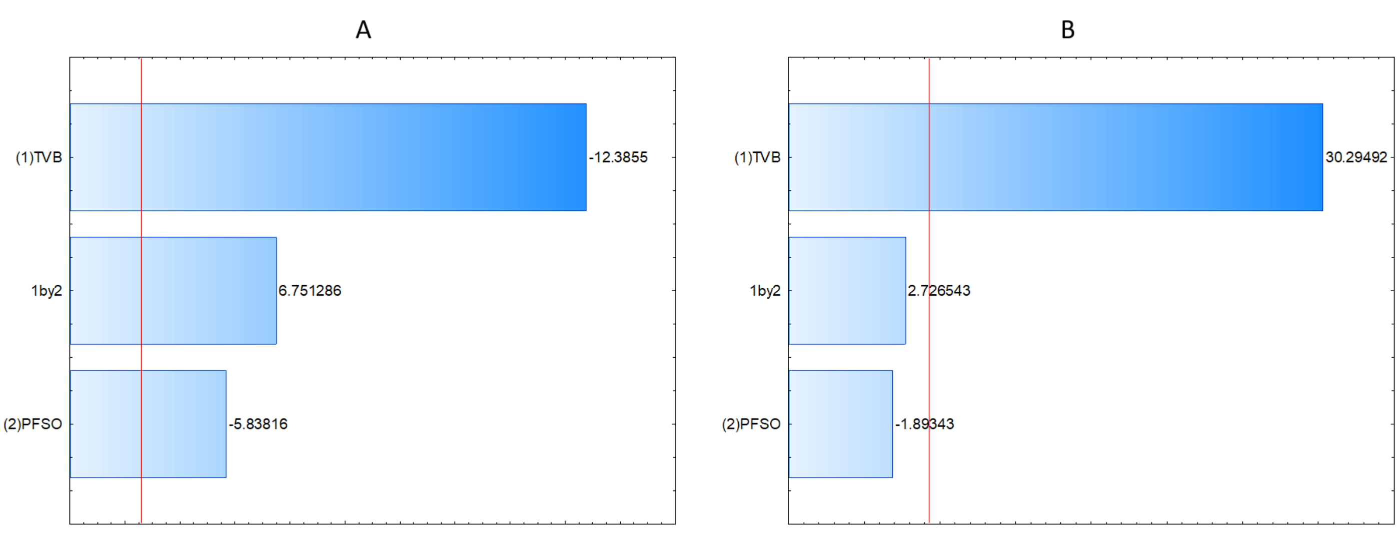
Assays	TVB (%)	PFSO (%)	Surface tension (mN/m)	Biomass yield (g/L)
1	3	3	45.53	3.17
2	9	3	35.68	6.81
3	3	9	39.05	2.56
4	9	9	36.15	6.92
5	6	6	41.03	4.88
6	6	6	41.95	4.62
7	6	6	41.09	4.71

peptone, and yeast extract is commonly described (Yuan et al., 2006; Méndez-Castillo et al., 2017). In this sense, the reuse of agro-industrial waste such as TVB and PFSO as alternative substrates can make the production process more economical and sustainable.

The effects of TVB and PFSO on surface tension were statistically evaluated through ANOVA and illustrated in the Pareto chart (Figure 2A). Both substrates had a significant negative effect on the dependent variable, demonstrating that increasing them independently reduced surface tension, consistent with biosurfactant production in the medium. TVB showed the strongest effect (p=0.0065), and PFSO also had a significant effect (p=0.0281). Their interaction was statistically significant (p=0.0212), indicating complementary roles of the substrates. TVB, rich in protein and nitrogen, likely provided readily assimilable nitrogen and amino acids, essential for cell growth and the synthesis of the peptide portion of the biosurfactant molecule. Meanwhile, PFSO supplied fatty acids that served as carbon sources and precursors for lipid fraction synthesis. Synergistic effects between protein- and lipid-rich substrates have been shown to enhance the production of amphipathic secondary metabolites in filamentous fungi, reinforcing the relevance of both components for future studies aimed at optimizing biosurfactant production under the evaluated conditions. Furthermore, it is important to consider that although the significant reduction in surface tension is primarily attributed to biosurfactant production, other extracellular metabolites may also exert minor effects on the overall tensiometric behavior.

The pronounced effect of TVB indicates its suitability as a low-cost substrate for biosurfactant production. Similarly, previous studies have reported the feasibility of using fish-processing residues as substrates for biosurfactant production, highlighting the superior surface activity of bacterial biosurfactants relative to fungal metabolites (Alvarado-Ramírez et al., 2023). For example, *Coynebacterium aquaticum* cultivated in fish waste reduced surface tension to 27.8 mN/m (Martins and Martins, 2018), while *Bacillus subtilis* grown on fish waste-based peptones achieved values ranging from 26 to 30 mN/m (Zhu et al., 2020; Hu et al., 2021). In contrast, fungal biosurfactants generally exhibit a less pronounced performance, with reductions around 35–40 mN/m. Recently, Costa et al. (2025) used tilapia viscera wastewater for biosurfactant production by *P. citrinum* UCP 1183, resulting in a surface tension of 36 mN/m. Hence, the value of 35.68 mN/m obtained in the current study falls within the lower–intermediate range reported for fungal systems and demonstrates competitive performance compared to other waste-based fungal biosurfactants. Although the reduction does not reach the lowest values typically observed for bacterial lipopeptides, such as surfactin, the results confirm the technical feasibility of using TVB as a sustainable substrate, reinforcing its potential within a circular bioeconomy framework. Further optimization strategies are required to enhance surface activity.

In other hand, it should be noted that our study did not include a conventional synthetic reference medium based on defined carbon and nitrogen sources. Therefore, the results primarily demonstrate the feasibility of TVB as a renewable substrate, rather than providing a direct quantitative comparison with standard media. Comparative essays will be necessary in future research to further assess the relative performance of this residue-based medium. Additionally, future studies that monitor dissolved oxygen and pH profiles may provide deeper insights into the metabolic behavior of *P. rubens*, therefore contributing to further process optimization and yield enhancement.



(1) TVB: tilapia viscera broth; (2) PFSO: post-frying soybean oil; 1by2: interaction between factors 1 (TVB) and 2 (PFSO).
Figure 2 – Pareto charts showing the standardized effects of TVB and PFSO concentrations, as well as their interaction, on (A) surface tension and (B) biomass yield, according to the results from the full-factorial design.

Production of biomass by *P. rubens* UCP 1213 using agro-industrial substrates

In this study, fungal biomass production was assessed under FFD conditions as this parameter is commonly used as an indicator of microbial growth. The results in Table 3 indicate that P. rubens adapted well to agro-industrial substrates and showed robust growth across all FFD conditions. Additionally, the Pareto diagram (Figure 2B) showed a significant positive effect of TVB (p=0.001088) on fungal biomass production, highlighting the potential of this substrate for developing alternative culture media for microbial growth.

Determination of the tensoactive properties of the biosurfactant

The reduction in interfacial tension is considered another parameter that confirms the surfactant properties of biosurfactants (Santos et al., 2022). In this context, an interfacial tension of 6.4 mN/m with *n*-hexadecane was observed for the biosurfactant produced under the selected conditions (medium containing 9% TVB and 3% PFSO). This result was considered excellent, as the literature indicates that effective interfacial tension values are typically below 18 mN/m (Ribeiro et al., 2020). To the best of our knowledge, this is the first study to evaluate the interfacial tension reduction capacity of biosurfactants produced by fungi of the genus *Penicillium*, thereby highlighting the promising potential of these fungal-derived surfactants.

In addition, the emulsification index (EI_{24}) is a commonly used parameter to assess the emulsifying properties of biosurfactants, based on their ability to maintain at least 50% of the original emulsion volume after 24 hours (Andrade et al., 2018; Pele et al., 2019; Santos et al., 2025). The cell-free metabolic liquid obtained under the selected condition showed EI_{24} values below 50% for all tested hydrophobic compounds (PFSO, 38.2%; motor oil, 40.9%; and burnt motor oil, 48.7%), indicating that the produced biosurfactant does not exhibit strong emulsifying activity. Similar findings were reported by Khan et al. (2023) for a biosurfactant produced by *P. chrysogenum* FP4, reinforcing that not all biosurfactants are good bioemulsifiers and, therefore, should be evaluated as distinct functional biomolecules (Uzoigwe et al., 2015; Rahman et al., 2019).

On the other hand, the Parafilm M test has been widely used as a rapid qualitative assay to detect surface-active compounds (Yaraguppi et al., 2020; Shatila et al., 2021). In this study, the metabolic liquid was evaluated using distilled water as a negative control and 2% SDS as a reference surfactant. As seen in Figure 3, the droplet formed by the metabolic liquid exhibited a larger diameter (7.5 mm) than those of distilled water (3.0 mm) and 2% SDS (6.0 mm), indicating a reduced surface tension and the presence of surface-active molecules. Previously, Pele et al. (2018) and Primeia et al. (2020) reported droplet diameter values ranging from 8.0 to 12.0 mm for other biosurfactant-producing microorganisms. However, this assay provides only qualitative information, and droplet diameter should not be interpreted as a robust indicator of biosurfactant performance. Moreover, the use of SDS as a reference must be considered with caution, since its physicochemical properties differ substantially from those of crude biosurfactant extracts. Therefore, the Parafilm M test was employed exclusively as a preliminary screening tool to confirm biosurfactant production.

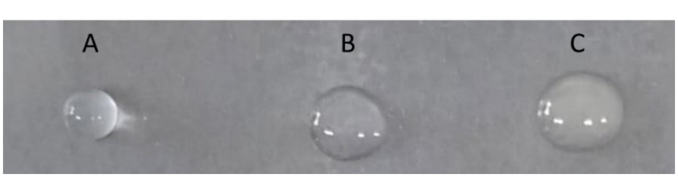


Figure 3 – Parafilm M test using (A) distilled water, (B) 2% sodium dodecyl sulfate, and (C) the cell-free metabolic liquid containing biosurfactant produced by *P. rubens* UCP 1213.

Biosurfactant recovery

For biosurfactant production, recovery can account for 70–80% of total process costs (Santos et al., 2016), making the choice of an efficient downstream strategy critical. Several recovery methods were evaluated, and acid precipitation proved to be the most effective, yielding 1.362 g/L (Table 4). In contrast, solvent extraction alone yielded negligible recovery, and even when combined with acid precipitation, the maximum yield was 0.568 g/L (70% ethanol), representing a 58.3% reduction relative to acid precipitation alone. This behavior is likely related to the predominantly lipopeptide nature of the biosurfactant and its strong affinity for the aqueous phase, which limits partitioning into organic solvents and may involve the formation of stable aggregates.

The biosurfactant yield obtained in this study (1.362 g/L) was highly competitive when compared to other protein-rich and animal-derived substrates reported in the literature. Quantitatively, our yield was 21.6% higher than the 1.12 g/L achieved by *Bacillus vallismortis* RMS25 using chicken feather waste (Pendyala et al., 2022) and significantly outperformed *B. subtilis* ATCC 21332, which produced only 0.274 g/L

Table 4 – Yield of biosurfactant produced by *P. rubens* UCP 1213 after recovery using different methods.

Recovery method	Biosurfactant yield (g/L)
Acid precipitation	1.362
Extraction with acetone (1:1, *v/v*)	-
Extraction with ethanol 95% (1:1, *v/v*)	-
Extraction with ethanol 70% (1:1, *v/v*)	-
Extraction with ethanol 70% (2:1, *v/v*)	-
Extraction with acetone (1:1, *v/v*) + acid precipitation	0.402
Extraction with ethanol 95% (1:1, *v/v*) + acid precipitation	0.470
Extraction with ethanol 70% (1:1, *v/v*) + acid precipitation	0.404
Extraction with ethanol 70% (2:1, *v/v*) + acid precipitation	0.568

of surfactin from tuna waste-based peptone (Hu et al., 2021), a nearly five-fold increase in our fungal system. Furthermore, while our results were lower than the 1.78 g/L reported for *Paenibacillus macerans* TKU029 on squid pen powder (Liang et al., 2014), they were 147% higher than the 0.55 g/L produced by *B. licheniformis* TKU004 using the same waste (Chen et al., 2012). These comparisons highlight that *P. rubens* UCP 1213, when cultivated in TVB, exhibits a robust biosynthetic capacity that rivals and often exceeds established bacterial benchmarks for waste-derived media. Consequently, these results confirm TVB as a competitive and sustainable substrate, although further optimization of downstream processing is essential for future industrial scale-up.

Preliminary characterization of the biosurfactant

The characterization of biosurfactants is important as it enables us to understand their properties (e.g., ionic charge and composition) and to guide their use across various industrial and environmental applications (Martins and Martins, 2018). The Zeta potential analysis carried out in the present study showed the anionic character (-27.8 mV) of the biosurfactant produced by *P. rubens* UCP 1213, in correspondence with the biosurfactants previously produced by *P. rugulosum* UCP 0143 and *P. spinulosum* UCP 1347 (Santos, 2021). Anionic biosurfactants have a negative charge in their polar portion, and several studies in the literature indicate their potential for breaking dormancy and inducing germination in vegetable seeds (Araújo et al., 2019; Santos et al., 2022).

Additionally, the Zeta potential indicates good colloidal stability (Lima et al., 2024), a desirable property for liquid agricultural formulations. In foliar applications, the Zeta potential influences spray stability and interaction with plant surfaces, which are typically negatively charged (Zhao et al., 2018). Therefore, the moderately negative charge of the biosurfactant may favor controlled wetting and spreading, enhancing adhesion and dispersion of active compounds on leaves.

On the other hand, the biochemical composition of the isolated biosurfactant (lipids 80%, proteins 13.22%, and carbohydrates 6.25%) indicated a predominantly lipopeptide nature, which was further supported by FTIR-ATR analysis (Figure 4). The spectrum exhibited a broad band at 3,277.50 cm^{-1}, attributed to N–H and O–H stretching vibrations, characteristic of peptide backbones and hydrogen-bonded hydroxyl groups. The intense bands at 2,924.18 and 2,853.81 cm^{-1} corresponded to asymmetric and symmetric C–H stretching of long aliphatic chains, confirming the lipid moiety. The absorption at 1,710.56 cm^{-1} was assigned to C=O stretching of ester or carboxylic groups, while the bands at 1,630.13 and 1,536.78 cm^{-1} were attributed to amide I and amide II vibrations, respectively, indicating peptide linkages. Additional signals in the 1,173–1,041 cm^{-1} region were associated with C–O and C–N stretching vibrations. The coexistence of characteristic lipid and peptide bands, coupled with the predominance of aliphatic and amide signals, is consistent with the typical FTIR profiles reported for lipopeptide biosurfactants, including surfactin-, iturin-, and fengycin-type molecules (Kumar et al., 2017; Barale et al., 2022; Wang et al., 2024), and with previous reports for biosurfactants produced by *Penicillium* spp. (Gautam et al., 2014; Landa-Faz et al., 2022). Although the preliminary characterization of the biomolecule strongly suggests a lipopeptide nature, advanced analytical techniques, such as mass spectrometry and NMR, would be necessary for definitive structural elucidation.

Assessment of biosurfactant phytotoxicity

Toxicity refers to the adverse effects of a substance on living organisms and depends on its physicochemical properties and concentration. In this context, plant-based assays are commonly used as preliminary tools to evaluate the potential phytotoxicity of biosurfactants (Selva Filho et al., 2023; Santos et al., 2025). In the present study, onion (*Allium cepa* L.) seeds were exposed to biosurfactant solutions at concentrations of 0.5 and 1%, and their toxicity was assessed based on the germination index (GI), integrating relative seed germination and relative root growth (Pele et al., 2019; Santos et al., 2022).

The results indicate the absence of acute phytotoxic effects for *A. cepa*, as GI values remained above the commonly accepted threshold of 80%, which is indicative of non-toxicity (Boutin et al., 2010) (Table 5). There were no inhibitory effects on seed germination or root growth at

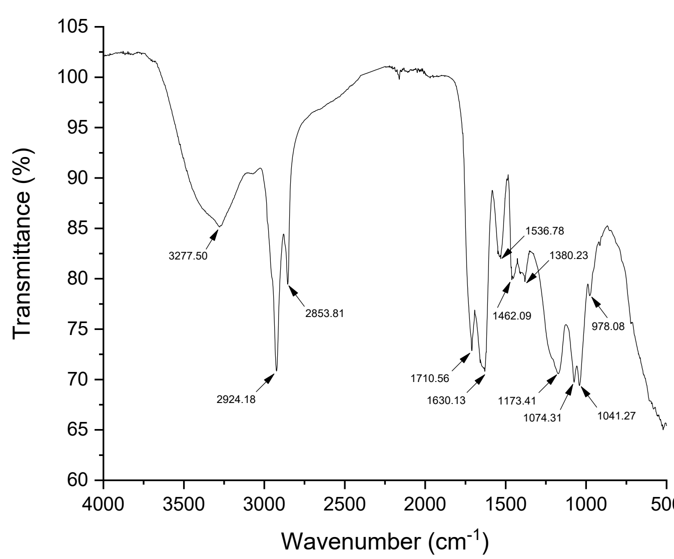


Figure 4 – Infrared spectrum of biosurfactant produced by *P. rubens* UCP 1213 in this study.

Table 5 – Phytotoxicity of biosurfactant produced by *P. rubens* UCP 1213 on onion seeds. Results are expressed as means±SDs (standard deviations, *n*=3), where means were considered statistically significant at $p<0.05$.

Biosurfactant concentration (%)	Relative seed germination (%)	Relative root elongation (%)	Germination index (%)
0.5	92.9±0.26	98.1±0.22	91.1±0.57
1.0	88.9±0.16	95.4±0.29	84.8±0.42

the tested concentrations. In fact, secondary roots were even observed in onion seeds treated with 1% biosurfactant, suggesting a positive impact on seed germination and development. However, these results should be interpreted cautiously, since the assay was conducted on only a single plant species, within a limited concentration range, and without dose–response analysis or reference phytotoxic controls. Therefore, the findings represent a preliminary assessment of environmental safety under the tested conditions; further ecotoxicological studies involving multiple plant species, soil systems, and wider concentration ranges are required before confirming agricultural applicability.

Conclusions

This study successfully demonstrated the valorization of tilapia processing waste as a sustainable nutrient source for biosurfactant production by *Penicillium rubens* UCP 1213. Integrating TVB and PFSO achieved a significant reduction in the surface tension (35.68 mN/m), producing an anionic biomolecule with predominantly lipopeptide nature, and indicated non-toxicity to onion seeds. Beyond the biotechnological efficiency, these findings offer a practical pathway for the aquaculture industry to transition toward a circular economy by transforming hazardous organic residues into high-value, eco-friendly solutions. By replacing synthetic surfactants with biodegradable fungal alternatives, this approach directly contributes to sustainable waste management and mitigates environmental impacts across the fish-processing chain, aligning with global demands for green chemistry and sustainable development.

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Authors' Contributions

Rodríguez, D. M.: conceptualization; data curation; formal analysis; investigation; methodology; software; writing – original draft; writing – review & editing. **Souza**, A.F.: formal analysis; investigation; methodology; writing — original draft; visualization. **Mendonça**, R. S.: methodology; software; writing — original draft. **Andrade**, R.F.S.: investigation; methodology; visualization. **Fernandes**, J.G.: conceptualization; supervision; validation; visualization. **Campos-Takaki**, G. M.: conceptualization; funding acquisition; project administration; resources; supervision; validation; visualization; writing – review & editing.

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