



## Article

# Production, Characterization and Application of Biosurfactant for Cleaning Cotton Fabric and Removing Oil from Contaminated Sand

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**Abstract:** Biosurfactants are a group of environmentally friendly amphiphilic molecules that are applicable in numerous industries as essential biotechnology products, such as food production, cleaning products, pharmacology, cosmetics, pesticides, textiles and oil and gas fields. In this sense, and knowing the potential of these biomolecules, the aim of this work was to produce a biosurfactant, characterize it regarding its chemical and surfactant properties and investigate its potential in the removal of contaminants and in the cleaning of cotton fabrics. The biosurfactant was initially obtained from the cultivation of the microorganism *Candida glabrata* UCP 1002 in medium containing distilled water with 2.5% residual frying oil, 2.5% molasses and 2.5% corn steep liquor agitated at 200 rpm for 144 h. The biosurfactant reduced the surface tension of water from 72 to 29 mN/m. The toxicity potential of the biosurfactant was evaluated using *Tenebrio molitor* larvae and demonstrated non-toxicity. The biosurfactant was applied as a degreaser of engine oil on cotton fabric, and showed 83% ( $2 \times$  CMC), 74% ( $1 \times$  CMC) and 78% ( $1/2 \times$  CMC) oil removal. Therefore, the biosurfactant produced in this work has promising surfactant and emulsifying properties with potential for application in various industrial segments.

**Keywords:** *Candida glabrata*; industry; detergents; contaminants



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## 1. Introduction

Biosurfactants are biomolecules that act as surface agents, capable of enhancing surface–surface interactions through micelle formation. They are naturally produced and/or synthesized by microorganisms and are frequently used across various industries [1]. These amphiphilic molecules with hydrophilic and hydrophobic moieties lower interfacial tension between fluids with different degrees of polarity. The hydrophilic part is generally composed of a carbohydrate, a cationic or anionic peptide or an amino acid, while the nonpolar hydrophobic tail can consist of a peptide, a protein and a saturated or unsaturated fatty acid [2].

Several microorganisms (filamentous fungi, bacteria, yeasts) can efficiently produce biosurfactants. However, the quantity and quality of the biosurfactant product rely on factors that play a fundamental role in their production, such as the microorganism used, the medium composition, substrate characteristics and other intrinsic and extrinsic factors of the microorganisms' growth, including carbon and nitrogen sources and their proportions, temperature, salinity, vitamins, oxygen availability, inhibitors, inducers, metabolic regulators, minerals, aeration and agitation [3–6]. Due to their biological origin, microbial surfactants possess excellent properties and characteristics such as environmental compatibility, biodegradability, low toxicity, high selectivity and specificity and stability in environments with different temperatures, pH values and extreme salinity. They can be formulated using renewable and economical materials, such as agro-industrial waste [7].

Despite displaying many advantageous properties, there are various challenges related to the production and commercialization of biosurfactants. However, biosurfactants have shown an increasing use trend due to their potential applications and development [8]. The global biosurfactant market is expected to reach USD 1.443 billion by 2026 [9], and the annual growth rate demonstrates massive demand and increasing applications of these surfactants across various sectors worldwide. Because of their ecological characteristics and promising properties, biosurfactants are widely used in agriculture and chemical processes, in the cleaning and detergent industries, in cosmetics and pharmaceuticals and in the food, leather, paper and textile industries [10,11].

In addition to these applications, biosurfactants are excellent candidates for bioremediation, as these natural products have the ability to enhance the solubility and bioavailability of hydrophobic contaminants. Bioremediation is a viable option for recovering areas contaminated by oily compounds with greater sustainability compared to physicochemical processes. The use of these natural surfactants has been extensively explored since they are capable of mobilizing, emulsifying and solubilizing compounds, enhancing biodegradation processes in soil, as well as enabling bioremediation and wastewater treatment [12–14].

Currently, biosurfactants are widely used in household cleaning products and clothing detergents due to their versatile properties, such as dispersing, wetting, foaming, emulsifying and reducing surface tension, as well as their antimicrobial and antibiofilm activities, which can be effective in various washing processes [15]. These bio-based surfactants present an alternative to synthetic detergents, which are difficult for bacteria to degrade in water, leading to the accumulation of these detergents in the environment and becoming a source of pollution in water flows [16]. As commercial detergents have active ingredients in the form of linear alkylbenzene sulfonate surfactants, which are petroleum derivatives containing benzene and paraffin, such products pose a significant environmental problem [16,17].

Various research works have shown the great potential of biosurfactants in remediating and cleaning environments contaminated by petroleum derivatives, as well as acting as textile detergents. Thus, the aim of this study was to produce a biosurfactant from *Candida glabrata* UCP 1002, characterize it in terms of its chemical and surfactant properties and investigate its efficiency in removing contaminants and as a biodetergent in cleaning cotton fabrics.

## 2. Materials and Methods

### 2.1. Microorganism

The yeast *Candida glabrata* UCP 1002 was obtained from the Culture Collection of the Multiuser Center for Analysis and Characterization of Biomolecules and Surfaces, located at the Catholic University of Pernambuco, Recife, Brazil, and was evaluated as biosurfactant producer. The cultures were preserved in test tubes containing yeast mold agar (YMA) media, stored at 5 °C.

## 2.2. Substrates for the Production Medium

Byproducts from agro-industrial processes were utilized as substrates for biosurfactant synthesis. Molasses was supplied by the São José Sugar Mill, situated in Igarassu, Pernambuco, Brazil. Corn steep liquor was obtained from Corn Products of Brazil, located in Cabo de Santo Agostinho, Pernambuco, Brazil. Waste frying oil was acquired from restaurants in the city of Recife, Pernambuco, Brazil.

## 2.3. Biosurfactant Production

*C. glabrata* UCP 1002 was placed in a distilled water medium supplemented with 2.5% residual frying oil, 2.5% corn steep liquor and 2.5% molasses. The pH of the medium was adjusted to 5.5, followed by incubation with a cell suspension of  $10^7$  cells/mL in a shaker at 200 rpm and 28 °C for 144 h, based on previously established conditions [18].

## 2.4. Determination of Surface Tension and Critical Micelle Concentration

Surface tension of the biosurfactant was determined using a tensiometer with a du Nouÿ ring (KSV Instruments Ltd., Sigma 700, Helsinki, Finland). The platinum ring was immersed in the cell-free metabolic liquid. The force required to pull the ring through the liquid/air interface was recorded. The calculation of the critical micelle concentration (CMC) was performed by measuring the surface tension of dilutions of the biosurfactant in distilled water until obtaining a constant surface tension. The CMC was derived from a surface tension vs. concentration of biosurfactant graph and expressed in g/L [18].

## 2.5. Biosurfactant Extraction

The extraction of the biosurfactant was performed using a one-to-four ratio of crude metabolic liquid to solvent (ethyl acetate). The procedure was performed twice, followed by the centrifugation of the solvent at 4500 rpm for 15 min. The organic phase was placed in a separating funnel. The sample was washed with a saturated sodium chloride (NaCl) solution, and any aqueous phase that appeared thereafter was discarded. The solvent was dried with sodium sulfate and filtered. Lastly, a heating plate was used to evaporate the organic phase and obtain the purified biosurfactant [18].

## 2.6. Emulsification Index

The calculation of emulsification index was performed using the method described by Cooper and Goldenberg [19]. First, 2 mL of a hydrocarbon was added to 2 mL of the cell-free broth in a test tube, which was vortexed for two minutes. After 24 h, the stability of the emulsion was assessed. Emulsion height was divided by total height of the mixture, and the result was multiplied by 100 for determination of the emulsification index.

## 2.7. Ionic Charge

A zeta potentiometer equipped with a Zeta-Meter 4.0 + ZM3-DG Direct Imaging system (Zeta Meter Inc., Harrisonburg, VA, USA) was used for the determination of the ionic charge of the isolated biosurfactant [18].

## 2.8. Fourier-Transform Infrared (FT-IR)

Spectroscopy (Spectrum 400, Perkin Elmer, Shelton, CT, USA) was used to characterize the chemical composition and structure of the semi-purified biosurfactant [18].

## 2.9. Nuclear Magnetic Resonance (NMR)

Spectroscopy was used to characterize the chemical composition and structure of the isolated biosurfactant employing a 300 MHz spectrometer (Agilent, Santa Clara, CA, USA) operating at 300.13 MHz. The semi-purified biosurfactant was dissolved in dimethyl sulfoxide (DMSO), and the NMR spectra were recorded at 25 °C. Chemical shifts ( $\delta$ ) relative to the tetramethylsilane range were expressed in ppm.

### 2.10. Toxicity Assay in *Tenebrio molitor*

Larvae of *Tenebrio molitor*, each weighing about 100 mg, were placed in Petri dishes in groups of five individuals. An amount of 10  $\mu$ L of biosurfactant at a concentration of 0.3 g/L (CMC) was administered to the larvae using a Hamilton syringe. Larval mortality, determined by a lack of movement, was monitored at 24, 48, 72 and 96 h. PSB was used as the negative control. Survival curves were plotted over time for the determination of the results, following Silva et al. [20].

### 2.11. Hydrophobic Compound Removal from Sand by Biosurfactant Static Test

Glass columns (measuring 55  $\times$  6 cm) were filled with approximately 200 g of sand contaminated with a hydrophobic pollutant at a concentration of 10% in a solution. A total of 200 mL of the biosurfactant solutions were added to the columns. The biosurfactant was used at concentrations corresponding to  $\frac{1}{2}\times$ , CMC and  $2\times$  CMC. The cell-free metabolic liquid and the chemical surfactant sodium dodecyl sulfate (SDS) were also tested. The control was a column with contaminated soil and 200 mL of distilled water without the biosurfactant. After 24 h of biosurfactant solution percolation, samples were collected to estimate the motor oil content by gravimetric analysis. Hexane was used to extract the residual motor oil into a separating funnel. Extraction was repeated twice to ensure complete oil recovery, followed by evaporation of the hexane and the determination of the weight of the oil removed [21].

### 2.12. Application of Biosurfactant as a Cleaning and Degreasing Agent for Oil on Fabric

The biosurfactant obtained from *Candida glabrata* UCP 1002 was used for cleaning and degreasing burnt motor oil impregnated into cotton fabric using a methodology adapted from Andrade et al. [22]. Clean white cotton fabric cut into pieces measuring 2  $\times$  2 cm was impregnated with burnt motor oil obtained from the automotive industry. The cotton fabric sample was impregnated with a drop of burnt motor oil, and after the fabric absorbed it, the sample was immersed in aqueous biosurfactant solutions at concentrations of 0.15 g/L ( $\frac{1}{2}\times$  CMC), 0.3 g/L ( $1\times$  CMC) and 0.6 g/L ( $2\times$  CMC). The washing of the cotton fabric occurred at an agitation speed of 150 rpm over time 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 the cleaning and degreasing of burnt motor oil by the biosurfactant from *C. glabrata* was examined by optical microscopy. The percentage of burnt motor oil removed from the cotton fabric by the action of the biosurfactant and other conditions tested was determined according to Equation (1):

$$W = [m_{\text{total}} - m_i / m_{\text{total}}] \times 100 \quad (1)$$

in which  $m_{\text{total}}$  = total mass of oil applied and  $m_i$  = residual oil in fabric after treatment.

## 3. Results

### 3.1. Biosurfactant Production and Yield

One of the advantages of formulating natural surfactants using residues is the sustainable production. A variety of renewable sources are used in fermentation processes, such as byproducts from food processing and agro-industrial processes [23]. In addition to the significant cost reduction achieved by using these residues, the large amount of lipids and carbohydrates in these byproducts are a great source of primary or supplementary carbon for the growth of microorganisms [24,25].

Various factors can exert an impact on the synthesis, quantity and quality of the biosurfactant produced. Such variables differ depending on the specific microorganism, growth environment and type of biosurfactant being formulated. Particularly, carbon and nitrogen sources are of great importance. The concentration of carbon (sugars, hydrocarbons or organic acids) and the nitrogen source significantly impact production and can affect yield.

Nitrogen sources can also influence metabolism and cell growth. Other important parameters that can influence microorganism metabolism and biosurfactant synthesis include pH levels of the growth medium, temperature, aeration and oxygen [26–28].

The microbial surfactant developed in this study was formulated using distilled water supplemented with 2.5% residual frying oil, 2.5% molasses and 2.5% corn steep liquor. The corn steep liquor acted as a nitrogen source, as it contains various amino acids, organic salts and vitamins. Molasses was used as one of the carbon sources, as its main components include sucrose, fructose, glucose and fermentable sugars, as well as vitamins and minerals. The residual frying oil was used as a supplementary carbon source for biomolecule synthesis during fermentation [18]. After 114 h of cultivation at 200 rpm and 28 °C, the biosurfactant produced by *C. glabrata* UCP 1002 reduced the surface tension of water from 72 to 29 mN/m, and after the isolation process, a yield of 9.0 g/L was obtained.

According to the literature, various microorganisms are used for biosurfactant production. Wu et al. [29] studied a biosurfactant obtained from *Bacillus subtilis* and after fermentation, the surface tension of the cell free metabolic liquid was reduced to 25.6 mN/m, and a yield of 1369 mg/L was obtained. Chotard et al. [30] produced a biosurfactant using *Mucor plumbeus* UBOCC-A-111133 and reduced the surface tension of the medium to 31 mN/m.

According to Cooper and Goldenberg [19], a microorganism is promising for biosurfactant production if it can reduce the surface tension to 40 mN/m or less.

### 3.2. Surface Tension and Critical Micelle Concentration

A surface-active agent is a compound with surfactant activity, which indicates its ability to adsorb to interfaces and reduce the surface tension of water. Surface tension is an important parameter in various physical phenomena, such as wetting, catalysis, adsorption and distillation, and is directly involved in the creation of many industrial products. The phenomenon of surface tension reduction occurs when a surfactant is mixed with water, and the water/air interface becomes occupied by surfactant monomers, with the hydrophilic group pointing toward the water and the hydrophobic chain toward the air. When surface tension reduction occurs, the surfactant molecules pack together tightly at the interface. When this packing reaches its maximum, spherical aggregates, micelles, vesicles and bilayers are formed. This is called critical micelle concentration (CMC) [18,31].

The biosurfactant produced by *C. glabrata* UCP1002 lowered the surface tension of water to 29 mN/m, demonstrating excellent surface tension reducing capacity. Micelle formation occurred when the concentration of 0.3 g/L was reached.

In a study involving a biosurfactant produced by the yeast *Starmerella bombicola* ATC 222214, Selva Filho et al. [32] obtained a reduction in surface tension to 33.2 mN/m, with a CMC of 2.0 g/L of biosurfactant.

Lima et al. [33] evaluated the surface tension reduction capacity of a natural surfactant produced by *Candida lipolytica* UCP0988 in a medium containing 2.5% residual frying oil, 2.5% corn steep liquor and 4.0% molasses. Surface tension was lowered to 25 mN/m and the CMC was 0.5 g/L.

### 3.3. Determination of Emulsification Index

Emulsification activity refers to the ability of a biosurfactant to generate turbidity due to suspended hydrocarbons in a liquid system [34]. The emulsification index is an important method used to support the selection of potential biosurfactant producers and serves as a qualitative screening test [35]. Bioemulsifiers have applications in the cosmetic, petroleum, textiles, agriculture and food industries [36]. The biosurfactant produced showed a good emulsification index, as the molecules facilitated the mixing of immiscible substances for the hydrocarbons tested, particularly for burnt motor oil, with 100% emulsification, followed by 50% for corn oil, 48% for cottonseed oil, 43% for soybean oil and 33% for canola oil (Table 1).



**Table 1.** Emulsification index of different hydrocarbons using the biosurfactant from *C. glabrata* cultivated in distilled water supplemented with 2.5% residual frying oil, 2.5% corn steep liquor and 2.5% molasses.

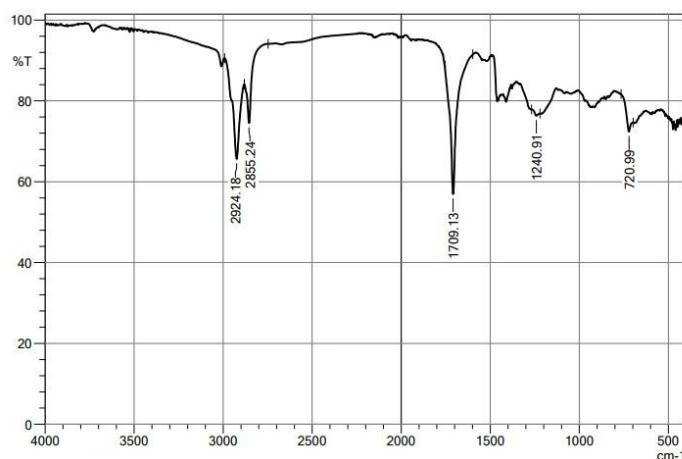
IE (%) Motor Oil	IE (%) Corn Oil	IE (%) Cottonseed Oil	IE (%) Soybean Oil	IE (%) Canola Oil
100 ± 1.3	50 ± 1.1	48 ± 1.2	43 ± 1.5	33 ± 1.7

### 3.4. Determination of Ionic Charge

The Zeta potentiometer revealed that the biosurfactant produced by *C. glabrata* had a negative charge in the hydrophilic area of  $-81.4$  ZPmV and  $89.506$   $\mu\text{S}/\text{cm}$  at  $25.7$   $^{\circ}\text{C}$ , suggesting an anionic surfactant. Previous studies have documented anionic properties of other biosurfactants produced by the *Candida* genus using a Zeta potentiometer (Zeta Meter, Inc., Harrisonburg, VA, USA) [33,37].

### 3.5. Fourier-Transform Infrared Spectroscopy (FT-IR)

The FT-IR spectrum is shown in Figure 1. The main functional groups identified in the sample include a carbonyl group (C=O) with a peak at  $1709$   $\text{cm}^{-1}$ , strongly suggesting that the molecule may contain a ketone, aldehyde or carboxylic acid. C-H groups from aliphatic chains appear at  $2855$   $\text{cm}^{-1}$  and  $2924$   $\text{cm}^{-1}$ , which are typical of saturated hydrocarbon chains such as alkanes or part of a molecule containing long methyl and methylene chains.

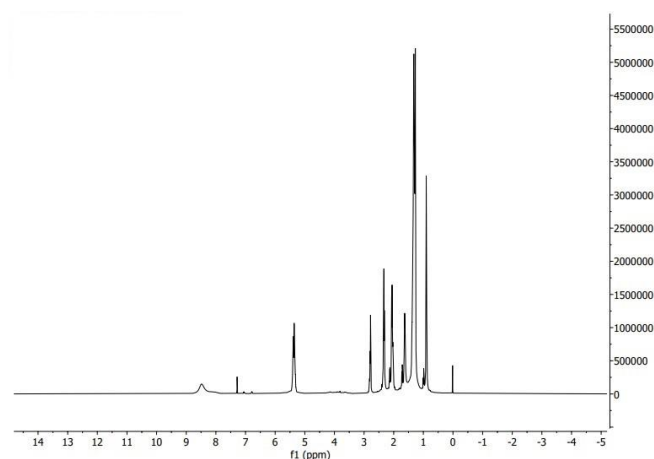


**Figure 1.** FT-IR spectrum of the semi-purified biosurfactant produced by *C. glabrata* UCP 1002 in distilled water with 2.5% residual frying oil, 2.5% corn steep liquor and 2.5% molasses.

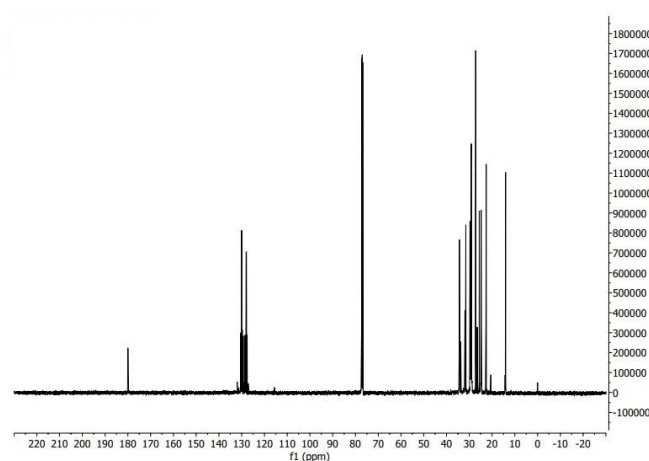
### 3.6. Nuclear Magnetic Resonance Spectroscopy

The  $^1\text{H}$  NMR analysis (Figure 2) of the semi-purified biosurfactant revealed the presence of hydrogens attached to saturated carbons in the region from 0 to 3 ppm, suggesting the presence of saturated aliphatic chains commonly found in fatty acids or lipids. Hydrogens from alkenes or near electronegative groups are present in the region between 4 and 6 ppm, aromatic hydrogens between 6 and 8 ppm and the regions from 9 to 10 ppm are typical of aldehydes or carboxylic acids.

In the  $^{13}\text{C}$  NMR spectrum (Figure 3), the region from 0 to 50 ppm suggests the presence of long aliphatic chains, characteristic of fatty acids. Signals typically associated with carbons in aliphatic chains are found between 0 and 100 ppm. The region between 100 and 150 ppm indicates carbons in aromatic rings or double bonds (alkenes), and the region from 150 to 200 ppm corresponds to carbons in carbonyl groups such as ketones and carboxylic acids.



**Figure 2.**  $^1\text{H}$  NMR spectrum of biosurfactant isolated from *C. glabrata* in distilled water supplemented with 2.5% residual frying oil, 2.5% corn steep liquor and 2.5% molasses.



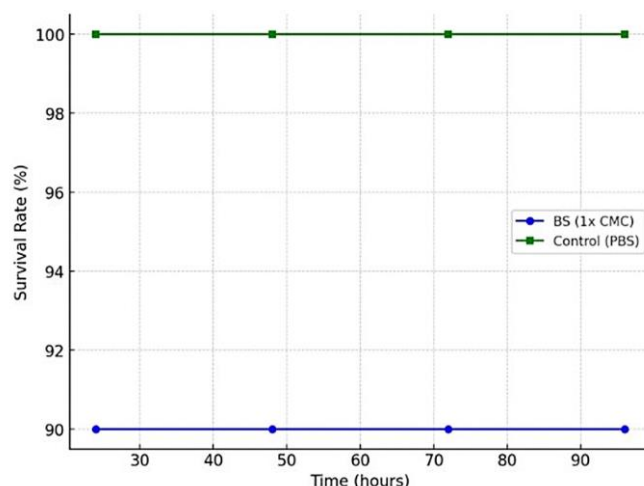
**Figure 3.**  $^{13}\text{C}$  NMR spectrum of biosurfactant isolated from *C. glabrata* in distilled water supplemented with 2.5% residual frying oil, 2.5% corn steep liquor and 2.5% molasses.

### 3.7. *Tenebrio molitor* Toxicity Assay

After 96 h of observation, no significant difference in survival rates was found between the control group and groups tested with the formulated biosurfactant. The survival rate was 90%, suggesting that the biosurfactant at the tested concentration did not cause significant toxicity to *Tenebrio molitor* larvae, according to the survival curve (Figure 4). Lima et al. [38] conducted a similar test and used a biosurfactant produced by *C. lipolytica*; no mortality was observed in larvae subjected to the tested concentrations, demonstrating this test is a simple, economical and viable model for evaluating toxic effects in living organisms.

### 3.8. Hydrophobic Compound Removal from Sand by Biosurfactant in Static Assay

The contamination of soil and marine environments has contributed to the increased research into bioremediation. Physical and chemical methods are often used to remove hydrocarbons from environments; however, these techniques present many disadvantages when compared to biological techniques. Bioremediation emerges as a more economical, efficient and environmentally friendly alternative, capable of mineralizing pollutants or transforming them into less harmful substances. To choose which in situ or ex situ bioremediation technique is most appropriate, preliminary analyses of the environmental conditions, type of pollutant, composition of the solid to be treated, removal costs and treatment duration are necessary [39].



**Figure 4.** Toxicity effects of the biosurfactant on larvae of *Tenebrio molitor* compared to negative control (phosphate-buffered saline).

In this study, the formulated biosurfactant was employed as an agent for removing hydrophobic compounds adsorbed on sand. It was demonstrated that the biosurfactant produced by *C. glabrata* removed the contaminant under all the tested biosurfactant conditions (Table 2), as well as when using the metabolic liquid (crude biosurfactant). The removal rate varied between 92.1%, 97%, 95.7% and 97.8% for  $\frac{1}{2} \times$  CMC,  $1 \times$  CMC,  $2 \times$  CMC and metabolic liquid, respectively, surpassing the removal rate achieved by SDS (chemical surfactant).

**Table 2.** Removal of hydrophobic contaminants from sand in static assay.

Solutions	Oil Removal (%)
Control (distilled water)	28.3 ± 1.3
Metabolic liquid	97.8 ± 1.1
Biosurfactant concentration at $\frac{1}{2} \times$ CMC	92.1 ± 1.4
Biosurfactant concentration at $1 \times$ CMC	97.0 ± 1.1
Biosurfactant concentration at $2 \times$ CMC	95.7 ± 1.2
SDS (Sodium Dodecyl Sulfate)	77.2 ± 1.6

Selva Filho et al. [40] also conducted a static assay for the removal of oil adsorbed to sand in packed columns using plant extract from *Eichhornia crassipes* and achieved success in their results, with 55%, 57% and 68% removal rates at  $\frac{1}{2} \times$  CMC,  $1 \times$  CMC and  $2 \times$  CMC concentrations, respectively. Lima et al. [33] studied the effects of the biosurfactant obtained by *C. lipolytica* for oil compound solubilization and showed good removal rates for the contaminant: 20%, 33% and 50% for  $\frac{1}{2} \times$  CMC,  $1 \times$  CMC and  $2 \times$  CMC concentrations.

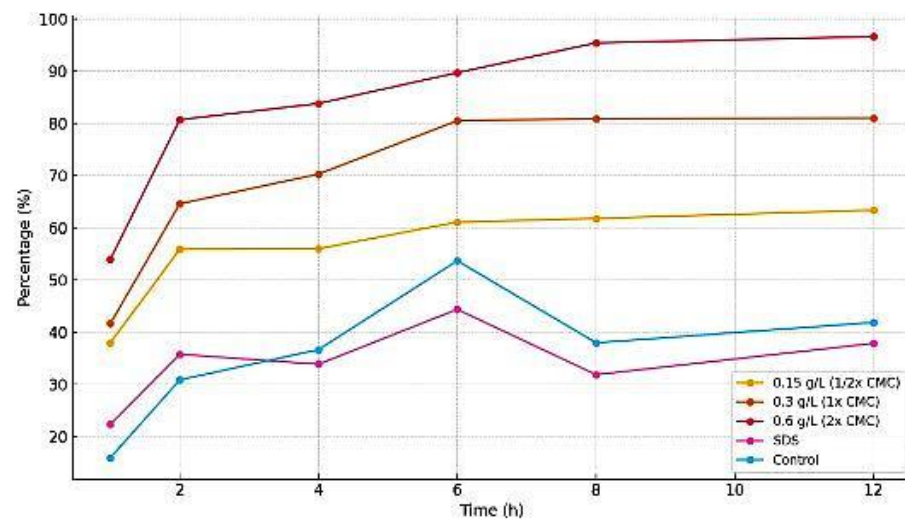
Biosurfactants alter the wettability of soil particles by adsorbing the hydrophobic component on the soil surface and interacting with the hydrophilic component in the aqueous phase. The separation of the contaminant is also improved by the repulsive behavior between the polar head of the biosurfactant and the soil particle [41,42].

### 3.9. Use of Biosurfactant as a Fabric Oil Cleaning and Degreasing Agent

Modern detergents contain softeners, chemical surfactants, oxidizing agents and other harmful components, including emerging contaminants derived from petroleum. These contaminants represent a significant environmental challenge due to the persistence of these products and the potential contamination of surface and groundwater, in addition to subsequent health issues. Considering this problem, the scientific community and industries have been seeking to create more environmentally friendly products using bio-based surfactants [17,22].

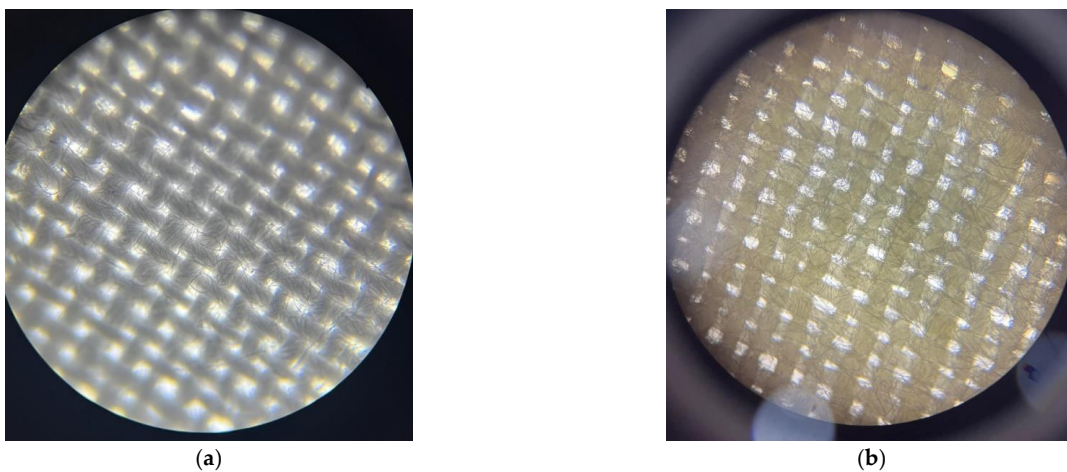


This study investigated the ability of the biosurfactant produced by *C. glabrata* to serve as a degreasing and cleaning agent on cotton fabrics impregnated with burnt motor oil. The washing test was conducted over six time periods to observe the best removal rates. According to the results obtained, the microbial surfactant was able to remove more than 50% of the hydrophobic compound at all tested times at a concentration of 0.6 g/L ( $2\times$  CMC), reaching up to 96.6% after a period of 12 h of washing. Initial results show that there was removal in the first hour of cleaning, with removal percentages of 38%, 41% and 55% for  $\frac{1}{2}\times$  CMC (0.15 g/L),  $1\times$  CMC (0.3 g/L) and  $2\times$  CMC (0.6 g/L) concentrations, respectively. Over time, the removal rates increased significantly, reaching values of 63%, 81% and 96% at the end of 12 h using biosurfactant solutions at  $\frac{1}{2}\times$  CMC (0.15 g/L),  $1\times$  CMC (0.3 g/L) and  $2\times$  CMC (0.6 g/L) concentrations, respectively. Figure 5 shows the washing kinetics of the cotton fabrics.



**Figure 5.** Results of cleaning and degreasing of cotton fabric stained with burnt motor oil using biosurfactant produced by *C. glabrata*.

The studied biosurfactant demonstrated excellent efficiency in removing the contaminant when compared to its chemical counterpart (SDS), whose highest removal rate occurred after 6 h of washing, reaching 44.4% removal. After washing, the cotton fabrics were also evaluated for their structural integrity and observed under an optical microscope, showing that the fibers were intact (Figure 6).



**Figure 6.** Evaluation of the structural integrity of cotton fabrics (a) clean fabric without oil; (b) fabric after washing.

Andrade et al. [22] investigated the capability of a biosurfactant produced by *C. echinulata* to clean cotton fabric stained with burnt motor oil, achieving excellent results with a removal rate of 86% after 1 h of washing. Anidu et al. [43] investigated the cleaning ability of a biosurfactant produced by the S62A strain of *B. anthracis* to clean and degrease fabrics, with maximum oil removal rates results of 78%, 72% and 64% for polyester, cotton and chiffon fabrics stained with 10 mg/mL of critical micelle concentration, respectively.

#### 4. Conclusions

The present results demonstrated that the biosurfactant produced by the yeast *Candida glabrata* UCP 1002 presents promising characteristics both in terms of production efficiency and application. The biosurfactant demonstrated excellent ability to reduce water surface tension, forming micelles at low concentrations, indicating its high surfactant efficiency. Additionally, its ability to emulsify different types of oils, including burnt motor oil, emphasizes its potential for industrial use in bioremediation processes and in the formulation of eco-friendly cleaning products such as textile detergents. The use of agro-industrial waste as substrates for biosurfactant production contributes to the sustainability of the process, making it economically viable and environmentally friendly. Another important point is the low toxicity observed in the tests with *Tenebrio molitor*, which reinforces the safety of the biosurfactant for environmental and industrial applications. Its effectiveness in removing hydrophobic compounds from cotton fabrics and contaminated sand, which was comparable and, in some cases, superior to synthetic surfactants, suggests that the biosurfactant could be an efficient and sustainable alternative to synthetic detergents. Thus, this study significantly contributes to the validation of the utilization of biosurfactants in industrial and environmental solutions, with economic and ecological advantages. It is expected that in the future, after further and more in-depth studies, the production of this biomolecule can expand significantly to meet the demands for sustainable alternatives in the cleaning products industry, bioremediation and other environmental applications. Furthermore, through the development of more efficient purification and extraction processes, it is expected that large-scale production of this biosurfactant will compete directly with synthetic surfactants.

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