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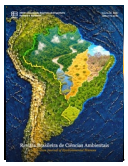

Biosurfactant from *Candida guilliermondii* UCP 1592 for cleaning and degreasing applications

Biossurfactante de *Candida guilliermondii* UCP 1592 para aplicações de limpeza e desengorduramento

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ABSTRACT

Biosurfactants are surface-active molecules produced by microorganisms, whose amphiphilic structure enables them to reduce surface tension and stabilize emulsions. This study evaluated the production, characterization, stability, toxicity, and cleaning potential of a biosurfactant synthesized by *Candida guilliermondii* UCP 1592. The compound was produced in a medium containing distilled water, 2.5% corn steep liquor, and 5% residual frying oil, under agitation for 144 h. The biosurfactant reduced the surface tension of water from 72 to 30 mN/m and achieved a production yield of 18 g/L. It effectively emulsified 100% of burnt motor oil, forming a stable emulsion. Toxicity tests using *Tenebrio molitor* and *Artemia salina* revealed no toxic effects, with 100% survival. In cleaning tests, the biosurfactant removed 100% of oil from glass surfaces, even at concentrations below its critical micelle concentration (CMC). For oil removal from cotton fabrics, it reached efficiencies of 91, 70, and 57% at 2 CMC (0.6 g/L), 1 CMC (0.3 g/L), and 0.5 CMC (0.15 g/L), respectively. These results demonstrate the biosurfactant's high effectiveness, safety, and environmental compatibility, highlighting its potential as a sustainable alternative to synthetic surfactants for cleaning and oil removal applications.

Keywords: microbial surfactant; yeasts; surface tension; oil dispersion; emulsification.

RESUMO

Biossurfactantes são moléculas com atividade superficial produzidas por microrganismos, cuja estrutura anfifílica lhes permite reduzir a tensão superficial e estabilizar emulsões. Este estudo avaliou a produção, caracterização, estabilidade, toxicidade e potencial de limpeza de um biossurfactante sintetizado por *Candida guilliermondii* UCP 1592. O composto foi produzido em meio contendo água destilada, 2,5% de milhocina e 5% de óleo residual de fritura, sob agitação por 144 horas. O biossurfactante reduziu a tensão superficial da água de 72 para 30 mN/m e apresentou rendimento de 18 g/L. Emulsificou eficientemente 100% do óleo de motor queimado, formando uma emulsão estável. Testes de toxicidade com *Tenebrio molitor* e *Artemia salina* não revelaram efeitos tóxicos, com 100% de sobrevivência observada. Nos testes de limpeza, o biossurfactante removeu 100% do óleo de superfícies de vidro, mesmo em concentrações abaixo de sua Concentração Micelar Crítica (CMC). Na remoção de óleo de tecidos de algodão, alcançou eficiências de 91, 70 e 57% nas concentrações de 2 CMC (0,6 g/L), 1 CMC (0,3 g/L) e 0.5 CMC (0,15 g/L), respectivamente. Esses resultados demonstram a eficácia, segurança e compatibilidade ambiental do biossurfactante, destacando seu potencial como alternativa sustentável aos surfactantes sintéticos em aplicações de limpeza e remoção de óleo.

Palavras-chave: surfactante microbiano; leveduras; tensão superficial; dispersão de óleo; emulsificação.

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Introduction

Biosurfactants are amphiphilic biomolecules that act as surface-active agents, enhancing interactions between interfaces through micelle formation. These molecules are synthesized and secreted by various microorganisms, including filamentous fungi, bacteria, and yeasts, and have broad applications across multiple industries, such as washing detergents, oil spill cleanup operations, soil remediation and flushing, treatment of wastewater, heavy metal remediation, preparation of fibers, dyeing and printing, and finishing of textiles (Sarubbo et al., 2022; Chabhadiya et al., 2024).

These surface-active compounds are classified by their chemical composition and molecular weight. They typically consist of a hydrophilic portion (e.g., amino acids or sugars) and a hydrophobic fatty acid chain. Low-molecular-weight biosurfactants (500–1500 Da), like glycolipids and lipopeptides, are effective in reducing surface tension, while high-molecular-weight types, such as lipoproteins and polysaccharides, act mainly as bioemulsifiers (Drakontis and Amin, 2020; Sharma et al., 2021; Balakrishnan et al., 2023).

These biomolecules have attracted growing interest from both scientific and industrial communities due to their promising and distinctive properties. Among their key features are the ability to reduce surface and interfacial tension, as well as biodegradability, bioavailability, structural diversity, low toxicity, and environmental compatibility, supporting their wide-ranging applicability across different sectors (Heldin and Hossam, 2023).

These natural compounds find applications in sectors such as agriculture, chemicals, cosmetics, pharmaceuticals, food, cleaning, leather, paper, and textiles (Ribeiro et al., 2020; Abbot et al., 2022; Hernández et al., 2023; Datta et al., 2024; Markam et al., 2024; Sani et al., 2024). Additionally, they hold great potential in bioremediation, particularly in remediating hydrocarbon-contaminated areas (Silva et al., 2024a). In the cleaning sector, biosurfactants are widely used in laundry detergents and household products due to their emulsifying, dispersing, foaming, and surface tension-reducing properties (Farias et al., 2021b). Moreover, they have shown significant potential in hydrocarbon bioremediation and exhibit antimicrobial and anti-biofilm activities, making them effective in various washing processes (Lourenço et al., 2024).

The growing demand for sustainable and natural alternatives has driven significant advancements in biosurfactant development, aiming to enhance market competitiveness (Thakur et al., 2024). The global biosurfactant market is projected to grow at an annual rate of 11.0%, reaching USD 2.3 billion by 2028, up from USD 1.3 billion in 2023, with the personal care and cosmetics industries as primary drivers. Europe currently leads biosurfactant research and production, followed by North America and Asia (Markets and Markets, 2024).

The increasing interest in the use of biosurfactants reflects their recognized potential as environmentally responsible alternatives in various industrial applications, particularly in replacing synthetic surfactants. This shift aligns with the United Nations Sustainable Development Goals and underscores global efforts to mitigate environmental impacts while promoting a balance between economic growth and ecological preservation (Sarubbo et al., 2022). Their use reduces water pollution and toxicity risks, contributing to greener industrial practices and the development of a sustainable economy (Qamar and Pacifico, 2023; Yumnan et al., 2024).

Recent research has highlighted the potential of biosurfactants produced by yeasts in cleaning environments contaminated by petroleum derivatives and as detergents in the textile industry (Silva et al., 2024b; Oliveira et al., 2025). Species such as *Candida albicans* and *Yarrowia lipolytica* have been widely studied for their ability to produce compounds like sophorolipids, which stand out due to their emulsifying properties, low toxicity, and biodegradability, making them promising for various industrial applications (Gaur et al., 2019; Aslam et al., 2024; Barros et al., 2024).

In this context, the present study aimed to produce, characterize, and evaluate the physicochemical properties, environmental stability, toxicity, and cleaning potential of the biosurfactant produced by *Candida guilliermondii* UCP 1592, with a focus on its application for surface cleaning and oil removal.

Materials and Methods

Microorganism and inoculum preparation

The yeast *C. guilliermondii* UCP 1592, stored in the Culture Bank of the Catholic University of Pernambuco (Brazil), was used as a biosurfactant producer. Cultures were maintained at 5°C in slanted tubes containing Yeast Mold Agar (YMA), composed of yeast extract (3 g/L), D-glucose (10 g/L), tryptone (5 g/L), and agar (20 g/L) dissolved in distilled water. The inoculum was standardized by transferring the culture to YMA medium to obtain a young culture. It was then cultivated in flasks with Yeast Mold Broth, sterilized in an autoclave (121°C for 20 min), and incubated under agitation (150 rpm, 28°C, 24 h). The cell density was adjusted to 10^6 cells/mL using a Neubauer chamber and added to the production medium at 5% (v/v).

Production of biosurfactant

The fermentations for biosurfactant production were carried out in 2 L Erlenmeyer flasks containing 1 L of medium formulated with distilled water supplemented with 2.5% corn steep liquor (a bioproduct of corn production, kindly provided by Corn Products S.A. do Brasil, located in Cabo de Santo Agostinho, Pernambuco, Brazil) and 5.0% residual frying oil (acquired from restaurants in the city of Recife, Pernambuco, Brazil). After adjusting the medium pH to 5.5, the production medium was sterilized in an autoclave at 121°C for 20 min, followed by inoculation with a cell suspension of 10^6 cells/mL and incubation at 28°C under orbital shaking at 200 rpm for 144 h (Silva et al., 2023).

Growth curve

Aliquots were collected at intervals of 2, 4, 6, 8, 12, 24, 30, 36, 48, 60, 72, 96, 120, and 144 h during fermentation. These samples were centrifuged (4,500 rpm, 9°C for 15 min, Megafuge 16R centrifuge, Thermo Fisher Scientific, Waltham, MA, USA) and filtered using Whatman No. 1 filters to obtain cell-free broths, which were used to measure surface tension and pH. To determine biomass by dry weight, 100 mL of culture was centrifuged at 4,500 rpm for 15 min, and the supernatant was discarded. After centrifugation, the biomass was dried in an oven at 105°C for 24 h and then weighed. To determine production yield, 100 mL of the sample was subjected to extraction using the metabolic liquid containing cells, with the organic solvent ethyl acetate (Lima et al., 2024).

Isolation of biosurfactant

The extraction of the biosurfactant present in the medium was carried out using the organic solvent ethyl acetate. The metabolic liquid, without prior centrifugation, was mixed with the solvent at a 1:4 (v/v) ratio. This process was repeated twice. Then, the solvent was centrifuged at 4,500 rpm for 15 min. The resulting organic phase was transferred to a separatory funnel, discarding the possible aqueous phase. This separation was facilitated by adding a saturated sodium chloride (NaCl) solution. To remove any water residues, sodium sulfate was used, and the material was subsequently filtered. Finally, the organic phase was heated on a hot plate to evaporate the solvent, resulting in the isolated biosurfactant (Silva et al., 2023).

Characterization of biosurfactant

To elucidate the chemical composition of the biosurfactant, Fourier-transform infrared spectroscopy (FTIR, KBr) and nuclear magnetic resonance (NMR) were employed. The purified biosurfactant was dissolved in deuterated chloroform ($CDCl_3$) and analyzed in a 500/300 MHz spectrometer (Agilent, Santa Clara, CA, USA) operating at 300.13 MHz to determine chemical shifts (δ) in the 1H and 13C NMR spectra on a ppm scale relative to tetramethylsilane. The infrared spectroscopy analysis was performed using an FT-IR spectrometer (Spectrum 400, Perkin Elmer, Shelton, CT, USA) in the spectral range of 4,000 to 400 cm^{-1}.

Determination of surface tension and critical micelle concentration

The surface tension of the biosurfactant was measured in the cell-free metabolic liquid after centrifugation. At room temperature, the du Noüy ring method was employed using a Sigma 700 Tensiometer (KSV Instruments Ltd., Helsinki, Finland). To determine the critical micelle concentration (CMC), 0.1 g of the isolated biosurfactant was weighed and diluted to an initial concentration of 5 g/L. From this solution, successive dilutions were performed using distilled water. The surface tensions of the dilutions were then quantified using the du Noüy ring method (Silva et al., 2023).

Determination of emulsification activity

The emulsifying activity of the formulated biosurfactant was determined using the method described by Cooper and Goldenberg (1987). A total of 2 mL of a hydrocarbon was added to 2 mL of the cell-free broth in a test tube, which was vortexed for 2 min. After 24 h, the stability of the emulsion was assessed. Emulsion height was divided by the total height of the mixture, and the result was multiplied by 100 for determination of the emulsification index.

Motor oil dispersion test in water

The crude and isolated biosurfactants (at 0.5, 1, and 2 CMC) were evaluated for their dispersion capacity. An oil spill was simulated in the laboratory by contaminating water samples with used motor oil, following the methodology of Morikawa et al. (2000). The diameter of the clear zone on the oil surface was measured in the oil displacement area. Distilled water was used as a negative control, and the chemical surfactant sodium dodecyl sulfate (SDS) was used as a positive control.

Assessment of stability of biosurfactant (effects of pH, NaCl, and temperature)

The stability of the biosurfactant was evaluated based on its surface tension and emulsifying capacity under three different conditions: pH, temperature, and salinity. For this, the effects of different temperatures (0, 5, 28, 70, 100, and 120°C), NaCl concentrations (2.0–10.0%), and pH levels (2–12) were tested separately on the biosurfactant. The pH was adjusted using HCl or NaOH as appropriate. The biosurfactant was then analyzed for surface tension and emulsification activity (Silva et al., 2024c).

Toxicity of biosurfactant to *Tenebrio molitor*

Tenebrio molitor larvae, each weighing approximately 100 mg, were randomly distributed into groups of five individuals. A volume of 10 µL of biosurfactant was injected into the ventral membrane between the second and third abdominal segments (from tail to head) at concentrations of 0.5 CMC, 1 CMC, and 2 CMC. Larval viability was assessed over 24, 48, 72, 96, and 120 h by evaluating the absence of movement after mechanical stimulation. Larvae inoculated with phosphate-buffered saline (PBS) served as negative controls. The results were plotted as survival curves over time (Silva et al., 2020).

Toxicity of biosurfactant to *Artemia salina*

Toxicity to microcrustaceans was tested using *Artemia salina*, a commonly used marine bioindicator. For the test, 1 L of saline solution was prepared by dissolving 33.3 g of sea salt. After incubation, 10 microcrustaceans were placed in biosurfactant solutions at concentrations of 0.5 CMC, 1 CMC, and 2 CMC in distilled water (Meyer et al., 1982).

Application of the biosurfactant as a cleaning and degreasing agent

Cleaning of glass surface contaminated with oil

The biosurfactant obtained from *C. guilliermondii* UCP 1592 was used for cleaning and degreasing used motor oil present on a smooth surface (glass slide), following the methodology of Farias et al. (2021b). Part of the surface of a glass slide with a known mass was uniformly contaminated with 100 µL of heavy oil. The slides were submerged in solutions containing 0.5 CMC, 1 CMC, and 2 CMC for 3 min. The negative control was performed with distilled water. The removal rate was calculated as follows (Equation 1):

$$I = 100 \times ([Wc - Ww])/([Wc - Wi]) \quad (1)$$

Where:
Wc: the weight of the contaminated test sample, ;
Ww: the weight of the test sample after washing;
Wi: the initial weight of the test sample.

Cleaning and degreasing of oil on cotton fabric

The biosurfactant obtained from *C. guilliermondii* UCP 1592 was used for cleaning and degreasing used motor oil impregnated in cotton fabric using a methodology adapted from Andrade et al. (2018). Clean white cotton fabrics, cut into 2×2 cm pieces, were impregnated with a drop of used motor oil, and after the fabric absorbed it, the sample was immersed in aqueous solutions of biosurfactant at concentrations of 0.5, 1, and 2 CMC. The fabric was washed at an agitation speed of 150 rpm for periods of 1, 2, 4, 6, 8, and 12 h in an orbital shaker. The positive control was SDS, and the negative control was distilled water. The fabric was rinsed with 100 mL of distilled water for 1 h of agitation, followed by natural drying. The structure of the cotton fabric fibers before and after cleaning and degreasing the oil was examined by optical microscopy. The percentage of used motor oil removed from the cotton fabric by the action of the biosurfactant and other tested conditions was determined according to the Equation 2:

$$W = (mtotal - mi/mtotal) \times 100 \quad (2)$$

Where:
mtotal: total mass of oil applied;
mi: residual oil in the fabric after treatment.

Statistical analysis

The data obtained were statistically analyzed using the software Statistica (version 7.0). Analysis of variance (ANOVA) was applied to verify significant differences between groups, followed by Tukey's test. All experiments were conducted in triplicate, and results are presented as mean±standard deviation (n=3). A 95% confidence interval was considered, adopting a significance level of 5% ($p<0.05$).

Results and Discussion

Growth curve and biosurfactant production

Biosurfactant production remains limited by high costs, primarily due to expensive culture media. To improve economic viability, the use of agro-industrial residues as alternative substrates and the optimization of fermentation parameters—such as time, temperature, and substrate type—are crucial strategies for enhancing yield and sustainability (Begum et al., 2023; Ambechada and Umrania, 2024).

In this study, *C. guilliermondii* UCP 1592 showed significant growth and biosurfactant production in a medium based on corn steep liquor and residual frying oil, with a reduction in surface tension from 72 to 30 mN/m after 144 h. Biomass production started after 2 h of fermentation, reaching its peak at 144 h with a maximum biosurfactant production of 18 g/L. Moreover, the pH remained stable throughout the production, ranging from 5.1 to 5.9 during the 30 and 144-h cultivation times, respectively (Figure 1).

The production kinetics observed in this study were consistent with previous reports involving *Candida* species. For example, *Candida lipolytica* produced 12 g/L of biosurfactant and reduced surface tension to 28 mN/m after 6 days in a medium with residual soybean oil, corn steep liquor, and molasses (Lima et al., 2024). Similarly, *Candida bombicola* yielded 12.5 g/L under flask conditions, with a 17 times increase upon scale-up to a 50 L bioreactor (Pinto et al., 2022).

The results, though broadly comparable, show variations in biosurfactant yield due to methodological differences. In this study, the combination of residual frying oil and corn steep liquor provided a richer nutrient matrix, enhancing the metabolic activity and biosurfactant synthesis by *C. guilliermondii*. Residual frying oil, in particular, offers high lipid availability, which is crucial for glycolipid biosurfactant production. The *C. guilliermondii* UCP 1592 strain may also possess specific metabolic advantages, such as acid pH tolerance and efficient lipid assimilation, contributing to the higher yield. In contrast, the lower yields reported by Pinto et al. (2022) and Lima et al. (2024) could be related to differences in strain performance, substrate composition, or process conditions like agitation and oxygen transfer. These findings emphasize that both strain selection and precise control of fermentation parameters are critical for biosurfactant productivity. Moreover, the stable pH observed in this study may have supported microbial metabolism by preventing the inhibition of biosynthetic pathways.

Surface tension and critical micelle concentration

The biosurfactant produced from *C. guilliermondii* UCP 1592 exhibited a significant ability to reduce the surface tension of water from 72 to 30 mN/m. This biosurfactant achieved a yield of 18 g/L, demonstrating its productive potential. The analysis of surface tension behavior as a function of biosurfactant concentration showed that the CMC was reached at 0.3 g/L, resulting in a surface tension of 25.83 mN/m (Figure 2).

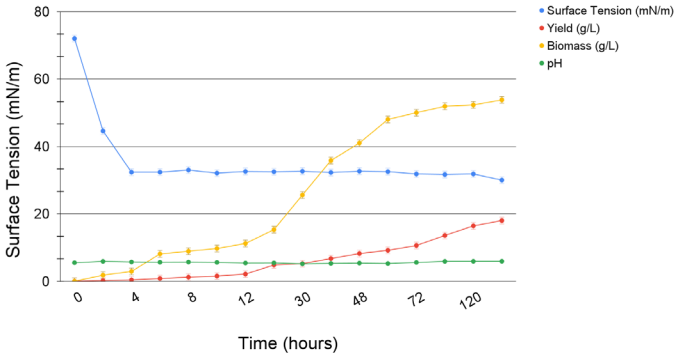

Figure 1 – Growth curve, pH, and biosurfactant production of C. *guilliermondii* UCP 1592 grown in distilled water supplemented with 2.5% corn steep liquor and 5% residual frying oil, at 28°C, 200 rpm, for 144 h.

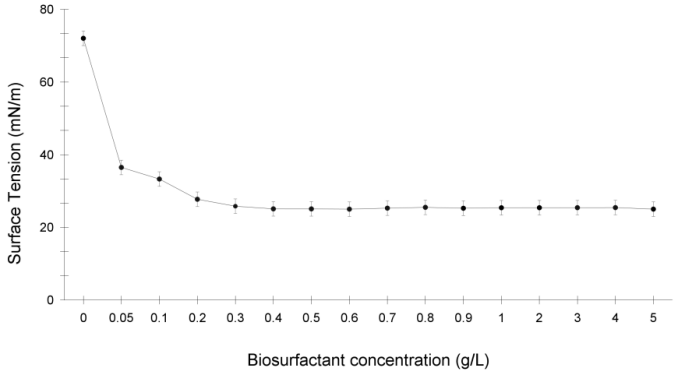

Figure 2 – Determination of the Critical Micelle Concentration of the biosurfactant produced by *Candida guilliermondii* UCP 1592 grown in a medium supplemented with 2.5% corn steep liquor and 5% residual frying oil, for 144 h at 200 rpm, with 5% inoculum at 28°C.

Studies by Souza et al. (2023) reported a surface tension of 32.18 mN/m and a CMC of 0.72 g/L when using *C. guilliermondii* in a medium supplemented with crude cottonseed oil and sucrose. Lima et al. (2024) observed a CMC of 0.5 g/L and a surface tension of 25 mN/m in biosurfactant produced by *C. lipolytica*. Using *C. bombicola*, Silva et al. (2023) achieved a surface tension reduction to 29 mN/m, with a CMC of 0.5%.

Additional studies demonstrate the versatility of biosurfactants produced by various *Candida* species under different cultivation conditions, reinforcing the potential of these microorganisms in producing biosurfactants with significant properties. For instance, studies by Rocha Junior et al. (2018) indicated that *Candida tropicalis* UCP0996 in a medium supplemented with canola oil and glycerol resulted in a CMC of 0.5% and a surface tension of 28 mN/m. Later, using the same strain, Almeida et al. (2021) reported a CMC of 0.06% and a reduction in surface tension from 70.0 to 25.6 mN/m for the biosurfactant produced in a medium formulated with corn steep liquor, molasses, and residual frying oil.

The results highlight the crucial role of the culture medium in surface tension reduction efficiency. The consistent achievement of low CMCs and reduced surface tension in various *Candida* species confirms the potential of these biosurfactants for industrial applications requiring high efficiency at low concentrations. The CMC is a key parameter, representing the minimum biosurfactant amount needed for maximum surface tension reduction and micelle formation, allowing performance comparison among surfactants (Johnson et al., 2021; Turchi et al., 2022).

Emulsification index

The biosurfactant produced by *C. guilliermondii* UCP 1592 demonstrated an emulsification capacity of 100±0.0% burnt motor oil after 24 h, indicating maximum and consistent emulsifying activity across all replicates. This result is comparable to the study by Silva et al. (2024c), which obtained the same result with the biosurfactant produced by *Candida glabrata* UCP 1002, and to Selva Filho et al. (2024), who achieved 95.43% emulsification for exhaust motor oil with the biosurfactant produced by the yeast *Starmerella bombicola* ATCC 222214, respectively.

Motor oil dispersion test in water

The dispersant potential of the biosurfactant produced by *C. guilliermondii* UCP 1592 after 144 h of fermentation was evaluated. Figure 3 presents the dispersion of burnt motor oil using the crude biosurfactant (cell-free broth) at concentrations of 0.5 CMC, 1 CMC, and 2 CMC, alongside its synthetic counterpart, SDS. The oil dispersion activity of the biosurfactant was quantitatively assessed by measuring the diameter of the dispersion halos formed on an oil-covered surface. The results showed that the biosurfactant at 2 CMC exhibited the largest halo (12.0 cm), indicating a strong surface activity. The diameters for the other treatments were as follows: cell-free broth—6.2 cm; 1 CMC—6.0 cm; 0.5 CMC—4.0 cm; and SDS—1.8 cm. Statistical analysis confirmed that the halo diameter at 2 CMC was significantly larger than all other treatments ($p<0.05$), including SDS, demonstrating the superior dispersant potential of the biosurfactant. These quantitative results support the visual evidence and highlight the dose-dependent behavior of the biosurfactant.

Stability of biosurfactant

The cell-free broth produced by *C. guilliermondii* UCP 1592 after 144 h of cultivation was analyzed under various environmental conditions, simulating variations in pH, temperature, and salinity. The parameters used for evaluation included emulsification capacity and surface tension, aiming to identify the performance and stability of the biosurfactant under different conditions.

According to ANOVA followed by Tukey's test ($p<0.05$), pH had a statistically significant effect on both surface tension and emulsification capacity. At acidic pH (2–4), surface tension values were significantly higher (>30 mN/m), whereas under neutral and alkaline conditions (pH 6–12), values remained lower (<30 mN/m), indicating greater surface activity in these environments. Emulsification was also highest at acidic pH, with significantly elevated E24 values ranging from 82 to 94% (Table 1).

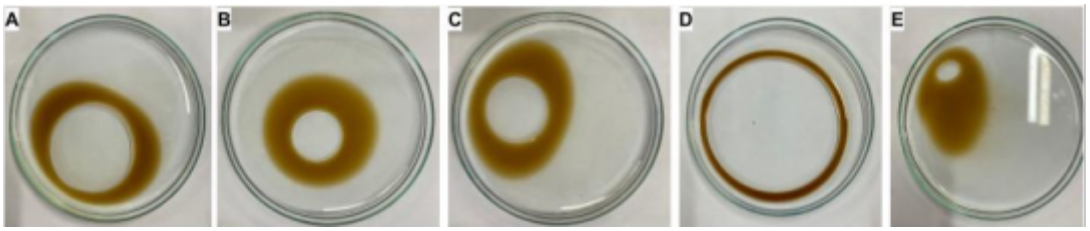

Figure 3 – Dispersion of burnt motor oil by the biosurfactant produced by *Candida guilliermondii* UCP 1592 and by SDS: (A) cell-free broth; (B) 0.5 CMC; (C) 1 CMC; (D) 2 CMC; (E) SDS.

Table 1 – Surface tension and stability of burnt motor oil emulsification of the biosurfactant produced by *Candida guilliermondii* UCP 1592 cultivated in distilled water supplemented with steep corn liquor (2.5%) and residual frying oil (5%) with variations in NaCl concentration, temperature, and pH.

pH	Surface tension (mN/m)	Emulsification (%)	Temperature (°C)	Surface tension (mN/m)	Emulsification (%)	NaCl (%)	Surface tension (mN/m)	Emulsification (%)
2	32±1.41^{b}	89±1.4^{e}	0	33±1.5	70±1.6^{a}	2	28±1.8	60±2.1^{a}
4	32±1.9^{b}	94±1.7^{f}	5	33±1.7	80±1.1^{c}	4	28±1.4	52±1.4^{b}
6	30±1.6^{b}	82±1.3^{d}	28	33±1.2	80±1.5^{c}	6	28±1.5	52±1.8^{b}
8	26±1.31^{a}	70±1.4^{c}	70	33±1.6	82±1.3^{d}	8	28±1.6	51±1.5^{bc}
10	28±1.5^{ab}	70±1.2^{b}	100	33±1.8	84±1.5^{e}	10	28±1.2	50±1.9^{c}
12	28±1.2^{ab}	61±1.5^{a}	120	33±1.4	79±1.4^{b}	12	28±1.6	51±1.2^{bc}

The data represent mean±standard deviation (n=3). Different letters within the same column indicate statistically significant differences between levels of each factor, according to Tukey's test ($p<0.05$), following one-way ANOVA.

Temperature variations (0–120 °C) did not significantly affect surface tension ($p>0.05$), with values remaining stable at approximately 33 mN/m across all temperatures. However, emulsification capacity was significantly influenced by temperature, with the highest E24 values (84%) observed at 100°C and the lowest (70%) at 0°C, according to Tukey's test (Table 1).

For salinity, the addition of NaCl reduced surface tension to 28 mN/m, with no statistically significant differences observed among the tested concentrations (2–12% NaCl). In contrast, emulsification capacity was significantly affected ($p<0.05$), with decreasing E24 values as salt concentration increased, ranging from 60% at 2% NaCl to 50% at 10% NaCl (Table 1).

These results demonstrate that the emulsifying capacity of the biosurfactant is significantly influenced by environmental factors such as pH, temperature, and salinity, while surface tension is mainly affected by variations in pH, with temperature and salinity showing no statistically significant impact. These findings have important implications for its performance under different environmental conditions, suggesting that, for practical applications, controlling and adjusting specific environmental variables—particularly pH—may be strategic tools to maximize the performance and stability of the biosurfactant in diverse contexts (Lima et al., 2024; Silva et al., 2024c).

Structural characterization of biosurfactant

The biosurfactant produced by the yeast *C. guilliermondii* UCP 1592 was structurally characterized using NMR and FTIR Spectroscopy to predict its possible chemical nature.

The ^{1}H NMR spectrum suggested the presence of hydrogens in alkyl groups attached to saturated carbons around the 1–2 ppm peaks. The presence of multiple peaks or coupled signals suggests the presence of functional groups close to each other, typical of hydrocarbon chains interacting with one another (Figure 4A). The ^{13}C NMR spectrum revealed significant peaks between 10 and 70 ppm (indicating carbons in alkyl groups), around 120–150 ppm (indicating carbons in aromatic rings or unsaturated carbons in double bonds), and a strong peak at approximately 180 ppm, which is indicative of the presence of carbonyl carbons (C=O), suggesting the presence of functional groups such as esters, carboxylic acids, or ketones (Figure 4B).

The FTIR spectroscopy (Figure 5) revealed that the peaks at 2,924 and 2,855 cm^{-1} are attributed to C-H stretching vibrations in CH2 and CH3 groups, which are common in long alkyl chains. A peak corresponding to the carbonyl functional group (C=O) was found at 1,710.96 cm^{-1}. Peaks at 1,411 and 722 cm^{-1} may indicate bending deformations in aliphatic chains. This suggests that the biosurfactant is an amphiphilic compound with a polar head (ester group) and an apolar tail (aliphatic chain).

The analysis of the spectra suggests that the biosurfactant studied has a typical structure of amphiphilic compounds, with a hydrophobic tail consisting of a long aliphatic chain and a polar head, likely containing an ester group. This structural organization is responsible for its ability to reduce surface tension, an essential characteristic of biosurfactants. Therefore, it can be concluded that the biosurfactant is possibly a fatty acid metabolized by the microorganism, as evidenced by the presence of aliphatic chains, carbonyl groups, and characteristic double

bonds. These findings are consistent with other studies in the literature, which have reported similar amphiphilic profiles and structural characteristics for biosurfactants produced by *Candida* species and related yeasts (Almeida et al., 2021; Lima et al., 2024; Silva et al., 2024a).

Evaluation of biosurfactant toxicity

Toxicity to Tenebrio molitor

The *Tenebrio molitor* larvae, known as yellow mealworm larvae, have been used as a model to evaluate the toxicity of biosurfactants due to their sensitivity to environmental changes and biochemical stimulus (Lima et al., 2024; Silva et al., 2024c). This study analyzed the toxicity of the biosurfactant produced by *C. guilliermondii* UCP 1592 and achieved a survival rate of 100±0.0% after 120 h of testing, the same result obtained with the negative control (PBS) (Figure 6). No physical changes were identified in the larvae, such as color alterations, which is an indicator frequently associated with stress processes or toxic exposure (Figure 7). These results suggest that the tested biosurfactant did not present toxic effects on *T. molitor* larvae. This outcome is similar to those reported by Lima et al. (2024) and Silva et al. (2024c), who also assessed the toxicity of biosurfactants produced by *C. lipolytica* and *C. glabrata*, respectively.

Toxicity to Artemia salina

Artemia salina is a microcrustacean widely recognized as a bioindicator in toxicity assays due to its easy handling under laboratory conditions, low operational cost, and short life cycle—characteristics that make it efficient and reliable for toxicological studies (Meyer et al., 1982).

In the present experiment, *A. salina* larvae were exposed to solutions containing different concentrations of the biosurfactant, corresponding to 0.5 CMC (0.15 g/L), 1 CMC (0.3 g/L), and 2 CMC (0.6 g/L), for a period of 24 h. The results showed a 100±0.0% survival rate at all tested concentrations, demonstrating the complete absence of toxicity of the compound under the evaluated conditions.

Application of biosurfactant in cleaning surfaces contaminated with burnt motor oil

This study investigated the potential of the biosurfactant produced by *C. guilliermondii* UCP 1592 as a degreasing and cleaning agent on a surface made of glass, as well as on cotton fabrics impregnated with burnt motor oil.

The glass surface cleaning test, conducted with concentrations of 0.5, 1, and 2 CMC, demonstrated the high efficiency of the biosurfactant produced by *C. guilliermondii* UCP 1592, which was able to solubilize 100±0.0% of the motor oil at all tested concentrations. This result highlights its effectiveness even at concentrations below the CMC, reinforcing its potential for practical applications. Comparatively, Barata et al. (2024) reported a 98.42% oil removal rate on glass surfaces using a biosurfactant from *Bacillus invictae* UCP 1617, while Farias et al. (2021a) achieved 100% removal with a biosurfactant from *Pseudomonas* spp. cultivated in a mineral medium containing 5.0% glycerol and 2.0% glucose. These results indicate that the biosurfactant evaluated in the present study performs at a level comparable to the best results previously described in the literature.

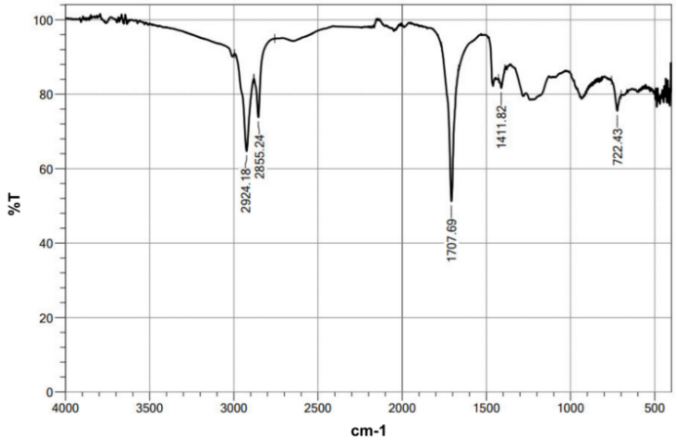

Figure 5 – FTIR spectrum of the purified biosurfactant from *Candida guilliermondii* UCP 1592.

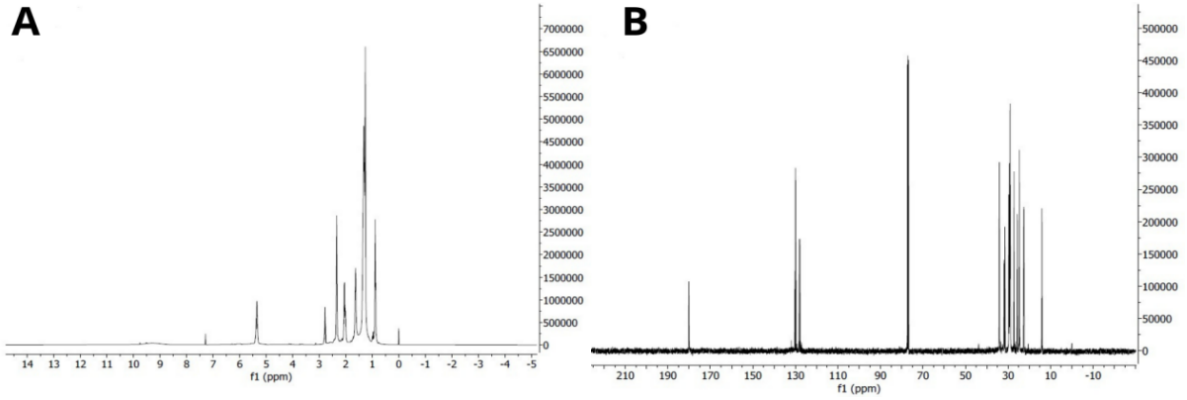

Figure 4 – (A) ^{1}H NMR and (B) ^{13}C NMR spectra of the purified biosurfactant from *Candida guilliermondii* UCP 1592.

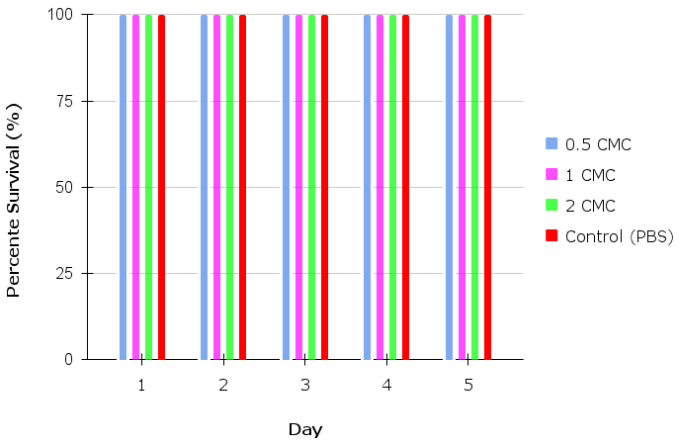

Figure 6 – Survival of *Tenebrio molitor* larvae after 120 h of exposure to biosurfactant at concentrations of 0.5, 1, and 2 CMC. Phosphate-buffered saline (PBS) was used as the negative control.

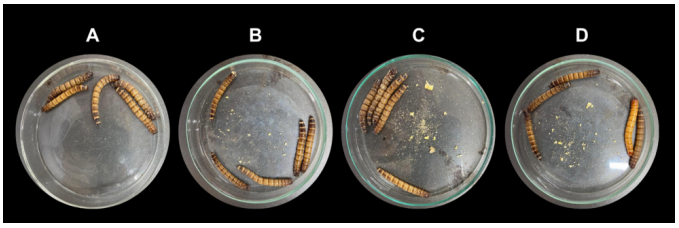

Figure 7 – Representative images of *Tenebrio molitor* larvae following exposure to phosphate-buffered saline (PBS) and varying concentrations of the biosurfactant in the toxicity assay. (A) Negative control (PBS); (B) 0.5 CMC; (C) 1 CMC; (D) 2 CMC.

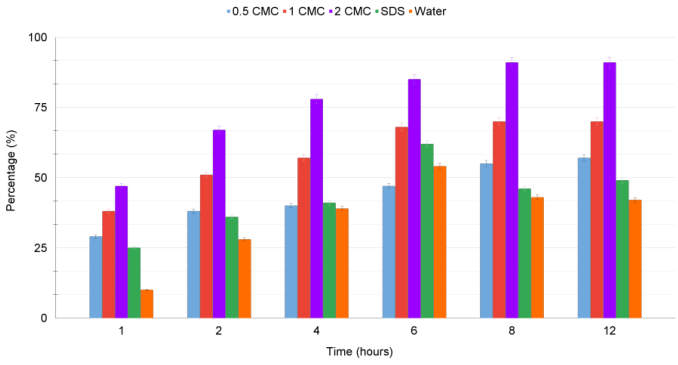

Figure 8 – Percentage of burnt motor oil removal from cotton fabrics using biosurfactant produced by *Candida guilliermondii* UCP 1592.

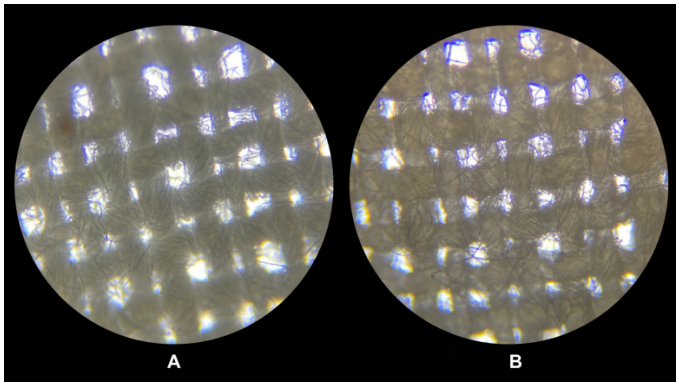

Figure 9 – Evaluation of the structural integrity of cotton fabrics: (A) clean fabric without oil; (B) fabric after washing

The results of washing cotton fabrics contaminated with burnt motor oil using the produced biosurfactant are presented in Figure 8. Initial findings showed oil removal within the first hour of cleaning, with removal percentages of 29%, 38%, and 47% for concentrations of 0.5 CMC (0.15 g/L), 1 CMC (0.3 g/L), and 2 CMC (0.6 g/L), respectively, reaching 57%, 70%, and 91% for the same concentrations after 12 h.

The test demonstrated the high potential of the biosurfactant as an agent for removing oil impregnated in cotton fabrics, with the most significant performance observed at the 2 CMC concentration. This concentration resulted in oil removal rates of 67%, 78%, and 85% at 2, 4, and 6 h, respectively. The removal behavior remained consistent throughout the experiment, reaching 91% removal after 12 h. The 1 CMC concentration also showed promising results, with over 50% removal after just 2 h of washing and reaching 70% by the end of the experiment.

While SDS initially exhibited emulsifying performance comparable to the biosurfactant at 1 CMC, its efficiency declined significantly after 6 h, dropping below 50% by the end of the test. This reduction may be attributed to the instability of SDS micelles under prolonged exposure, potentially due to environmental factors such as ionic strength or pH fluctuations that can lead to micelle disintegration or reduced surface activity. In contrast, the biosurfactant maintained stable emulsification over time, likely due to its more complex amphiphilic structure and natural adaptability, which confer greater micelle stability and resilience. These differences highlight the superior performance and consistency of the biosurfactant, suggesting it may have a more robust mechanism of action and better environmental tolerance compared to the synthetic surfactant SDS.

Finally, water was the least efficient agent, achieving only around 25% removal at its peak performance (4 h) and showing a subsequent decline. This confirms that water alone has a low capacity to remove oil impregnated in fabrics. After the final rinsing step with water alone, all cotton fabrics were examined by optical microscopy. No visible physical alterations in fiber structure were observed, indicating that structural integrity was fully maintained (Figure 9).

In a previous study, Silva et al. (2024c) investigated the ability of the biosurfactant produced by *C. glabrata* in a medium supplemented with 2.5% residual frying oil, 2.5% molasses, and 2.5% corn steep liquor to act as a degreasing and cleaning agent for cotton fabrics impregnated with burnt motor oil. The results showed that the biosurfactant was able to remove more than 50% of the hydrophobic compound in all tested periods, reaching up to 96.6% removal after 12 h of washing at a concentration of 0.6 g/L (2 CMC). Similarly, Andrade et al. (2018)

reported an 86% removal efficiency using a biosurfactant produced by *Cunninghamella echinulata* after just 1 h of washing.

When comparing these findings with the results of the present study, the biosurfactant produced by *C. guilliermondii* UCP 1592 achieved up to 91% oil removal after 12 h at the same concentration (2 CMC), which is slightly lower than the value reported by Silva et al. (2024c). However, the present biosurfactant demonstrated consistent efficiency across time points, including 67% removal after just 2 h, 78% after 4 h, and 85% after 6 h, indicating a progressive and stable action. This behavior suggests a controlled and sustained degreasing effect, which may be advantageous for certain applications requiring prolonged contact times.

Moreover, unlike Andrade et al.'s (2018) results, which showed high removal within 1 hour, the present biosurfactant stands out for maintaining high performance over extended washing periods, which may reflect greater stability or stronger interaction with fabric-bound oil residues. These differences could be attributed to variations in biosurfactant structure, production substrates, or oil-fabric interactions, and highlight the need to evaluate not only removal rates but also performance dynamics over time. Thus, although the absolute removal values are similar, the biosurfactant from *C. guilliermondii* shows distinguishing features such as efficiency at lower concentrations, time-dependent performance stability, and potential cost-effectiveness, reinforcing its applicability for fabric cleaning processes in industrial and domestic settings.

Conclusions

The results obtained in this study demonstrated that the biosurfactant produced by *C. guilliermondii* UCP 1592 presents significant potential, particularly due to its production efficiency and surface-active properties. The compound was effective in reducing the surface tension of water and formed micelles at low concentrations, indicating high surfactant capacity. Moreover, its ability to emulsify used motor oil under the tested conditions suggests promising applicability in bioremediation and sustainable cleaning formulations. While these findings highlight specific practical uses, further studies under a broader range of environmental conditions and with diverse hydrophobic compounds are necessary to fully explore and validate the biosurfactant's versatility across industrial applications. Therefore, this biosurfactant emerges as a promising candidate for eco-friendly technologies, in alignment with sustainable and responsible industrial practices. Continued research will be essential to assess long-term stability and scalability.

Authors' Contributions

Silva, Y. A.: conceptualization, data curation, formal analysis, investigation, methodology, software, validation, visualization, writing – original draft, review & editing. **Ferreira**, H. S.: investigation, methodology, writing – original draft. **Albuquerque**, A. P. C.: methodology. **Borba**, G. H. A.: methodology. **Sarubbo**, L. A.: conceptualization, project administration, funding, resources, supervision, validation, writing – review. **Luna**, J. M. L.: conceptualization, formal analysis, funding, project administration, resources, supervision, validation, visualization, writing – review & editing.

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