

Article

Production and Optimization of Biosurfactant Properties Using *Candida mogii* and Licuri Oil (*Syagrus coronata*)

Peterson F. F. da Silva ¹, Renata R. da Silva ², Leonie A. Sarubbo ^{3,4} and Jenyffer M. C. Guerra ^{1,*}

¹ Centro de Tecnologia e Geociências, Programa de Pós-Graduação em Engenharia Química, Departamento de Engenharia Química, Universidade Federal de Pernambuco (UFPE), Avenida Professor Moraes Rego, n. 1235, Cidade Universitária, Recife 50670-901, PE, Brazil; peterson.fsilva@ufpe.br

² Rede Nordeste de Biotecnologia (RENORBIO), Universidade Federal Rural de Pernambuco (UFRPE), Rua Dom Manuel de Medeiros, s/n—Dois Irmãos, Recife 52171-900, PE, Brazil; renatabiology2015@gmail.com

³ Instituto Avançado de Tecnologia e Inovação (IATI), Rua Potyra, n. 31, Prado, Recife 50070-280, PE, Brazil; leonie.sarubbo@unicap.br

⁴ School of Technology and Communication, Catholic University of Pernambuco (UNICAP), Rua do Príncipe, n. 526, Boa Vista, Recife 50050-900, PE, Brazil

* Correspondence: jenyffer.campos@ufpe.br

Abstract: Optimizing biosurfactant (BS) production is key for sustainable industrial applications. This study investigated BS synthesis by *Candida mogii* using licuri oil, a renewable carbon source rich in medium-chain fatty acids. Process optimization was conducted via central composite design (CCD), adjusting concentrations of licuri oil, glucose, NH_4NO_3 , and yeast extract. The predictive model achieved an R^2 of 0.9451 and adjusted R^2 of 0.8812. Under optimized conditions, *C. mogii* lowered water surface tension from $71.04 \text{ mN}\cdot\text{m}^{-1}$ to $28.66 \text{ mN}\cdot\text{m}^{-1}$, with a critical micelle concentration (CMC) of $0.8 \text{ g}\cdot\text{L}^{-1}$. The biosurfactant displayed high emulsification indices, exceeding 70% for canola, licuri, and motor oils, suggesting strong potential as an industrial emulsifier. FTIR and NMR analyses confirmed its glycolipid structure. Bioassays showed no toxicity to *Lactuca sativa* seeds, ensuring environmental safety, while antimicrobial tests demonstrated efficacy against *Staphylococcus aureus* and *Escherichia coli*, indicating its suitability as a biocidal agent. This work positions *C. mogii* BS from licuri oil as a promising alternative for bioremediation, biotechnology, and antimicrobial uses.



Citation: da Silva, P.F.F.; da Silva, R.R.;

Sarubbo, L.A.; Guerra, J.M.C.

Production and Optimization of Biosurfactant Properties Using *Candida mogii* and Licuri Oil

(*Syagrus coronata*). *Foods* **2024**, *13*, 4029.

<https://doi.org/10.3390/foods13244029>

Academic Editor: Moktar Hamdi

Received: 6 November 2024

Revised: 4 December 2024

Accepted: 7 December 2024

Published: 13 December 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Surfactants play a central role in industry due to their amphiphilic properties, characterized by the presence of hydrophilic and hydrophobic segments within their molecules [1]. This amphiphilic structure is a key factor in reducing surface and interfacial tension between immiscible substances, making them highly applicable across various sectors [2,3]. Based on their origin, they are classified as natural or synthetic. Synthetic surfactants, derived from fossil fuels, raise concerns due to their potential adverse effects on health and the environment, associated with toxicity and low biodegradability [4]. In this context, biosurfactants (BS) emerge as a sustainable alternative to address these issues [5].

Defined as natural products obtained from microbial metabolism [6], biosurfactants have seen remarkable growth, with the market projected to reach USD 6.3 billion by 2026 [7]. These microbiologically derived compounds offer advantages such as biodegradability, low toxicity, selectivity, and production from renewable and low-cost raw materials [8].

Sourced from various organisms, including bacteria, fungi, and yeasts, biosurfactants exhibit a wide range of chemical structures and distinct properties [9]. In the current scenario, yeasts have been used for surfactant production due to their GRAS (Generally Recognized as Safe) status, enabling broad applications [10]. Within this group, the *Candida* genus has been extensively explored for its industrial significance, with BS produced by *C.*

tropicalis used in oil removal from contaminated areas [11], and those from *C. bombicola* and *C. utilis* used as food additives [12,13].

However, *Candida mogii*, a yeast with well-documented metabolic versatility, remains unexplored for biosurfactant production. Known for its efficient xylitol production from xylose and its adaptability to diverse fermentation conditions [14,15], it presents a compelling candidate for biosurfactant synthesis.

Currently, high production costs result in low economic competitiveness, due to the use of expensive substrates and inefficient product recovery methods. Optimizing the production medium composition is a promising approach, potentially reducing costs by up to 40% [16–18], leading to the maximization of desirable factors such as yield and surface tension reduction [19]. For instance, the application of Central Composite Design (CCD) reduced surface tension from 61 to 34 mN·m⁻¹ for BS produced by *Bacillus mycoides*, while the yield of the surfactant produced by *C. lipolytica* increased from 0.59 g·L⁻¹ to 7.27 g·L⁻¹ using a 2⁴ factorial design [20,21].

New natural carbon sources are often investigated to understand how microorganisms respond to them and thus improve yields [22]. Media containing vegetable oils present promising substrates [23]. Within this context lies licuri oil (*Syagrus coronata*), an edible oil traditionally consumed in Northeastern Brazil, which is primarily composed of medium-chain fatty acids, making it an excellent candidate for producing lipid-derived biomolecules. Beyond its nutritional value, licuri oil is a key product of local agroecosystems and rural livelihoods, with potential to generate added value through biotechnological applications [18]. Souza et al. [24] demonstrated that the oil in question was non-toxic, making it safe for pharmacological and food applications.

Considering the need to research new substrates and producing strains for the viability of biomolecule production, this study aimed to explore *Candida mogii* as a novel biosurfactant-producing strain by optimizing its production medium using licuri oil, a renewable and edible carbon source, while evaluating its phytotoxicity and antimicrobial activity.

2. Materials and Methods

2.1. Materials

The chemical reagents and culture media used in this study were of analytical grade. The crude oil, obtained via mechanical pressing of *Syagrus coronata* almonds, was provided by the Cooperativa de Produção da Região do Piemonte da Diamantina (COOPES), located in the city of Capim Grosso, Bahia, Brazil (11°22'51" S, 40°00'46" W).

2.2. Maintenance and Growth of the Microorganism

The yeast *Candida mogii* UFPEDA 3968 was obtained from the culture collection of the Mycology Department at the Federal University of Pernambuco. The microorganism was maintained at a temperature of 5 ± 1 °C on a yeast mold agar (YMA) medium composed of (% w/v) yeast extract, 0.3%; malt extract, 0.3%; tryptone, 0.3%; glucose, 1.0%; and agar, 2.0% [25].

2.3. Yeast Inoculation

The yeast inoculum standardization was performed by transferring the culture to a tube containing the YMA medium to obtain a young culture at 28 ± 1 °C. Subsequently, the sample was placed in a 250 mL Erlenmeyer flask with 100 mL of yeast mold broth (YMB) medium composed of (% w/v) yeast extract, 0.3%; malt extract, 0.3%; tryptone, 0.3%; and glucose, 1.0%. It was then incubated aerobically with orbital shaking at 200 rpm at 28 ± 1 °C for 24 h in a C25KC shaker incubator (New Brunswick Scientific, Edison, NJ, USA).

2.4. Biosurfactant Production Medium

The culture medium for biosurfactant production used licuri oil and glucose in a mineral base containing (% *w/v*) NH₄NO₃, 0.1%; KH₂PO₄, 0.01%; MgSO₄·7H₂O, 0.5%; FeCl₃, 0.01%; and NaCl, 0.01%, with the pH adjusted to 5.7, in 250 mL Erlenmeyer flasks containing 100 mL of medium [26]. The incubation conditions were maintained for 168 h at 200 rpm, using a 1% inoculum with a concentration of 10⁷ cells/mL, quantified in a Neubauer chamber.

To identify the ideal production conditions, a Central Composite Rotational Design (CCRD) was used, configured in the software Design Expert Stat-Ease® 360 (State-Ease Inc., Minneapolis, MN, USA), considering the following factors: licuri oil concentration (A), glucose (B), ammonium nitrate (C), and yeast extract (D). The factor levels varied between minimum (-1), central (0), maximum (+1), and axial points ($\pm\alpha$), with the specific concentrations indicated in Table 1. Biosurfactant production was evaluated by the ability to reduce surface tension (ST_{red}) in cell-free culture, considered the dependent variable. The total number of experiments considered in the approach was 27, including 3 repetitions for the central point, to account for the magnitude of possible random errors.

Table 1. Values of the input variables used in the Central Composite Rotational Design (CCRD).

Variables	Levels				
	-1.41	-1	0	+1	+1.41
A (%)	0.50	2.00	3.50	5.00	6.50
B (%)	1.00	4.00	7.00	10.0	13.0
C (%)	0.05	0.20	0.35	0.50	0.65
D (%)	0.05	0.20	0.35	0.50	0.65

The dependent variable ST_{red} was calculated from the following relation:

$$ST_{red} = ST_w - ST_{exp}, \quad (1)$$

where ST_w is the measurement of the surface tension of water (standard procedure during equipment calibration), and ST_{exp} is the measured surface tension obtained by the equipment.

The results were analyzed to develop an appropriate model for surface tension reduction. An analysis of variance (ANOVA) was performed to assess the significant impact of the independent factors on biosurfactant production, using F and *p* values. The goodness of fit of the polynomial model was determined by the R² and adjusted R² coefficients. The software was used to identify factor-level combinations that optimized surface tension reduction, employing the quadratic model:

$$Y = \beta_0 + \sum_{i=1}^4 \beta_i X_i + \sum_{i=1}^4 \beta_{ii} X_i^2 + \sum_{i=1}^3 \sum_{j=i+1}^4 \beta_{ij} X_i X_j + \epsilon, \quad (2)$$

where Y is the predicted response (ST_{red}), X represents the independent factors, i.e., (X₁ = A, X₂ = B, X₃ = C, X₄ = D), β_0 is the intercept, β_i are the coefficients for individual factors, β_{ii} are the quadratic coefficients, and β_{ij} are the coefficients for the interactions between factors (for example, β_{12} for AB).

2.5. Surface Tension Measurement

The surface tension measurements were performed in cell-free broth, after removing the cells by centrifugation at 4500 rpm for 10 min in a centrifuge (Rotina 420R, Hettich Zentrifugen, Tuttlingen, Germany) at 4 ± 1 °C. For this procedure, the Du-Nuoy ring method was used with a Sigma 70 tensiometer (KSV Instruments LTD, Helsinki, Finland) at room temperature (25 ± 1 °C).

2.6. Growth Curve

To determine the optimal production time, the growth profile was analyzed based on the optimized composition that resulted in the greatest reduction in surface tension (ST_{red}). The culture was performed in 1 L Erlenmeyer flasks containing 500 mL of the production medium, under orbital shaking at 200 rpm, for a total of 216 h at $28 \pm 1^\circ\text{C}$. Samples were collected at 0, 2, 6, 12, 24, and up to 216 h (at regular 24 h intervals after the first day of fermentation) to determine biomass ($\text{g}\cdot\text{L}^{-1}$), surface tension ($\text{mN}\cdot\text{m}^{-1}$), and yield ($\text{g}\cdot\text{L}^{-1}$) [27]. The determinations were performed in triplicate.

2.7. Emulsification Index (E_{24})

The emulsification index for the composition that showed the best reduction in surface tension was determined against canola, licuri, soybean, diesel, kerosene, and used motor oils, following the method described by Cooper and Goldenberg [28]. For this, 2 mL of hydrocarbon was added to 2 mL of the cell-free metabolic liquid in a test tube, followed by shaking for 2 min. The stability of the emulsion was evaluated after 24 h and calculated as follows:

$$E_{24} = \frac{h_e}{H} \times 100, \quad (3)$$

where h_e denotes the height of the emulsion column, and H represents the total height of the column formed by the mixture of the two liquids; the result is expressed as a percentage. A higher E_{24} percentage indicates a more stable emulsion, while a lower one suggests reduced stability.

2.8. Biosurfactant Isolation

The biosurfactant was isolated from the non-centrifuged culture medium, i.e., the entire broth obtained after 168 h of fermentation, using ethyl acetate in a 1:4 (v/v) ratio with the culture medium. The organic phase was collected, and the process was repeated two more times with the aqueous phase to ensure maximum extraction. Then, the organic phase was centrifuged for 15 min at 4500 rpm and filtered under vacuum. The filtrate was returned to the separation funnel, with the addition of saturated sodium chloride (NaCl) to facilitate the removal of the residual aqueous phase. Anhydrous magnesium sulfate (MgSO_4) was added to the organic phase, followed by filtration and drying in an oven at $50 \pm 1^\circ\text{C}$ [17].

2.9. Critical Micelle Concentration

The critical micelle concentration (CMC) was determined by measuring the surface tension of dilutions of the isolated biosurfactant in distilled water until a constant surface tension was reached. The CMC value was obtained from the graph relating surface tension and biosurfactant concentration, expressed in $\text{g}\cdot\text{L}^{-1}$ [12].

2.10. Determination of Ionic Charge and Particle Size

The determination of the ionic charge and particle size of the biosurfactant was conducted using the Malvern Zetasizer Nano ZS90 (Malvern Instruments Inc., Worcestershire, UK), which allows for accurate measurements of the zeta potential and the average particle diameter in the sample.

2.11. Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR analysis was performed using the IRSpirit spectrophotometer (Shimadzu, Kyoto, Japan) with a total attenuated reflectance accessory (QATR-S). The measurements were made in percentage transmittance (%T) mode with Happ-Genzel apodization, covering the mid-infrared range of 400 to 4000 cm^{-1} . Each spectrum was obtained with 42 scans at a resolution of 16 cm^{-1} .

2.12. Nuclear Magnetic Resonance Spectroscopy (NMR)

The analyses of ^1H and ^{13}C NMR were performed on a Bruker Avance-NEO-600 NMR spectrometer (Bruker Corporation, Billerica, MA, USA), operating at 500 MHz for the hydrogen nucleus. Twenty milligrams of the isolated biosurfactant were prepared, dissolved in 500 μL of deuterated chloroform (CDCl_3 ; Sigma-Aldrich, Taufkirchen, Germany), maintaining the temperature at 298.1 K. The chemical shift scale (δ) was referenced in ppm, using tetramethylsilane (TMS) as an internal standard.

2.13. Phytotoxicity Assay

The phytotoxicity of the biosurfactant was evaluated in a static assay, focusing on the seed germination and root growth of *Lactuca sativa* (lettuce), following the methodology described by Tiquia and collaborators [29]. Solutions of both crude and isolated biosurfactant were prepared in distilled water at concentrations of $1/2$ CMC (0.25%), 1 CMC (0.5%), and 2 CMC (1.0%). The assay was conducted in sterilized Petri dishes lined with filter paper, where seeds, previously treated with sodium hypochlorite, were placed (10 seeds per dish) along with 5 mL of each test solution. After five days of incubation in the dark, the germination rate and root lengths were measured, as described in the study:

$$\text{Relative Seed Germination (RSG, \%)} = \left(\frac{G_e}{G_c} \right) \times 100 \quad (4)$$

$$\text{Relative Root Length (RRL, \%)} = \left(\frac{L_e}{L_c} \right) \times 100, \quad (5)$$

$$\text{Germination Index} = \left(\frac{\text{RSG} \times \text{RRL}}{100} \right), \quad (6)$$

where G_e , G_c , L_e , and L_c represent the quantities of germinated seeds and the root lengths in the tested samples (subscript e) and control (subscript c), respectively.

2.14. Antimicrobial Activity Potential

A disk diffusion assay was conducted to evaluate the antibacterial activity of the biosurfactant. Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria were tested at a concentration of $10^8 \text{ CFU} \cdot \text{mL}^{-1}$. Filter paper disks impregnated with the biosurfactant in solutions of different concentrations: $0.4 \text{ g} \cdot \text{L}^{-1}$ ($1/2$ CMC); $0.8 \text{ g} \cdot \text{L}^{-1}$ (1 CMC); $1.6 \text{ g} \cdot \text{L}^{-1}$ (2 CMC); fermentation metabolic liquid and sterile water (negative control) were applied to Mueller–Hinton Agar (Merck, Darmstadt, Germany) plates. After 24 h of incubation at $30 \pm 1^\circ\text{C}$, the inhibition zones were measured in duplicate, following the CLSI protocol [30,31].

2.15. Statistical Analysis

The data were statistically analyzed using Statistica® software (version 12), followed by an analysis of variance (ANOVA). All experiments were performed in triplicate, and the results were expressed as means \pm standard deviations (SD), with a 95% confidence interval.

3. Results and Discussion

3.1. Biosurfactant Production

Table 2 presents the experimental setup established by the Design Expert Stat-Ease® 360 software, including the surface tension measurements and the experimental and predicted values of the response variable (ST_{red}). The obtained data highlight the potential of *Candida mogii* as a biosurfactant producer, demonstrating a substantial reduction in the surface tension of water, from $71.179 \pm 0.332 \text{ mN} \cdot \text{m}^{-1}$ to values ranging between 28.397 ± 0.101 and $48.848 \pm 0.216 \text{ mN} \cdot \text{m}^{-1}$. This variation range underscores the significant influence of the substrate composition on the biosurfactant production by the studied strain.

The effectiveness of a biosurfactant is determined by its ability to reduce surface tension; a greater reduction indicates higher effectiveness [32]. The experimental design indicated configuration 12, composed of (%) licuri oil (5), glucose (4), NH₄NO₃ (0.5), and yeast extract (0.5), as the composition that most effectively reduced the surface tension of the medium, reaching a minimum value of 28.397 ± 0.508 mN·m⁻¹ (ST_{red} of 42.782 mN·m⁻¹). This result highlighted the yeast as comparable to other *Candida* strains known for their efficient reduction in surface tension, such as *C. tropicalis*, which was able to reduce the surface tension of water from 72 mN·m⁻¹ to 30 mN·m⁻¹ [11]; *C. bombicola*, which achieved 29 mN·m⁻¹ [33]; and *C. lipolytica*, which showed an excellent value of 25 mN·m⁻¹ [27].

Table 2. Central Composite Rotational Design (CCRD) with observed and predicted STred.

Run	A (%)	B (%)	C (%)	D (%)	STexp	STred (Observed)	STred (Predicted)
1	5.00	4.00	0.50	0.20	45.244	25.783	26.564
2	2.00	10.0	0.20	0.20	35.603	34.921	34.065
3	2.00	4.00	0.50	0.20	42.707	28.472	26.428
4	2.00	4.00	0.20	0.50	37.063	32.576	31.603
5	3.50	7.00	0.35	0.35	36.258	30.321	31.291
6	3.50	13.0	0.35	0.35	40.081	32.145	30.512
7	3.50	7.00	0.35	0.35	44.847	31.438	31.291
8	2.00	10.0	0.20	0.50	40.347	31.098	31.768
9	5.00	10.0	0.20	0.50	47.587	31.265	32.167
10	5.00	4.00	0.20	0.20	33.577	23.592	23.732
11	6.50	7.00	0.35	0.35	45.396	31.254	30.296
12	5.00	4.00	0.50	0.50	28.397	42.782	42.496
13	2.00	4.00	0.50	0.50	44.044	34.116	35.119
14	5.00	4.00	0.20	0.50	39.914	37.602	36.601
15	3.50	7.00	0.05	0.35	46.688	30.924	31.393
16	2.00	10.0	0.50	0.20	40.258	26.332	28.784
17	0.50	7.00	0.35	0.35	42.068	29.111	29.760
18	3.50	1.00	0.35	0.35	39.925	31.265	32.589
19	3.50	7.00	0.35	0.35	39.914	32.115	31.291
20	5.00	10.0	0.50	0.50	39.034	30.921	32.33
21	5.00	10.0	0.50	0.20	40.885	24.491	24.323
22	5.00	10.0	0.20	0.20	38.39	26.775	27.223
23	3.50	7.00	0.35	0.05	48.848	22.331	22.088
24	2.00	4.00	0.20	0.20	35.39	25.935	25.976
25	3.50	7.00	0.35	0.65	39.064	35.789	35.722
26	3.50	7.00	0.65	0.35	40.585	32.786	32.008
27	2.00	10.0	0.50	0.50	39.741	30.832	29.550

The coded variables represent the concentrations of licuri oil (A), glucose (B), ammonium nitrate (C), and yeast extract (D). STexp denotes the experimental value obtained during the measurement of surface tension using the tensiometer. STred denotes the surface tension reduction, calculated as the difference between the surface tension of water (71.02 mN·m⁻¹) and STexp in each configuration.

The efficiency in surface tension reduction (ST_{red}) as a function of the independent variables was modeled with a second-order polynomial equation, based on 15 terms. The equation, adjusted to the experimental data, considered the coded variables A, B, C, and D, representing different concentrations of medium components. This approach allowed for the identification of the individual and interactive influence of each variable on the response, providing a predictive model to optimize the formulation, as shown:

$$\text{ST}_{\text{red}} = 31.291 + 0.134A - 0.519B + 0.154C + 3.409D - 1.149AB + 0.595AC + 1.810AD - 1.433BC - 1.981BD + 0.766CD - 0.316A^2 + 0.065B^2 + 0.102C^2 - 0.597D^2, \quad (7)$$

The analysis of variance (ANOVA) for the quadratic model of surface tension reduction (ST_{red}) is presented in Table 3 and revealed that the model was highly significant, with an F-value of 14.78 and $p < 0.0001$, indicating a strong fit to the experimental data. Among

the terms, the main factor D (yeast extract) and the interactions AB, AC, AD, BC, and BD showed significant influence ($p < 0.05$), suggesting that these terms had a considerable impact on the response. The lack of fit absence ($p = 0.261$) confirmed the model's adequacy, ensuring accurate data representation and reliable predictions.

Table 3. ANOVA analysis of the quadratic model for surface tension reduction.

Source	Sum of Squares	df	Mean Square	F-Value	p-Value
Model	482.70	14	34.478	14.775	<0.0001 s
A—Licuri oil	0.43068	1	0.43068	0.18456	0.6751 ns
B—Glucose	6.4719	1	6.4719	2.7734	0.1217 ns
C—Ammonium nitrate	0.56703	1	0.56703	0.24299	0.6309 ns
D—Yeast extract	278.85	1	278.85	119.50	<0.0001 s
AB	21.139	1	21.139	9.0588	0.0108 s
AC	5.6656	1	5.6656	2.4279	0.1451 ns
AD	52.443	1	52.443	22.473	0.0004 s
BC	32.864	1	32.864	14.083	0.0027 s
BD	62.794	1	62.794	26.909	0.0002 s
CD	9.3866	1	9.3866	4.0224	0.0679 ns
A ²	2.1293	1	2.1293	0.91246	0.3583 ns
B ²	0.089298	1	0.089298	0.038267	0.8481 ns
C ²	0.22281	1	0.22281	0.095483	0.7626 ns
D ²	7.5920	1	7.5920	3.2534	0.0964 ns
Lack of fit	26.361	10	2.6361	3.2119	0.26069 ns
Pure error	1.6415	2	0.82074		
$R^2 = 0.9451$; $R^2_{adj} = 0.8812$; adeq. precision = 17.924					

s indicates significant difference at $p < 0.05$. ns indicates no significant difference at $p > 0.05$.

The fit statistics reinforced the adequacy of the model, with $R^2 = 0.9451$ and $R^2_{adj} = 0.8812$, indicating a robust explanation of the data variability. The precision level of 17.924, well above the minimum desired (4), suggested an excellent signal-to-noise ratio, indicating that the model was reliable for exploring the design space [34]. As seen in Figure 1, the normal probability plot of externally studentized residuals shows points aligning closely with the red line, indicating residuals reasonably followed a normal distribution, with minimal outliers [35].

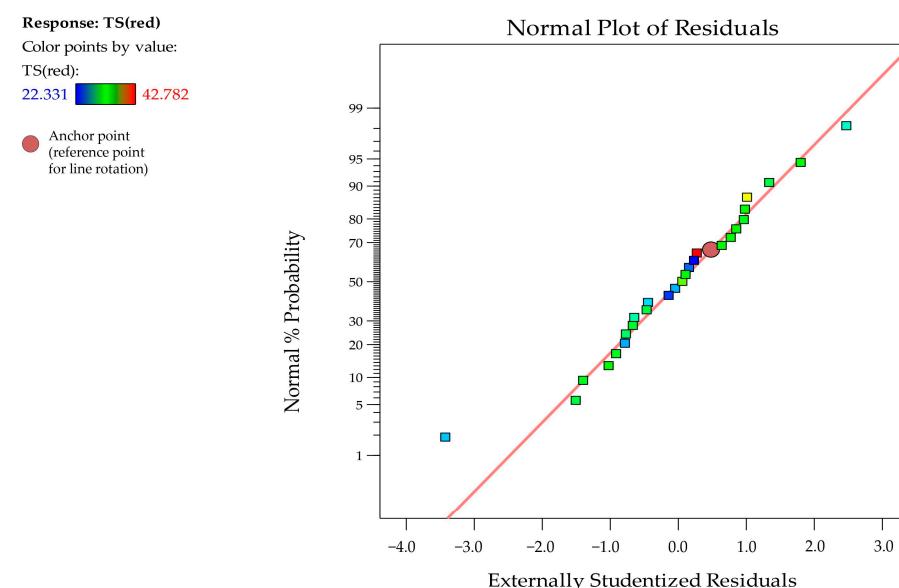


Figure 1. Normal plot of residuals indicating the normal distribution of the model applied to optimize the reduction in surface tension by *C. mogii*.

Figure 2 illustrates the Pareto chart with the main effects of each factor influencing the reduction in surface tension. Negative values for the interactions BD, BC, and AB suggested that the combination of these factors decreased their effect on the response variable. In other words, when variables B (glucose) and D (yeast extract), B and C (ammonium nitrate), or A (licuri oil) and B interacted, the combined effect of these interactions resulted in a smaller reduction in surface tension than if these variables acted individually or in other combinations. On the other hand, the positive values observed for variables D and AD indicated that these factors had an effect that enhanced the effectiveness of the surface tension reduction when interacting. This means that yeast extract (D) alone and in interaction with licuri oil (A) contributed positively to the desired response, potentially maximizing the surface tension reduction and standing out as favorable conditions for the experiment's goal. It can also be noted that the quadratic terms (Q) did not show significant effects on surface tension reduction, a conclusion that could be directly verified from the ANOVA table.

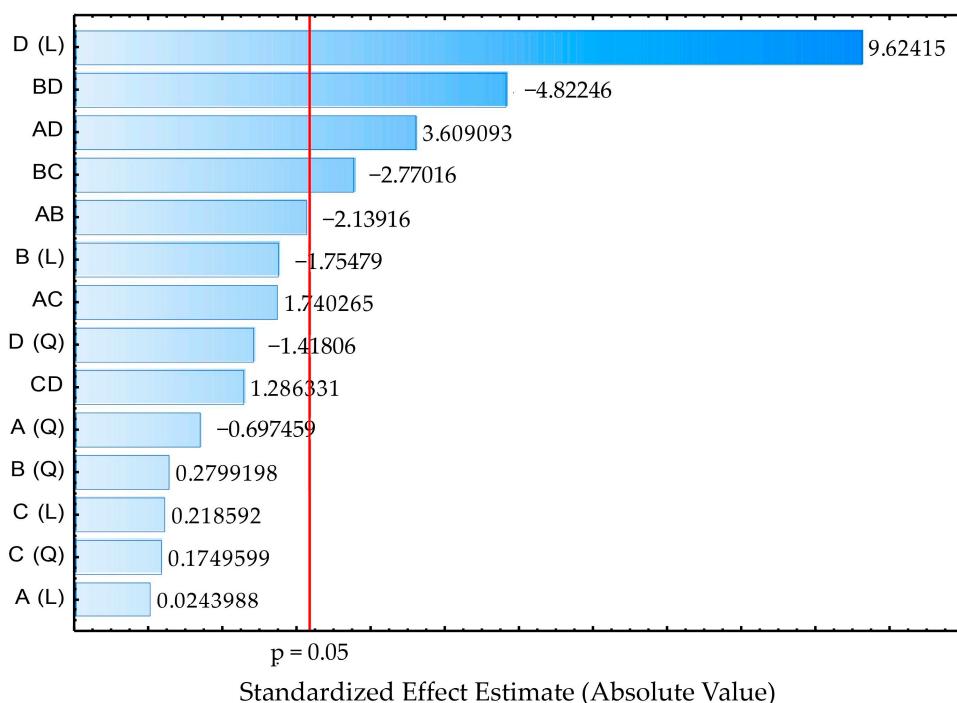


Figure 2. Pareto chart for STred according to the statistical analysis of the CCD carried out to evaluate the effect of independent variables' concentration in the culture medium for the biosurfactant production.

The goal of optimization in this study was to achieve a greater reduction in surface tension by setting the concentrations of licuri oil, glucose, ammonium nitrate, and yeast extract to optimized values. The desirability function, proposed by Derringer and Suich [36], aims to find optimal conditions for each individual variable so that the response lies within an acceptable range. In this context, each individual desirability were adjusted to achieve a global desirability index that maximized STred following Equation (7) found for the model. With a desirability index of 0.9095, the best composition for the medium was (%) licuri oil (6.2), glucose (8.45), NH_4NO_3 (0.62), and yeast extract (0.62), resulting in an STred of $42.782 \text{ mN}\cdot\text{m}^{-1}$. The behavior of the predictive values for each independent factor and desirability is represented in Figure 3.

Since the quadratic terms did not show any significant influence on the response, we could evaluate the interaction between variables through the response surface plot, providing a quicker and more direct visual analysis, as shown in Figure 4. That analysis could reveal both synergistic and antagonistic effects between the variables.

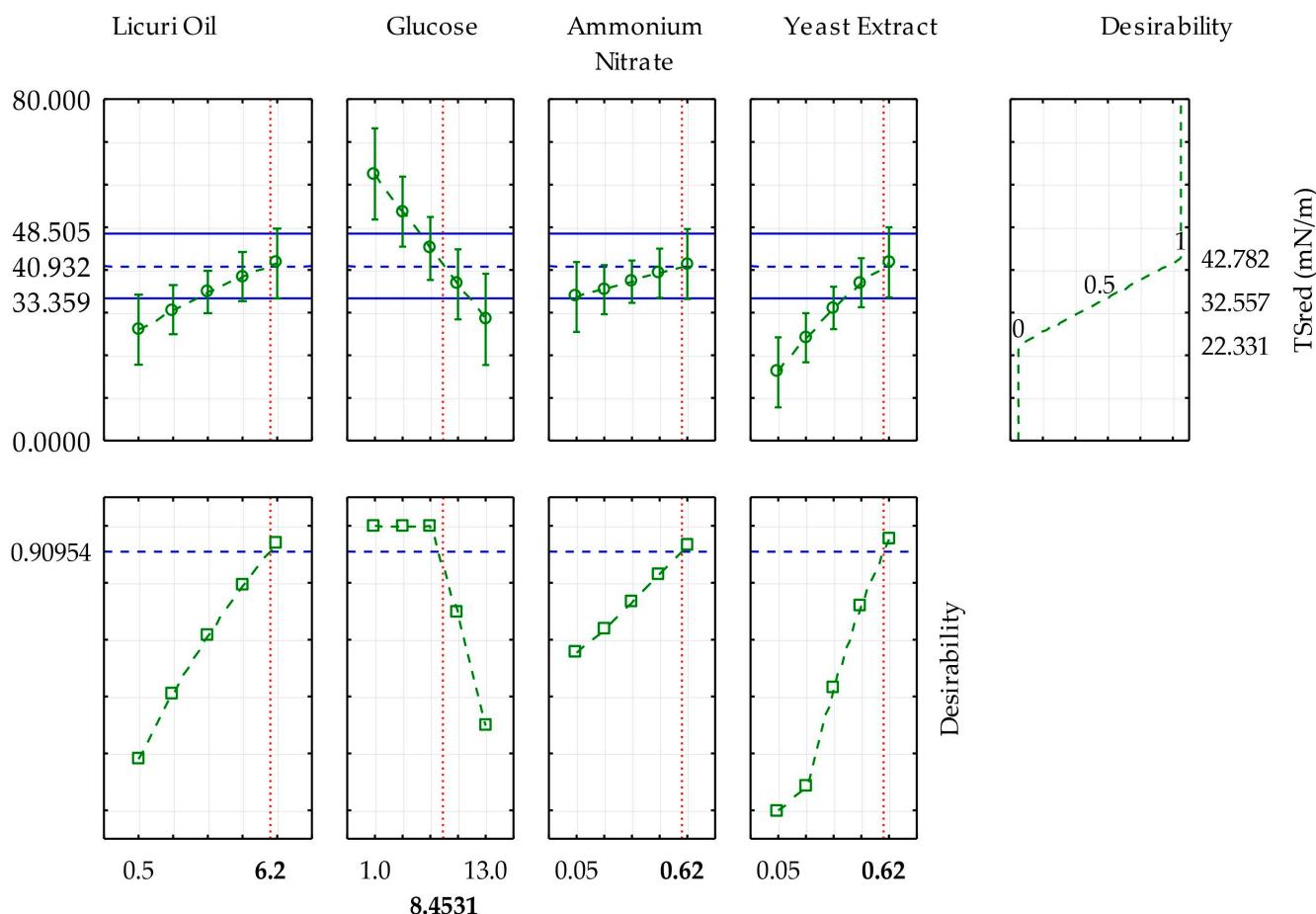


Figure 3. Profiles for predicted values and desirability for ST_{red} based on the statistical analysis of the CCD adopted in this study.

Synergistic interactions, such as the increase in response with higher levels of licuri oil and yeast extract (Figure 4C), suggest that these components may act complementarily, enhancing the desired effect. The influence of that interaction was also evidenced in the Pareto chart presented. Licuri oil likely served as an effective carbon source due to its fatty acid composition [24], which could stimulate key biosynthetic pathways involved in the production of lipid-based biosurfactants, such as glycolipids. The fatty acids in licuri oil are metabolized via β -oxidation, yielding acetyl-CoA, a critical precursor for biosurfactant synthesis [37].

On the other hand, antagonistic interactions, where the response decreases with the increase in certain pairs, indicate competition or inhibition, such as between licuri oil and glucose (Figure 4A), licuri oil and ammonium nitrate (Figure 4B), and between glucose and ammonium nitrate (Figure 4D) under certain conditions. Figure 4E,F relate the effects of interactions between glucose and ammonium nitrate with yeast extract. We can observe that in all cases, increasing the concentration of this nitrogen source to values greater than 0.3% favored the reduction in surface tension. Furthermore, the association between yeast extract and NH_4NO_3 at maximum concentrations achieved the highest levels of ST_{red} .

The nitrogen source also showed a significant effect on BS production by *S. marcescens*, where the combined effects of peptone (0.4%) and ammonium sulfate (0.5%) were able to reduce the surface tension to $28.4 \text{ mN}\cdot\text{m}^{-1}$ [34]. In the production of surfactants by *P. aeruginosa* MA01, concentrations ranged between 0.3–0.9% and 0.1–0.4% of sodium nitrate and yeast extract, respectively, for both non-optimized and optimized media, significantly increasing process yield. Yeast extract is an excellent nitrogen source for biosurfactant production due to its high bioavailability of amino acids and proteins, stimulation of

metabolic pathways for secondary metabolite production, and broad compatibility with different microbial strains [38,39].

The validation of the statistical model was conducted in the subsequent stage, with the evaluation of the growth curve and determination of the optimal production time.

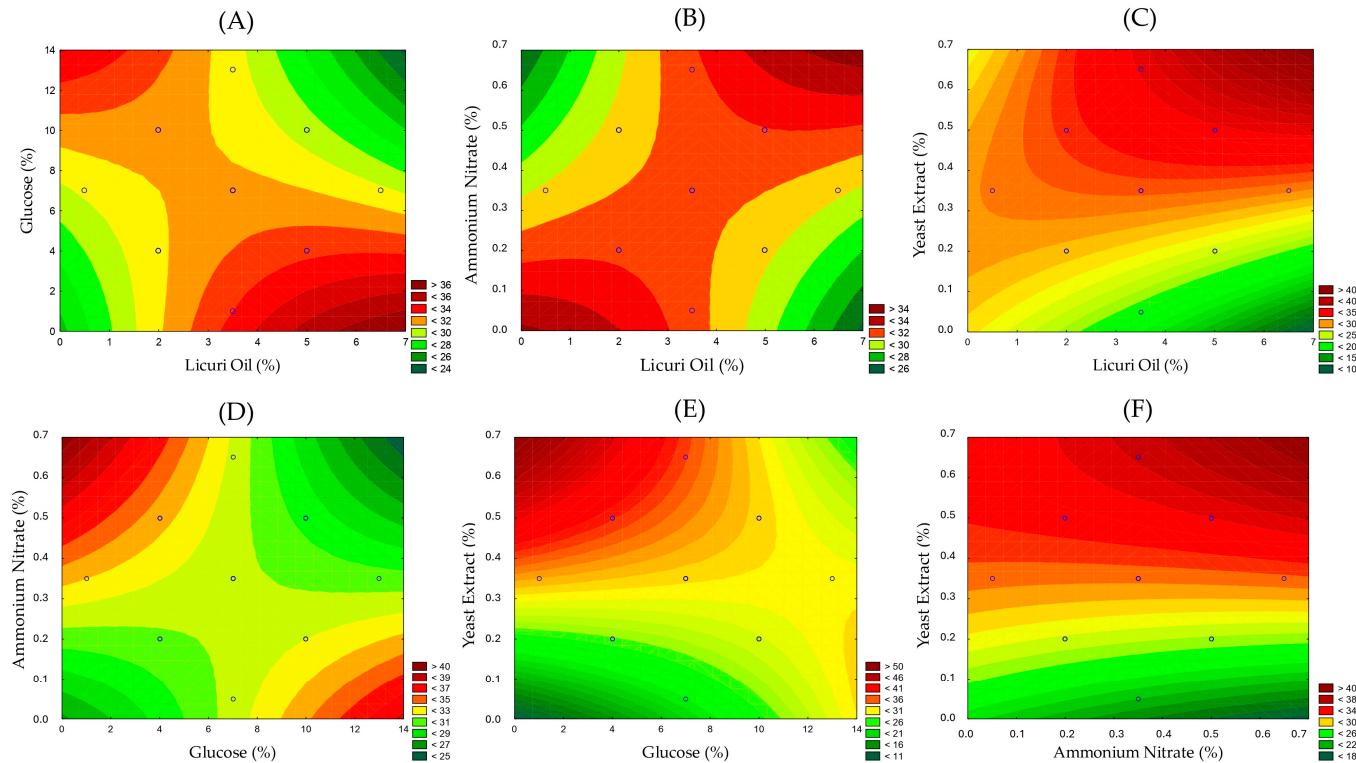


Figure 4. Response profiles for the interactions between variables: (A) licuri oil and glucose, (B) licuri oil and ammonium nitrate, (C) licuri oil and yeast extract, (D) glucose and ammonium nitrate, (E) glucose and yeast extract, and (F) ammonium nitrate and yeast extract.

3.2. Growth Curve Analysis

Figure 5 shows the evaluation of the biomass concentration, yield, and surface tension reduction in the production of BS by *Candida mogii* during the pre-established fermentation period (216 h) in the optimized medium.

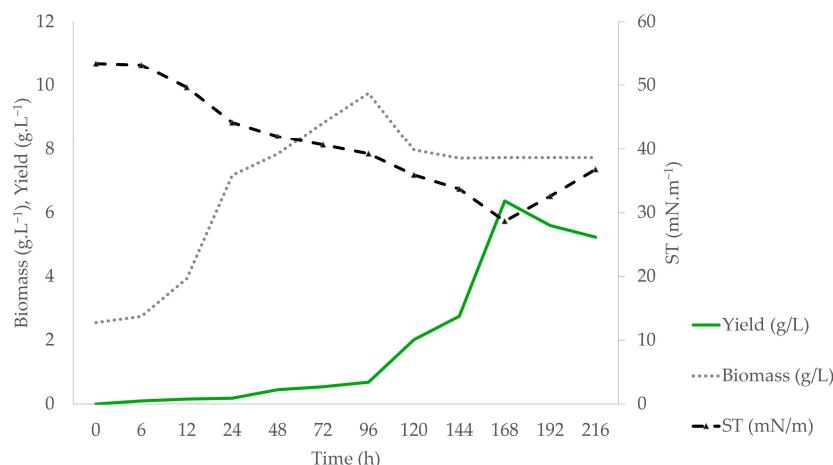


Figure 5. Temporal changes in biomass, yield and surface tension during cultivation of *C. mogii* in mineral medium.

The biomass representative curve reached its maximum point ($9.74 \pm 0.46 \text{ g}\cdot\text{L}^{-1}$) within the first 96 h of fermentation, likely while there was still glucose in the medium. The low complexity of the hydrophilic source increases carbon availability, promoting its initial use by the cells as an energy source. As this source is depleted, a reduction in cell growth occurs, leading to the stationary phase, when carbon begins to be obtained from the fatty acid chains present in licuri oil. The hydrophobic nature of oils extends resistance to biodegradation due to their low solubility in water, which increases their adsorption to cell surfaces and reduces their availability for degrading microorganisms [40].

The balance between hydrophilic and hydrophobic sources in the culture medium is crucial for the quantity and characteristics of the biosurfactants (BS) produced. In this study, a 40:50 ratio was used, resulting in a maximum efficiency of $6.36 \pm 0.81 \text{ g}\cdot\text{L}^{-1}$ at 168 h, with a productivity of $37.85 \text{ mg}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$. Other studies have reported different optimal ratios for biosurfactant production: 10:10 (glucose/soap waste) with a rate of $49.96 \text{ mg}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$; 100:100 (glucose/sunflower oil waste) with $216 \text{ mg}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$; and 100:30 (glucose/rapeseed oil) with $1.64 \text{ g}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$. These findings highlight the importance of the selection and proportion of carbon sources in the characteristics of the produced biosurfactants [41–43].

Although there are no records on the biosurfactant production by *C. mogii*, other species of the *Candida* genus, such as *C. bombicola* and *C. tropicalis* [12,44], have demonstrated a strong capacity to reduce surface tension. In this study, *C. mogii* achieved a reduction in surface tension from 71.042 ± 0.021 to $28.662 \pm 0.563 \text{ mN}\cdot\text{m}^{-1}$ after 168 h in an optimized medium, highlighting its potential in biosurfactant production. Biosurfactant production began in the logarithmic phase and peaked during the decline phase, similar to what was observed in *C. catenulata* [41] and *C. tropicalis* [44], which reported a greater surface tension reduction in the stationary phase. Studies with another *C. tropicalis* strain showed higher yields already in the logarithmic phase, indicating that biosurfactant production and surface tension reduction are not necessarily linked to cellular growth [45].

3.3. Emulsification Index and Critical Micelle Concentration

The data on the emulsification activity of the metabolic liquid obtained from fermentation by *Candida mogii* in mineral medium supplemented with licuri oil against different hydrocarbons are presented in Table 4.

Table 4. Emulsification index (E_{24}) for BS produced by *C. mogii* against various hydrocarbons.

Hydrocarbon	E_{24} (%)	Hydrocarbon	E_{24} (%)
Canola oil	75.43 ± 0.61	Waste engine oil	80.48 ± 7.74
Licuri oil	82.38 ± 15.49	Diesel	8.81 ± 0.74
Soybean oil	5.79 ± 0.13	Kerosene	3.28 ± 0.05

Experiments were performed in triplicate, and the results represent the mean \pm standard deviation of two independent experiments.

The emulsification index determines the effectiveness of the biosurfactant as a bioemulsifier. Although emulsifying and dispersing additives do not necessarily reduce the surface tension of water or hydrocarbons, they help decrease the interfacial energy between the phases [46]. To be considered a good emulsifying agent, the ability to form stable emulsions must remain above 50% for at least 24 h [47]. Among the oils tested, only canola, licuri, and motor (residual) oils showed a satisfactory emulsification activity. The values were higher than those reported by Pinto et al. (E_{24} of $59.0 \pm 0.9\%$) [12] and Durval et al. (E_{24} of $65.8 \pm 1.5\%$) [48] for canola oil, using *C. bombicola* and *B. cereus*, respectively. For waste engine oil, *C. mogii* showed more satisfactory results than those found by Almeida et al. using *C. tropicalis* (E_{24} close to 70%) [11].

The surface tension behavior as a function of biosurfactant concentration in the medium is shown in Figure 6. As indicated in the graph, a concentration of $0.8 \text{ g}\cdot\text{L}^{-1}$ was able to reduce the surface tension of the medium to the maximum value of 25.58 ± 0.31 ,

which is the CMC for the biosurfactant produced by *C. mogii* in medium supplemented with licuri oil.

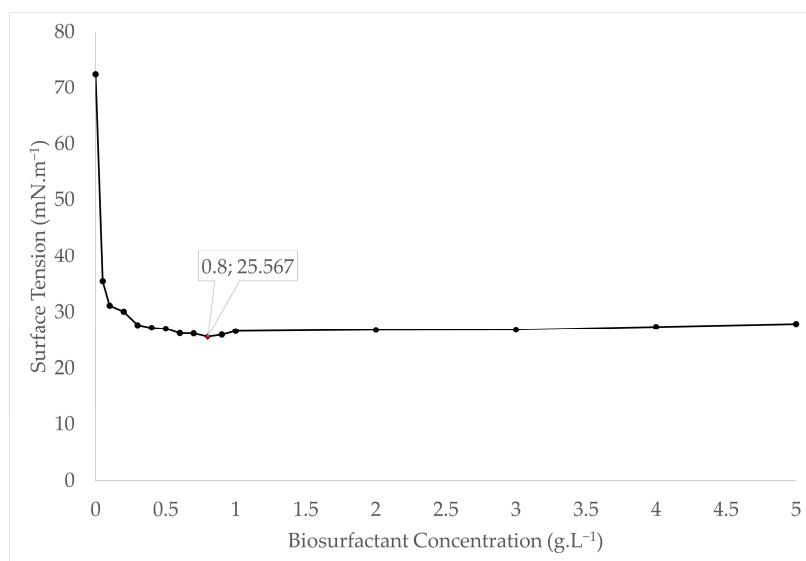


Figure 6. Critical micelle concentration of the biosurfactant from *C. mogii*.

The CMC is a crucial parameter for evaluating the surface activity of a biosurfactant and its solubility in aqueous media. This parameter is influenced by the structure and composition of the biosurfactant, as well as factors such as temperature, ionic strength, and the presence of organic additives. The CMC found in this study was very close to the values achieved by *Starmerella bombicola* ($0.6\text{ g}\cdot\text{L}^{-1}$), *Candida utilis* ($0.6\text{ g}\cdot\text{L}^{-1}$), and *Yarrowia lipolytica* ($1.2\text{ g}\cdot\text{L}^{-1}$) reported in the literature [13,49,50].

3.4. Biosurfactant Characterization

The biomolecule produced by *C. mogii* exhibited an ionic charge of -98.9 mV (0.136 mS/cm , $24.9\text{ }^\circ\text{C}$), classifying it as an anionic surfactant. Similar results were reported for other *Candida* species [27,33]. A zeta potential of -98.9 mV suggested that the particles were highly stable in suspension, which is desirable in many biosurfactant applications, such as in emulsion formation or dispersion stabilization [51]. The particle size distribution by intensity showed that most particles had a size around 194.6 nm , with smaller populations of larger particles.

The isolated and purified biosurfactant was subjected to FT-IR and NMR analyses (Figures 7 and 8). In the FT-IR spectrum, the band at 3402.45 cm^{-1} is associated with the hydroxyl group, indicating the presence of functional groups that may be involved in hydrogen bonding interactions. The bands at 2924.18 cm^{-1} and 2853.81 cm^{-1} correspond to the C-H stretching characteristic of alkanes, suggesting a hydrophobic structure. The presence of carbonyl groups was confirmed by the band at 1743.60 cm^{-1} , which can be attributed to esters or carboxylic acids. Additionally, the band at 1620.08 cm^{-1} can be interpreted as a C=C stretching or a N-H bending, indicating the possibility of double bonds or amine groups. Finally, the band at 1408.95 cm^{-1} suggests C-H bending or C-O stretching.

The ^1H NMR spectrum (Figure 8A) exhibited a characteristic signal at 7.28 ppm corresponding to the solvent used (deuterated chloroform). Signals between 5.20 and 5.40 ppm indicated alkene protons, suggesting unsaturation in the molecule. In the range of 4.10 – 5.20 ppm , signals suggested protons on carbons bonded to oxygen, as seen in esters, ethers, or vinyl protons (C=C-H). Shifts near 2.00 ppm corresponded to protons near electronegative groups, such as oxygen or nitrogen, indicating functional groups like esters

or amines. Finally, the range of 0.85–1.60 ppm showed methylene and methyl protons, consistent with hydrocarbon chains.

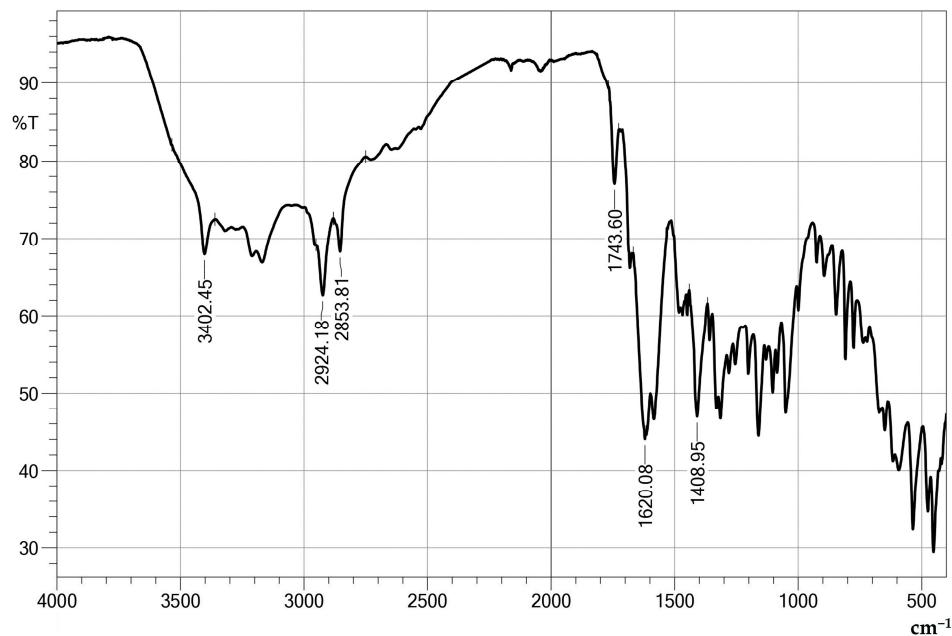


Figure 7. FT-IR spectrum of biosurfactant produced by *C. mogii* grown in an optimized medium containing licuri oil.

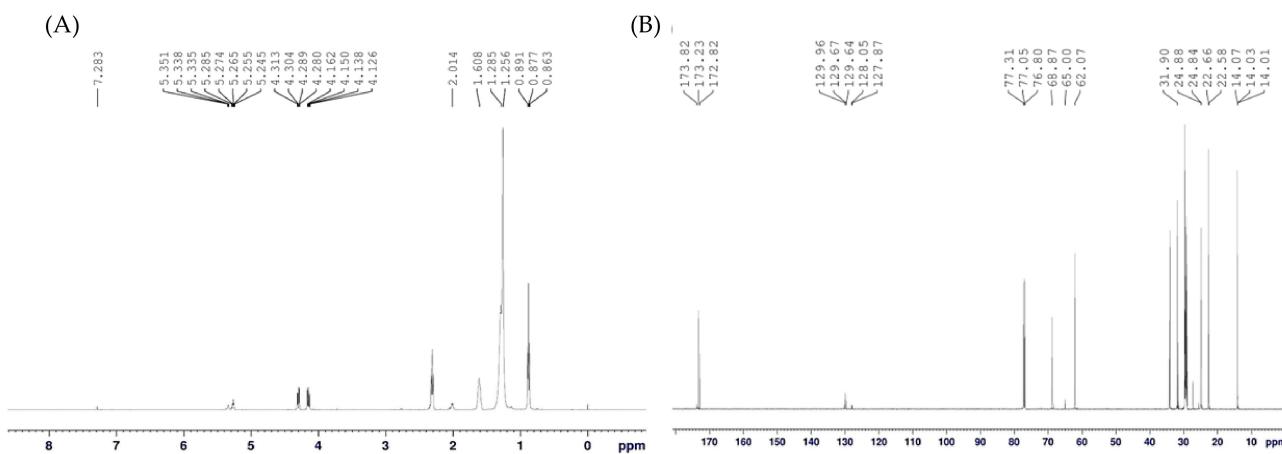


Figure 8. NMR spectrum of biosurfactant produced by *C. mogii* grown in an optimized medium containing licuri oil: (A) ^1H and (B) ^{13}C .

The ^{13}C NMR spectrum (Figure 8B) of the biosurfactant sample revealed several characteristic signals. A prominent peak between 77.3 and 77.5 ppm corresponded to the solvent (CDCl_3). In the carbonyl region (170–180 ppm), signals at 172.8–173.8 ppm indicated the presence of ester or carboxylic acid groups, typical of biosurfactants derived from fatty acids. Peaks in the aromatic or alkene region (120–140 ppm), particularly between 128.5 and 129.9 ppm, suggested the presence of sp^2 carbons, likely associated with unsaturated double bonds ($\text{C}=\text{C}$). The region spanning 20–60 ppm highlighted sp^3 carbons, including methylene ($-\text{CH}_2-$) groups and oxygenated carbons, such as those found in esters or glycosidic bonds (60–65 ppm). Lastly, a signal at 14.0 ppm corresponded to terminal methyl groups ($-\text{CH}_3$), confirming the presence of hydrocarbon chains in the structure.

Given that the spectra showed ester groups, aliphatic chains, and oxygen, it is possible to infer that the surfactant produced by the yeast *Candida mogii* belongs to the glycolipid group, as previously reported for other species of the genus [11,52,53].

3.5. Phytotoxicity

The assessment of phytotoxicity in biosurfactant development is crucial to ensure environmental safety and crop health. This study confirmed that the product did not harm plants, which is essential for agricultural applications [54]. The biosurfactant toxicity was tested on lettuce seeds (*Lactuca sativa*), with the results presented in Table 5.

Table 5. Biosurfactant toxicity test on *Lactuca sativa* seeds.

Concentration	Germination	Root Growth	GI
1/2 CMC	89.29% ^a	99.73% ^a	89.05% ^a
CMC	92.86% ^a	95.88% ^a	89.03% ^a
2× CMC	92.86% ^a	89.01% ^b	82.65% ^b

GI: germination index. Data with different indices (^a and ^b) indicate a statistically significant difference within the same column.

The results indicated that different concentrations of the biosurfactant produced by *Candida mogii* (½ CMC, 1 CMC, and 2× CMC) did not inhibit seed germination or root growth in lettuce. The observed germination indices remained above 80%, demonstrating that treatments with the biosurfactant did not negatively impact plant growth or health. Other biosurfactants also showed no negative effects on *Lactuca sativa*; for instance, mannosylerythritol lipids (MELs) at specific concentrations (158 mg·L⁻¹) may even have stimulated seed germination, root growth, and development, exhibiting a biostimulant effect without phytotoxicity. Additionally, biosurfactants produced by *Bacillus subtilis* showed no toxicity towards lettuce, supporting their safe use in environmental applications [55,56].

However, while the biosurfactant was non-toxic to *Lactuca sativa*, broader environmental safety requires evaluation. Future studies should assess its effects on aquatic species, such as *Artemia salina*, and soil microbiota to ensure its suitability for diverse applications and ecological balance.

3.6. Antimicrobial Activity

The presence of *Staphylococcus aureus* and *Escherichia coli* in foods and environments is frequently used as an indicator of contamination and hygienic-sanitary conditions. As Gram-positive and Gram-negative bacteria, respectively, they have shown resistance to multiple antibiotics, posing an additional challenge for infection control and food safety [57–59]. The diameters of inhibition zones obtained from the disk diffusion test are presented in Table 6, showing a concentration-dependent inhibition zone for both strains studied.

Table 6. The average diameter of inhibition zone against *S. aureus* and *E. coli* in response to the biosurfactant produced by *C. mogii* in medium supplemented with licuri oil.

Sample	Inhibition Zone of Growth (mm)	
	<i>S. aureus</i>	<i>E. coli</i>
Metabolic liquid	13	8
1/2 CMC	6	6
1 CMC	15	12
2 CMC	16	18
Negative control	-	-

The results presented in this study can be compared with EUCAST (The European Committee on Antimicrobial Susceptibility Testing) clinical breakpoints, which provide parameters for classifying bacterial sensitivity or resistance to conventional antimicrobials.

For example, the inhibition diameters for *E. coli* at 1 CMC (12 mm) and 2 CMC (18 mm) were close to the minimum values observed for known antibiotics, such as ampicillin and cephalexin, which typically present inhibition zones between 14 and 15 mm, depending on the strain [60]. Although they did not reach the potency of broad-spectrum antibiotics, such as benzylpenicillin, which shows inhibition zones greater than 26 mm against *S. aureus*, they appear promising for specific applications, such as pathogen reduction in agro-industrial systems or contact surfaces [61].

The metabolic liquid from fermentation alone was able to inhibit microorganism growth, which is crucial since the costs associated with extraction and purification in biosurfactant production represent a disadvantage in the process [62]. Other studies have demonstrated the effectiveness of glycolipids produced by *Candida* sp. against *E. coli* and *S. aureus* [10,41,61].

Minimum Inhibitory Concentration (MIC) tests will be necessary to identify the lowest concentration of the biosurfactant capable of completely inhibiting the growth of the sample. Also, future studies may include parallel tests with standard antibiotics to validate its competitiveness and explore potential synergies between the biosurfactant and other antimicrobials.

4. Conclusions

The results demonstrated the potential of the yeast *Candida mogii* in the production of glycolipids, indicating promising applications in bioremediation and biotechnology. The study revealed that that strain could reduce the surface tension of water from $71.04\text{ mN}\cdot\text{m}^{-1}$ to $28.66\text{ mN}\cdot\text{m}^{-1}$, particularly in the configuration optimized by the quadratic model, containing (%) licuri oil (6.2), glucose (8.45), NH_4NO_3 (0.62), and yeast extract (0.62). The emulsification capacity of the biosurfactants produced by *C. mogii* was effective, especially concerning canola, licuri, and waste engine oils, with emulsification indices exceeding 70% after 24 h, highlighting their potential as emulsifying agents for industrial applications. The developed polynomial model showed a good fit to the experimental data, with R^2 and adjusted R^2 values of 0.9451 and 0.8812, respectively, ensuring the model's reliability in predicting and optimizing biosurfactant production parameters. The biosurfactant exhibited no toxicity towards *Lactuca sativa* seeds and was identified as a promising antimicrobial agent against *S. aureus* and *E. coli*.

Further research is essential to confirm the bioproduct's safety for a broader range of organisms, strengthen the evidence of its antimicrobial efficacy through direct comparisons with standard antibiotics, and investigate its antibiofilm potential and underlying mechanisms of action. Future studies should also focus on scaling up production processes, testing the biosurfactant in contaminated environments, and conducting field trials to validate its effectiveness in real-world applications, ensuring its feasibility for industrial and environmental use.

Author Contributions: Conceptualization, P.F.F.d.S. and J.M.C.G.; methodology, P.F.F.d.S., R.R.d.S., L.A.S. and J.M.C.G.; validation, P.F.F.d.S. and J.M.C.G.; formal analysis, P.F.F.d.S. and J.M.C.G.; investigation, P.F.F.d.S. and J.M.C.G.; resources, J.M.C.G.; data curation, P.F.F.d.S.; writing—original draft preparation, P.F.F.d.S. and J.M.C.G.; writing—review and editing, P.F.F.d.S., R.R.d.S., L.A.S. and J.M.C.G.; visualization, J.M.C.G. and L.A.S.; supervision, J.M.C.G.; project administration, J.M.C.G.; funding acquisition, J.M.C.G. All authors have read and agreed to the published version of the manuscript.

Funding: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)—Finance Code 001.

Data Availability Statement: The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

Acknowledgments: The authors express their gratitude to the Bioprocess Laboratory of the Antibiotics Department at the Federal University of Pernambuco; Science and Technology Center of the Catholic University of Pernambuco (UNICAP); Center of Characterization and Analysis Central

analytic multiuser laboratory-UFPB (LMCA-UFPB) for obtaining the NMR spectra; Cooperativa de Produção da Região do Piemonte da Diamantina (COOPES) for providing the fixed oil of *S. coronata*; and to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil CAPES for providing scholarships.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Ahmar Siddiqui, M.; Parnthong, J.; Kungsanant, S.; Chavadej, S.; Chaiprapat, S. Influences of specific surfactant structures on biohydrogen production from oily wastewater in batch and continuous anaerobic dark fermentation. *Bioresour. Technol.* **2022**, *360*, 127617. [[CrossRef](#)] [[PubMed](#)]
2. Markandeywar, T.S.; Narang, R.K. Biosurfactant assisted synthesis and characterization of silver nanoparticles: In vitro investigation of their anti-oxidant, anti-inflammatory, anti-infective and wound healing potential. *Inorg. Chem. Commun.* **2024**, *165*, 112506. [[CrossRef](#)]
3. Campbell, J. Entrainment. In *Complete Casting Handbook*; Elsevier: Amsterdam, The Netherlands, 2015; pp. 17–90. [[CrossRef](#)]
4. Naveenkumar, R.; Baskar, G. Process optimization, green chemistry balance and technoeconomic analysis of biodiesel production from castor oil using heterogeneous nanocatalyst. *Bioresour. Technol.* **2021**, *320*, 124347. [[CrossRef](#)] [[PubMed](#)]
5. Miao, Y.; To, M.H.; Siddiqui, M.A.; Wang, H.; Lodens, S.; Chopra, S.S.; Kaur, G.; Roelants, S.L.K.W.; Lin, C.S.K. Sustainable biosurfactant production from secondary feedstock—Recent advances, process optimization and perspectives. *Front. Chem.* **2024**, *12*, 1327113. [[CrossRef](#)]
6. Marqués, A.M.; Pérez, L.; Farfán, M.; Pinazo, A. Green surfactants: Production, properties, and application in advanced medical technologies. In *Biosurfactants for a Sustainable Future: Production and Applications in the Environment and Biomedicine*; Sarma, H., Prasad, M.N.V., Eds.; Wiley: Hoboken, NJ, USA, 2021; ISBN 978-111967102-2; 978-111967100-8.
7. Ahalliya, R.M.; Raja, F.S.D.; Rangasamy, K.; Arumugam, V.; Palanisamy, S.; Saikia, K.; Rathankumar, A.K.; Al-Dhabi, N.A.; Arasu, M.V. Production cost of traditional surfactants and biosurfactants. In *Multifunctional Microbial Biosurfactants*; Kumar, P., Dubey, R.C., Eds.; Springer Nature: Berlin/Heidelberg, Germany, 2023; ISBN 978-303131230-4; 978-303131229-8.
8. Wojtowicz, K.; Steliga, T. The impact of a mixture of biosurfactants on the efficiency of the bioremediation of soil contaminated with petroleum hydrocarbons. *Nafta—Gaz* **2024**, *80*, 269–278. [[CrossRef](#)]
9. Kashif, A.; Rehman, R.; Fuwad, A.; Shahid, M.K.; Dayarathne, H.N.P.; Jamal, A.; Aftab, M.N.; Mainali, B.; Choi, Y. Current advances in the classification, production, properties and applications of microbial biosurfactants—A critical review. *Adv. Colloid Interface Sci.* **2022**, *306*, 102718. [[CrossRef](#)] [[PubMed](#)]
10. Araujo, J.M.M.; Monteiro, J.M.; Silva, D.H.d.S.; Veira, A.K.; Silva, M.R.C.; Ferraz, F.A.; Braga, F.H.R.; Siqueira, E.P.d.; Monteiro, A.d.S. *Candida krusei* M4CK produces a bioemulsifier that acts on melaleuca essential oil and aids in its antibacterial and antibiofilm activity. *Antibiotics* **2023**, *12*, 1686. [[CrossRef](#)] [[PubMed](#)]
11. Almeida, D.G.; da Silva, R.d.C.F.S.; Meira, H.M.; Brasileiro, P.P.F.; Silva, E.J.; Luna, J.M.; Rufino, R.D.; Sarubbo, L.A. Production, characterization and commercial formulation of a biosurfactant from *Candida tropicalis* UCP0996 and its application in decontamination of petroleum pollutants. *Processes* **2021**, *9*, 885. [[CrossRef](#)]
12. Pinto, M.I.S.; Guerra, J.M.C.; Meira, H.M.; Sarubbo, L.A.; de Luna, J.M. A biosurfactant from *Candida bombicola*: Its synthesis, characterization, and its application as a food emulsions. *Foods* **2022**, *11*, 561. [[CrossRef](#)]
13. Ribeiro, B.G.; de Veras, B.O.; dos Santos Aguiar, J.; Guerra, J.M.C.; Sarubbo, L.A. Biosurfactant produced by *Candida utilis* UFPEDA1009 with potential application in cookie formulation. *Electron. J. Biotechnol.* **2020**, *46*, 14–21. [[CrossRef](#)]
14. Stoklosa, R.J.; Nghiêm, N.P.; Latona, R.J. Xylose-enriched ethanol fermentation stillage from sweet sorghum for xylitol and astaxanthin production. *Fermentation* **2019**, *5*, 84. [[CrossRef](#)]
15. Sirisansaneeyakul, S.; Kop, B.; Tochampa, W.; Wannawilai, S.; Chaveesuk, R.; Lee, W.C. Sodium benzoate stimulates xylitol production by *Candida mogii*. *J. Taiwan Inst. Chem. Eng.* **2014**, *45*, 734–743. [[CrossRef](#)]
16. Gaur, V.K.; Sharma, P.; Sirohi, R.; Varjani, S.; Taherzadeh, M.J.; Chang, J.-S.; Yong Ng, H.; Wong, J.W.C.; Kim, S.-H. Production of biosurfactants from agro-industrial waste and waste cooking oil in a circular bioeconomy: An overview. *Bioresour. Technol.* **2022**, *343*, 126059. [[CrossRef](#)]
17. Karnwal, A. Biosurfactant production using bioreactors from industrial byproducts. In *Biosurfactants for a Sustainable Future: Production and Applications in the Environment and Biomedicine*; Sarma, H., Prasad, M.N.V., Eds.; Wiley: Hoboken, NJ, USA, 2021; ISBN 978-111967102-2; 978-111967100-8.
18. Lisboa, M.C.; Wiltshire, F.M.S.; Fricks, A.T.; Dariva, C.; Carrière, F.; Lima, Á.S.; Soares, C.M.F. Oleochemistry potential from brazil northeastern exotic plants. *Biochimie* **2020**, *178*, 96–104. [[CrossRef](#)]
19. Haala, F.; Dielentheis-Frenken, M.R.E.; Brandt, F.M.; Karmainski, T.; Blank, L.M.; Tiso, T. Doe-based medium optimization for improved biosurfactant production with *Aureobasidium pullulans*. *Front. Bioeng. Biotechnol.* **2024**, *12*, 1379707. [[CrossRef](#)]
20. Santos-Gandelman, J.F.; Cruz, K.; Crane, S.; Muricy, G.; Giambiagi-DeMarval, M.; Barkay, T.; Laport, M.S. Potential application in mercury bioremediation of a marine sponge-isolated *Bacillus cereus* strain pj1. *Curr. Microbiol.* **2014**, *69*, 374–380. [[CrossRef](#)]

21. Najafi, A.R.; Rahimpour, M.R.; Jahanmiri, A.H.; Roostaazad, R.; Arabian, D.; Ghobadi, Z. Enhancing biosurfactant production from an indigenous strain of *Bacillus mycoides* by optimizing the growth conditions using a response surface methodology. *Chem. Eng. J.* **2010**, *163*, 188–194. [[CrossRef](#)]
22. Darwesh, O.M.; Mahmoud, M.S.; Barakat, K.M.; Abuellil, A.; Ahmad, M.E. Improving the bioremediation technology of contaminated wastewater using biosurfactants produced by novel bacillus isolates. *Heliyon* **2021**, *7*, e08616. [[CrossRef](#)] [[PubMed](#)]
23. Selva Filho, A.A.P.; Faccioli, Y.E.; Converti, A.; da Silva, R.D.C.F.S.; Sarubbo, L.A. Maximization of the production of a low-cost biosurfactant for application in the treatment of soils contaminated with hydrocarbons. *Sustainability* **2024**, *16*, 7970. [[CrossRef](#)]
24. dos Santos Souza, T.G.; da Silva, M.M.; Feitoza, G.S.; de Melo Alcântara, L.F.; da Silva, M.A.; de Oliveira, A.M.; de Oliveira Farias de Aguiar, J.C.R.; do Amaral Ferraz Navarro, D.M.; de Aguiar Júnior, F.C.A.; da Silva, M.V.; et al. Biological safety of *Syagrus coronata* (mart.) Becc. Fixed oil: Cytotoxicity, acute oral toxicity, and genotoxicity studies. *J. Ethnopharmacol.* **2021**, *272*, 113941. [[CrossRef](#)]
25. da Silva, I.A.; de Almeida, F.C.G.; Alves, R.N.; Cunha, M.C.C.; de Oliveira, J.C.M.; Fernandes, M.L.B.; Sarubbo, L.A. The formulation of a natural detergent with a biosurfactant cultivated in a low-cost medium for use in coastal environmental remediation. *Fermentation* **2024**, *10*, 332. [[CrossRef](#)]
26. Campos, J.M.; Stamford, T.L.M.; Sarubbo, L.A. Characterization and application of a biosurfactant isolated from *Candida utilis* in salad dressings. *Biodegradation* **2019**, *30*, 313–324. [[CrossRef](#)] [[PubMed](#)]
27. Lima, B.G.A.; Santos, J.C.V.; Silva, R.R.; Caldas, M.C.F.; Meira, H.M.; Rufino, R.D.; Sarubbo, L.A.; Luna, J.M. Sustainable production of biosurfactant grown in medium with industrial waste and use for removal of oil from soil and seawater. *Surfaces* **2024**, *7*, 537–549. [[CrossRef](#)]
28. Cooper, D.G.; Goldenberg, B.G. Surface-active agents from two bacillus species. *Appl. Environ. Microbiol.* **1987**, *53*, 224–229. [[CrossRef](#)]
29. Tiquia, S.M.; Tam, N.F.Y.; Hodgkiss, I.J. Effects of composting on phytotoxicity of spent pig-manure sawdust litter. *Environ. Pollut.* **1996**, *93*, 249–256. [[CrossRef](#)]
30. Bona, E.A.M.D.; Pinto, F.G.d.S.; Fruet, T.K.; Jorge, T.C.M.; Moura, A.C. Comparison of methods for evaluation of antimicrobial activity and determination of minimum inhibitory concentration (mic) of aqueous and ethanol plant extracts. *Arq. Inst. Biol.* **2014**, *81*, 218–225. [[CrossRef](#)]
31. CLSI, Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Disk Susceptibility Testing*, 30th ed.; CLSI supplement M100; Clinical and Laboratory Standards Institute: Wayne, NY, USA, 2020.
32. Zajic, J.E.; Seffens, W.; Panchal, C. Biosurfactants. *Crit. Rev. Biotechnol.* **1983**, *1*, 87–107. [[CrossRef](#)]
33. da Silva, R.R.; Santos, J.C.V.; Meira, H.M.; Almeida, S.M.; Sarubbo, L.A.; Luna, J.M. Microbial biosurfactant: *Candida bombicola* as a potential mediator of environments contaminated by heavy metals. *Microorganisms* **2023**, *11*, 2772. [[CrossRef](#)]
34. Fadhile Almansoory, A.; Abu Hasan, H.; Idris, M.; Sheikh Abdullah, S.R.; Anuar, N.; Musa Tibin, E.M. Biosurfactant production by the hydrocarbon-degrading bacteria (hdb) *Serratia marcescens*: Optimization using central composite design (ccd). *J. Ind. Eng. Chem.* **2017**, *47*, 272–280. [[CrossRef](#)]
35. Anscombe, F.J. Graphs in statistical analysis. *Am. Stat.* **1973**, *27*, 17–21. [[CrossRef](#)]
36. Derringer, G.; Suich, R. Simultaneous optimization of several response variables. *J. Qual. Technol.* **1980**, *12*, 214–219. [[CrossRef](#)]
37. Yang, L.; Li, Y.; Zhang, X.; Liu, T.; Chen, J.; Wei, L.; Hua, Q. Metabolic profiling and flux distributions reveal a key role of acetyl-coa in sophorolipid synthesis by *Candida bombicola*. *Biochem. Eng. J.* **2019**, *145*, 74–82. [[CrossRef](#)]
38. Abbasi, H.; Sharafi, H.; Alidost, L.; Bodagh, A.; Zahiri, H.S.; Noghabi, K.A. Response surface optimization of biosurfactant produced by *Pseudomonas aeruginosa* ma01 isolated from spoiled apples. *Prep. Biochem. Biotechnol.* **2013**, *43*, 398–414. [[CrossRef](#)]
39. Taowkrue, E.; Songdech, P.; Maneerat, S.; Soontorngun, N. Enhanced production of yeast biosurfactant sophorolipids using yeast extract or the alternative nitrogen source soybean meal. *Ind. Crops Prod.* **2024**, *210*, 118089. [[CrossRef](#)]
40. Vedaraman, N.; Venkatesh, N. Production of surfactin by *Bacillus subtilis* MTCC 2423 from waste frying oils. *Braz. J. Chem. Eng.* **2011**, *28*, 175–180. [[CrossRef](#)]
41. Amiri, F.; Habibi, A. Free fatty acids in sunflower oil soap stock progressed the sophorolipid production by *Candida catenulata*. *Can. J. Chem. Eng.* **2024**, *102*, 53–64. [[CrossRef](#)]
42. Jadhav, J.V.; Pratap, A.P.; Kale, S.B. Evaluation of sunflower oil refinery waste as feedstock for production of sophorolipid. *Process Biochem.* **2019**, *78*, 15–24. [[CrossRef](#)]
43. Xu, F.; Chen, Y.; Zou, X.; Chu, J.; Tian, X. Precise fermentation coupling with simultaneous separation strategy enables highly efficient and economical sophorolipids production. *Bioresour. Technol.* **2023**, *388*, 129719. [[CrossRef](#)] [[PubMed](#)]
44. El-Shahed, M.M.S.; Mohamed, S.H.; Sadik, M.W.; Mabrouk, M.I.; Sedik, M.Z. Applications of *Candida tropicalis* bioactive biosurfactant produced using simple substrate medium. *Egypt. J. Bot.* **2022**, *62*, 371–387. [[CrossRef](#)]
45. Ankulkar, R.; Chavan, M. Characterisation and application studies of sophorolipid biosurfactant by *Candida tropicalis* ra1. *J. Pure Appl. Microbiol.* **2019**, *13*, 1653–1665. [[CrossRef](#)]
46. Ribeiro, B.G.; Guerra, J.M.C.; Sarubbo, L.A. Biosurfactants: Production and application prospects in the food industry. *Biotechnol. Prog.* **2020**, *36*, e3030. [[CrossRef](#)] [[PubMed](#)]
47. Bezerra, K.G.O.; Durvala, I.J.B.; Silva, I.A.; Almeida, F.C.G.; Melo, Y.T.F.; Rufino, R.D.; Sarubbo, L.A. Emulsifying capacity of biosurfactants from *Chenopodium quinoa* and *Pseudomonas aeruginosa* UCP 0992 with focus of application in the cosmetic industry. *Chem. Eng. Trans.* **2020**, *79*, 211–216. [[CrossRef](#)]

48. Durval, I.J.B.; Resende, A.H.M.; Ostendorf, T.A.; Oliveira, K.G.O.; Luna, J.M.; Rufino, R.D.; Sarubbo, L.A. Application of *Bacillus cereus* UCP 1615 biosurfactant for increase dispersion and removal of motor oil from contaminated sea water. *Chem. Eng. Trans.* **2019**, *74*, 319–324. [[CrossRef](#)]
49. Silva, I.A.; Fortunato, J.G.L.A.; Almeida, F.C.G.; Alves, R.N.; Cunha, M.C.C.; Rufino, R.D.; Fernandes, M.L.B.; Sarubbo, L.A. Production and application of a new biosurfactant for solubilisation and mobilisation of residual oil from sand and seawater. *Proceses* **2024**, *12*, 1605. [[CrossRef](#)]
50. Radha, P.; Suhazsini, P.; Prabhu, K.; Jayakumar, A.; Kandasamy, R. Chicken tallow, a renewable source for the production of biosurfactant by *Yarrowia lipolytica* MTCC9520, and its application in silver nanoparticle synthesis. *J. Surfactants Deterg.* **2020**, *23*, 119–135. [[CrossRef](#)]
51. Pinto, I.; Buss, A. Z potential as a measure of asphalt emulsion stability. *Energy Fuels* **2020**, *34*, 2143–2151. [[CrossRef](#)]
52. Lima, R.A.; Silva Andrade, R.F.; Antunes, A.A.; Casullo, H.A.; Jara, A.M.A.T.; Berguer, L.R.R.; de Campos-Takaki, G.M. *Biosurfactants Production by Candida Glabrata Strains Using Industrial Wastes as Carbon and Nitrogen Sources*; World Scientific Publishing Co.: Singapore, 2012; ISBN 978-981440504-1; 9814405035; 978-981440503-4.
53. Ganji, Z.; Beheshti-Maal, K.; Massah, A.; Emami-Karvani, Z. A novel sophorolipid-producing *Candida keroseneae* GBME-IAUF-2 as a potential agent in microbial enhanced oil recovery (meor). *FEMS Microbiol. Lett.* **2020**, *367*, fnaa144. [[CrossRef](#)]
54. Mendes, P.M.; Ribeiro, J.A.; Martins, G.A.; Lucia, T.; Araujo, T.R.; Fuentes-Guevara, M.D.; Corrêa, L.B.; Corrêa, É.K. Phytotoxicity test in check: Proposition of methodology for comparison of different method adaptations usually used worldwide. *J. Environ. Manag.* **2021**, *291*, 112698. [[CrossRef](#)]
55. Matosinhos, R.D.; Cesca, K.; Carciofi, B.A.M.; de Oliveira, D.; de Andrade, C.J. The biosurfactants mannosylerythritol lipids (mels) as stimulant on the germination of *Lactuca sativa* L. *Agriculture* **2023**, *13*, 1646. [[CrossRef](#)]
56. Nogueira Felix, A.K.; Martins, J.J.L.; Lima Almeida, J.G.; Giro, M.E.A.; Cavalcante, K.F.; Maciel Melo, V.M.; Loiola Pessoa, O.D.; Ponte Rocha, M.V.; Rocha Barros Gonçalves, L.; Saraiva de Santiago Aguiar, R. Purification and characterization of a biosurfactant produced by *Bacillus subtilis* in cashew apple juice and its application in the remediation of oil-contaminated soil. *Colloids Surf. B Biointerfaces* **2019**, *175*, 256–263. [[CrossRef](#)]
57. Joshi, S.G.; Paff, M.; Friedman, G.; Fridman, A.; Brooks, A.D. Control of methicillin-resistant *Staphylococcus aureus* in planktonic form and biofilms: A biocidal efficacy study of nonthermal dielectric-barrier discharge plasma. *Am. J. Infect. Control* **2010**, *38*, 293–301. [[CrossRef](#)] [[PubMed](#)]
58. Sun, J.; Warden, A.R.; Huang, J.; Wang, W.; Ding, X. Colorimetric and electrochemical detection of *Escherichia coli* and antibiotic resistance based on a p-benzoquinone-mediated bioassay. *Anal. Chem.* **2019**, *91*, 7524–7530. [[CrossRef](#)] [[PubMed](#)]
59. Kothe, C.I.; Pessoa, J.P.; Malheiros, P.S.; Tondo, E.C. Assessing the growth of *Staphylococcus aureus* and *Escherichia coli* on fruits and vegetables. *J. Infect. Dev. Ctries* **2019**, *13*, 480–486. [[CrossRef](#)] [[PubMed](#)]
60. EUCAST The European Committee on Antimicrobial Susceptibility Testing. Breakpoint Tables for Interpretation of MICs and Zone Diameters. Version 14.0. 2024. Available online: https://www.eucast.org/clinical_breakpoints (accessed on 3 December 2024).
61. Barrantes, K.; Araya, J.J.; Chacón, L.; Procupez-Schtirbu, R.; Lugo, F.; Ibarra, G.; Soto, V.H. Antiviral, antimicrobial, and antibiofilm properties of biosurfactants. In *Biosurfactants for a Sustainable Future: Production and Applications in the Environment and Biomedicine*; Sarma, H., Prasad, M.N.V., Eds.; Wiley: Hoboken, NJ, USA, 2021; Volume 5, pp. 245–268.
62. Haetinger, V.S.; Dalmoro, Y.K.; Godoy, G.L.; Lang, M.B.; de Souza, O.F.; Aristimunha, P.; Stefanello, C. Optimizing cost, growth performance, and nutrient absorption with a bio-emulsifier based on lysophospholipids for broiler chickens. *Poult. Sci.* **2021**, *100*, 101025. [[CrossRef](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.