Research Article

Promising Biosurfactant Produced by *Cunninghamella echinulata* UCP 1299 Using Renewable Resources and Its Application in Cotton Fabric Cleaning Process

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A biosurfactant was produced from *Cunninghamella echinulata* using sustainable technology for cleaning and degreasing of cotton fabric impregnated with burned motor oil. The surface tension was 32.4 mN/m on a medium containing instant noodle waste (2%), corn steep liquor (2%), and postfrying oil (0.5%) with a carbon/nitrogen ratio of 30:1, yield of 6.0 g·L⁻¹, emulsifier index of 81.4%, and dispersant property of 32.15 cm². The biosurfactant produced is a glycolipid constituted by carbohydrate (47.7%) and lipids (50.0%). The structure was confirmed by GC-MS (stearic acid in predominance with mass of 298 m/z), FTIR spectroscopy (polysaccharides in bands between 1025 and 1152 cm⁻¹ and fatty acids in bands between 2057 and 3100 cm⁻¹), ¹H NMR, and ¹³C NMR spectrum (carbohydrates in signal of 4.38 ppm and 77.0 ppm). The properties of cleaning and degreasing of burned engine oil in cotton fabric by biosurfactant of *C. echinulata* was evidenced by removal of 86% of oil. After use of the biosurfactant, the fibers were not damaged, which is important for structural integrity of cotton fabric after the wash. In addition, the biosurfactant did not show toxic effect. This study suggests that the biosurfactant from *C. echinulata* can be used in formulation of textile detergents, in particular for removal of hydrophobic residues from the automobile industry.

1. Introduction

The Modern detergents are complex mixtures containing chemical surfactants, softeners, oxidizing agents, and various enzymes among other ingredients [1]. They are contained in a wide range of industrial cleaning applications, including laundry detergents. Surfactants are one of these emerging contaminants derived from petroleum that pose environmental challenges. They are of interest because of their increasing industrial use. The industrial wastewater stream

containing surfactants that flows into environment is impacted by chemical compounds which are derived from petroleum, and hence, they need strong efforts for the removal of contaminants polluting environment. Thus, the environmental control legislation encourages the development of natural surfactants as an alternative to existing chemical products [2, 3].

Currently, with the advent of environmental and industrial sustainability, technologies are becoming increasingly essential for the industries all over the world [4]. In this

context, the biosurfactants are promising amphiphilic compounds, which are natural and biodegradable, produced by microorganisms. They are responsible for the most important and desired characteristic of a detergent, that is, the capacity of removal of dirt, for example, dirt caused by oil stains [5, 6].

The biosurfactant is capable of reducing the surface tension of the water, of natural lipophilic substances of the fabric (mineral oils, natural oils, and waxes), and of acquired lipophilic substances, allowing the infiltration of the biosurfactant in the fibers of the fabric and, consequently, the removal of the oil through the formation of micelles that interact with oil (apolar region) and water (polar region) [7].

The use of eco-friendly biosurfactants is more advantageous than using chemical surfactants because of their low toxicity, tolerance of extremes of temperature and pH, and high biodegradability. Above all, another advantage of them is the fact that they can be produced from renewable sources [8, 9].

Structures of biosurfactant such as glycolipids, lipopeptides, polymeric surfactants, and others can be formed according to the microorganism and substrates used as a source of carbon and nitrogen. Depending on the structure of biosurfactant, the interaction with the residue could be different [10, 11].

The biosurfactant production by waste conversion is a low-cost alternative and promising strategy for scale-up production. The source of carbon and nitrogen are hydrophilic compounds (e.g., glucose, lactose, and glycerol) and hydrophobic compounds (e.g., soybean oil and hexadecane), found in waste industrial food in higher levels as required, mainly carbohydrates, proteins, and lipids [12, 13].

This study was set out at investigating the potential of the biosurfactant from *Cunninghamella echinulata* in application as a textile detergent in fabric of cotton infiltrated with burned engine oil, that is, the waste obtained from the automobile industry is difficult for removal.

2. Materials and Methods

2.1. Materials for Biosurfactant Production

2.1.1. Microorganism. The Mucoralean fungus *Cunninghamella echinulata* UCP 1299 used in this study was isolated from Caatinga soil in Pernambuco, Brazil, and was obtained from the Culture Collection UCP, Catholic University of Pernambuco, registered in the World Federation for Culture Collections (WFCC).

2.1.2. Industrial Wastes. The industrial wastes used for biosurfactant production were the corn steep liquor obtained from corn products (Cabo, PE, Brazil), the instant noodle waste kindly provided by the instant noodle industry, and waste frying oil from informal food commerce. Table 1 shows the chemical composition of the instant noodle waste [14, 15], corn steep liquor [16], and postfrying oil [13].

2.2. Analytical Methods

2.2.1. Productive Process for Obtaining Biosurfactant. In production process for obtaining biosurfactants, *C. echinulata*

TABLE 1: Chemical compositions of renewable substrates instant noodle waste, corn steep liquor, and postfry soybean oil as components of the medium for biosurfactant production by *C. echinulata*.

<i>Instant noodle waste</i>	
Components	Quantity (mg)
Carbohydrates	51000
Proteins	9.400
Total fat	16.000
Sodium	1357
Thiamine	0.84
Riboflavin	0.91
Niacin	11
Pyridoxine	0.91
<i>Corn steep liquor</i>	
Main components	Quantity (%)
Amino acids	62.49%
<i>Postfry soybean oil</i>	
Components in fatty acids	Quantity (%)
Saturated	21.06
Monounsaturated	29.78
Polyunsaturated	55.97

was cultivated in Petri dishes containing Sabouraud agar medium (peptone 10 g·L⁻¹, dextrose 40 g·L⁻¹, and agar 18 g·L⁻¹) for growth of young mycelial mats. After incubation of the Petri dishes for 24 h at 28°C, 40 discs containing 10⁷ esporangioles/mL were used as inoculum in Erlenmeyer flasks containing 100 mL of medium constituted by different concentrations of instant noodle waste (0, 0.5, 1, and 2%) which had been supplemented with fixed concentrations of corn steep liquor (2%) and postfry oil (0.5%) at pH 5.5. The Erlenmeyer flasks were maintained in an orbital shaker at 150 rpm, 28°C for 96 h. After this period, the medium was filtered to separate the biomass from the metabolic liquid.

2.2.2. Growth Kinetics, pH, and Production. The kinetics of the growth and biosurfactant production by *Cunninghamella echinulata* were established for 168 h. Cell-free metabolic liquid aliquots were collected at intervals of 4 h, 12 h, and every 24 h. The biomass yield was calculated by gravimetry, and the results were expressed in g·L⁻¹. The pH and surface tension were measured using the metabolic liquid.

2.2.3. Extraction and Purification of the Biosurfactant. The biosurfactant was extracted from the cell-free supernatant using 70% ethanol in the ratio of 1 : 2 according to Bueno et al. [17]. The yield of crude biosurfactant that had been freeze-dried was determined, and the result was expressed in g·L⁻¹. The crude biosurfactant was purified by dialysis using a membrane (molecular weight of 8.000 Da) in distilled water at 4°C for 72 h and then freeze-dried. Next, the biosurfactant was analyzed by thin-layer chromatography (TLC) on a silica gel 60 plate (F254, Merck), using chloroform : methanol : water (65 : 15 : 2, v/v) as the solvent system, and developed for 3 h. The qualitative presence of carbohydrates, protein, and lipid was carried out by using anthrone, ninhydrin, and rhodamine B reagents, respectively [18].

2.2.4. Critical Micelle Concentration (CMC). The critical micelle concentration of the biosurfactant was investigated after the biosurfactant had been solubilized in water. Thus, the minimum concentration of biosurfactant required to reach the CMC was investigated for the different concentrations of biosurfactant in mg/mL: 0.01, 0.1, 0.3, 0.5, 1, 8, 10, 15, 20, and 25. Then, the surface tensions were measured in all the biosurfactant solutions tested. The CMC was reached after observing a constant value of surface tension as measured in an automatic tensiometer [19].

2.5. Chemical Composition of the Biosurfactant

2.5.1. Determination of Protein. The isolated biosurfactant was analyzed, and the protein content of proteins of this biomolecule was quantified using the Labtest kit (Labtest Diagnostica S.A.). For this analysis, bovine serum albumin was used as a standard.

2.5.2. Determination of Total Carbohydrates. The biochemical composition of isolated biosurfactant (1 mg) in total carbohydrates was determined by the phenol-sulfuric acid method using D-glucose as a standard [20].

2.5.3. Determination of Total Lipids. The total lipid content was quantified after extraction from biomass (1 g) using chloroform and methanol as solvents, following the methodology of Manocha et al. [21].

2.6. Biosurfactant Properties

2.6.1. Surface Tension (ST), Emulsification Index ($EI_{24}\%$), and Oil Spreading Test. Three methods were used to detect surfactant activity: measuring the surface tension (ST), making use of an Emulsification Index, and conducting the oil spreading test. The surface tension of the cell-free metabolic liquid was measured in accordance with the methodology of Kuyukina et al. [22] using a digital tensiometer equipped with a Du Noüy ring.

The emulsifying index was analyzed after mixing the cell-free metabolic liquid with the hydrophobic substrates (burnt engine oil, canola oil, soybean oil, postfry soybean oil, and diesel oil) according to the methodology described by Cooper and Goldenberg [23]. The Emulsification Index ($EI_{24}\%$) was defined as the height of the emulsion layer divided by the total height of the liquid column and expressed as a percentage. In test of oil spreading, the diameter of the clear zone on the oil surface was measured in the oil displacement area (ODA). Distilled water was used as a negative control, and the chemical surfactant sodium dodecyl sulfate (SDS) was used as a positive control. The oil spreading test was performed following the method established by Morikawa et al. [24], using burnt engine oil as the hydrophobic component.

2.6.2. Ionic Charge. The biosurfactant was solubilized in distilled water, and the contact angles of polar and nonpolar

nonaqueous liquids were combined with aqueous contact angle titrations to identify the ionic character by zeta potential in order to investigate the charge of the hydrophilic portion of the biosurfactant using a + 3.0 Zeta Meter system (model ZM3-DG, direct video Zeta Meter, Inc, USA) according to Lima et al. [6].

2.6.3. Thermal, NaCl Concentrations, and pH Stability. The stability of the biosurfactant produced was evaluated by determining the surface tension using the cell-free metabolic liquid that was submitted to different concentrations of NaCl (2%, 4%, 6%, 8%, 10%, 12%, 15%, and 20%), pH (2, 4, 6, 8, 10, and 12), and temperature (0°C, 4°C, 70°C, 100°C, and 120°C), according to Ghojavand et al. [25].

2.7. Biosurfactant Structural Characterization

2.7.1. Infrared Spectrometer with Fourier Transform (FT-IR). The chemical composition of the biosurfactant was confirmed using an infrared spectrometer with Fourier transform (FT-IR) recorded on a Bruker IFS 66 instrument, and the results were expressed in cm^{-1} in the region 4000–400 cm^{-1} . The infrared analysis was performed by mixing approximately 1 mg of purified biosurfactant with 100 mg of KBr discs [26].

2.7.2. Chromatography Coupled with Mass Spectrometry (GC-MS). The biosurfactant (10 mg) was subjected to methylation according to the methodology of Durham and Kloos [27] and dissolved in dichloromethane (CH_2Cl_2) for analysis. The Valcobond VB-5GC column had a length of 30 m, a thickness of 0.25 μm , and a diameter of 0.25 mm. The injector temperature was 290°C, the interface temperature 280°C, injection mode split, carrier gas helium, initial column internal pressure 52.8 kPa, column flow 1 mL/min, linear velocity 36.3 cm/second, split ratio 48, and full flow 50 mL/min. The initial temperature of the program was 50°C, and this was maintained for 2 min and then increased by 6°C per minute up to 280°C, held for 20 min at 280°C, and stabilized for 20 min. The mass spectrometer scan time was programmed each 3 min up to 60.37 min at a scanning rate of 350 m/z .

2.7.3. Nuclear Magnetic Resonance (NMR). NMR spectra ^1H and ^{13}C were determined by Varian VNMRs using 20 mg of purified biosurfactant dissolved in 500 μL of deuterated chloroform (Sigma Co.) at 500 MHz. Chemical shifts (δ) were expressed in parts per million (ppm) [28].

2.8. Biosurfactant Toxicity Test. Phytotoxic effect of the extracted biosurfactant of *C. echinulata* was tested in this study using seeds of onion (*Allium cepa* L.). Different concentrations of biosurfactant were prepared at concentration equal to the half of critical micelle concentration (CMC) value, equal to CMC, and twice the CMC. Distilled water was used for control, and the seeds were presterilized with sodium hypochlorite. For each concentration, 10 seeds

were placed in Petri dishes on sterilized filter paper discs. 5 mL of each sample was used to soak the filter paper discs. The plates were incubated for five days at a temperature of 28°C. Assays were performed in triplicates to achieve accuracy. Phytotoxicity can be determined by the germination of seeds (G), root growth (CR), and germination index (GI) in accordance with the equation proposed by Tiquia et al. [29].

2.9. Application of the Biosurfactant in Cleaning and Degreasing of Oil in Cotton Fabric

2.9.1. Cleaning and Degreasing Materials. The materials used for cleaning and degreasing of burned engine oil impregnated in cotton fabric were as follows: the biosurfactant obtained from *Cunninghamella echinulata* in the Nucleus of Research in Environmental Sciences and Biotechnology (Brazil) and a commercial detergent containing chemical surfactant (sodium dodecyl sulfate, SDS) used as a comparative. The clean white cotton fabric was cut into 2 × 2 cm pieces. The burned engine oil was obtained from the automobile industry and was used as dirty and degreasing compounds according to Bouassida et al. [30].

2.9.2. Washing Procedure. The sample of cotton fabric was immersed with 5 mL of burned engine oil. Posteriorly, the dirty cotton fabric was immersed in aqueous solution of the biosurfactant (1.5%). The washing occurred at a stirring speed of 150 rpm for 1 h. A commercial detergent was used as a positive control in cleaning and degreasing. The cotton fabric was rinsed twice with 50 mL of distilled water for 30 min with stirring followed by drying at room temperature [30].

2.9.3. Structural Evaluation of Fiber Fabric. The structure of fibers fabric before and after cleaning and degreasing of stain of burned engine oil by biosurfactant of *C. echinulata* was detected visually in the cotton fabric, by using optical microscopy (microscope Olympus BX50) with increase of 100x and by scanning electron microscopy (SEM) with electronmicrographs obtained from JEOL LV 5600, operating at 20 KV. In addition, the damage degree in the cotton fabric was evaluated by structural integrity of the fibers after the cleaning process by biosurfactants and commercial detergents.

2.9.4. Percentage of Removal of Burned Engine Oil. The percentage of burned engine oil removed of the fibers fabric by action of the biosurfactant of *C. echinulata* and by the commercial detergent was determined according to Equation (1) proposed by Grbavčić et al. [31]:

$$w = \frac{m_{\text{total}} - m_i}{m_{\text{total}}} \times 100, \quad (1)$$

where m_{total} is the total mass of inflicted oil and m_i is the residual oil on the cotton cloths after the treatment.

3. Results and Discussion

3.1. Eco-Friendly Strategy for Production of Biosurfactant from *C. echinulata*. The low commercialization of biosurfactants of microbial origin occurs generally due to the high cost of the substrates used in the production medium and low yield of the bioproduct at the end of the process. The food waste is a renewable and sustainable alternative for producing metabolites in the area of biotechnology [32]. In this context, special attention must be given for development of eco-friendly processes from use of cost-effective substrates for the growth of microorganisms and biosurfactant production.

The cost-effective strategy used in this study was the production microbiological of biosurfactant from culture of filamentous Mucoralean fungus *C. echinulata* in a medium containing instant noodle waste (2% INW) as the main carbon source in the medium supplemented with corn steep liquor (2% CSL) and waste postfry oil (0.5%).

The use of this smart technology from wastes for biosurfactant production by Mucoralean fungus favored the surface tension reduction from 72 mN/m to 32 mN/m in the medium containing elemental composition in mass percentages of carbon (C) and nitrogen (N) of about 44.64% and 1.74%, respectively, corresponding the C/N ratio of 30:1. Furthermore, the data show that it is the increase in the concentration of the instant noodle waste (INW) that generates the production of biosurfactant from *C. echinulata* (Table 2).

In addition, the biosurfactant produced by *C. echinulata* in conditions described was tested in potential for form emulsions using hydrophobic substrates. The results showed the formation of oil-type emulsions in water (O/W) from burnt motor oil (81.4%), canola oil (64.0%), soybean oil (60.2%), and postfry oil (76.0%) as substrates. Therefore, the data obtained suggest that *C. echinulata* is a promising fungus that can be used to produce this eco-friendly biomolecule.

The most sought-after properties in this biomolecule are to reduce the surface tension below 40 mN/m [33] and to obtain an emulsifying capacity above 50% [34]. Thus, the values of surface tension and the Emulsification Index obtained by the biosurfactant of *C. echinulata* produced in the medium containing wastes (2% INW, corn steep liquor 2% CSL and waste postfry oil 0.5% with C/N 30:1 ratio) were compared with those in the literature. This showed that the biosurfactant produced in this study is more effective at reducing the surface tension than either the biosurfactant produced by filamentous fungi or anionic surfactant SDS (sodium dodecyl sulfate) chemically synthesized from petroderivatives (Table 3).

In addition, having as reference the surface tension values (Table 3), the biosurfactant produced by *C. echinulata*, under the conditions described in this paper, has greater surface tension reduction potential (32 mN/m) when compared the reduction of surface tension (36 mN/m) of *C. echinulata* strain used by Andrade-Silva et al. [41]. They used another strain *Cunninghamella echinulata* UCP1295 for the production of biosurfactants, and several remarkable

TABLE 2: Eco-friendly biosurfactant production by *Cunninghamella echinulata* related to C/N ratio on surface tension (ST) and Emulsification Index (EI₂₄) evaluation.

C/N Ratio	Instant noodles waste (%)*	Surface tension (mN/m)	Emulsification Index (%)				
			Oils				
			Burned engine oil	Canola	Soybean	Postfry	Diesel
4:1	0.0	42.1	66.1	12.9	37.9	33.5	—
7:1	0.5	39.6	38.6	26.0	46.6	38.2	—
18:1	1.0	39.1	41.4	58.8	44.6	59.2	—
30:1	2.0	32.4	81.4	64.0	60.2	76.0	—

*Basal medium composition: corn steep liquor (2%) and postfrying oil (0.5%) with different concentrations of instant noodles waste.

TABLE 3: Comparison of biosurfactant production by filamentous fungi using different substrates with *Cunninghamella echinulata* evaluating the surface tension (ST) and Emulsification Index (EI₂₄) in this study.

Fungal strains	Carbon sources	Surface tension (mN/m)	Emulsification Index EI ₂₄ (%)	References
<i>Cunninghamella echinulata</i> UCP 1299	Instant noodle waste/waste frying oil	32.0	81.4	Present study
<i>Rhizopus arrhizus</i> UCP 1607	Soybean postfrying oil (5%)	31.8	82.6%	Pele et al. [35]
<i>R. microsporus</i> var. <i>microspores</i> UCP 1304	Soybean postfrying oil (5%)	34.7	94.8%	Pele et al. [35]
<i>R. microsporus</i> var. <i>chinensis</i> UCP 1296	Soybean postfrying oil (5%)	33.3	91.7%	Pele et al. [35]
<i>R. arrhizus</i> var. <i>arrhizus</i> UCP 1295	Soybean postfrying oil (5%)	35.0	69.0%	Pele et al. [35]
<i>Cunninghamella phaeospora</i> UCP 1303	Soybean postfrying oil (5%)	28.1	93.62%	Lins et al. [36]
<i>Phoma</i> sp.	Diesel	51.0	52.0	Lima et al. [37]
<i>Penicillium chrysogenum</i> SNP5	1% glucose and 5% glycerol	—	Oil and diesel up to 45% and 24%	Gautam et al. [38]
<i>Fusarium</i>	Sucrose	32	70	Qazi et al. [39]
Synthetic surfactant (control)	Petroderivatives	36.0	64.0	Christofi and Ivshina [40]

physiological responses controlled the culture of fungus. The main phenomenon is related to strong reduction of the transfer rate of oxygen from the gas into the liquid phase, causing oxygen-limited or microaerophilic conditions in the culture after a short period of cultivation.

3.2. Kinetics of Biosurfactant Production, Biomass, and pH. Figure 1 shows that *C. echinulata* reached the maximum growth after 60 h of cultivation in the exponential phase of growth. The biosurfactant started to be produced after the first 48 hours when there was a reduction in the surface tension of the medium from 72 mN/m of water to values around of 38 mN/m in medium with pH 5.5. However, the maximum biosurfactant production occurred in the stationary phase of growth in the medium with neutral pH, resulting in a minimum surface tension of 32 mN/m. The results obtained show that there was no relationship between the growth of *C. echinulata* and the production of the biosurfactant under the conditions created for this study.

3.3. Biosurfactant as Dispersant Agent. Many researchers have reported using the oil displacement area method to study the efficiency this technique for biosurfactant detection in the cell-free culture supernatant [11, 39].

According with Morikawa et al. [24] the area of displacement formed by presence of biosurfactant in solution is directly proportional to the potential of activity this biomolecule.

The ability of the biosurfactant produced by *C. echinulata* in the medium containing corn steep liquor (2%) and waste postfry oil (0.5%) supplemented with 2% of INW, corresponding to 30: 1 (C/N) ratio, after 96 h was investigated for the dispersant potential.

According to the results, the biosurfactant can be used as a dispersing agent of burnt engine oil because of its capacity to create a 32.15 cm² ODA (oil displacement area) (Figure 2(a)), while the synthetic surfactant SDS (positive control) was able to displace 63.58 cm² ODA (Figure 2(b)). When water was used as the negative control of displacement, dispersion was zero.

The result obtained in this study for oil displacement was similar to the result obtained by Andrade-Silva et al. [41] using different strains of *C. echinulata* with value of oil displacement of 37.36 cm² ODA. On the other hand, a lower result was obtained by the biosurfactant of *Fusarium* sp. which created an oil displacement area (ODA) in the range of 7–13 cm² [24]. Thus, the value obtained for the oil displacement in this work, compared to that obtained in the literature, proves the effective activity of biosurfactant of *C. echinulata* in the dispersion of hydrophobic compounds.

FIGURE 1: Growth profile, pH, and surface tension of *Cunninghamhamella echinulata* grown in the alternative medium constituted by instant noodle waste 2% (INW), corn steep liquor 2% (CSL), and soybean waste oil 0.5% (SWO).

FIGURE 2: Dispersion test of petroderivatives in water evaluated by oil displacement area (ODA): (a) oil and biosurfactant of *Cunninghamhamella echinulata*; (b) oil and chemical surfactant (SDS).

3.4. Stability of the Biosurfactant Evaluated by Determining Surface Tension. The ability of the biosurfactant to maintain its surfactant activity unaltered after exposure to the extreme conditions of temperature and different concentrations of sodium chloride and pH has been frequently investigated [42]. Thus, Figure 3 shows the biosurfactant stability exhibited by *C. chinulata* after thermal stability, NaCl concentration, and pH stability evaluated by determining surface tension.

The results showed that the biosurfactant obtained by *C. echinulata* in the medium containing instant noodle waste (2% INW) as the main carbon source and supplemented with corn steep liquor (2% CSL) and waste postfry oil (0.5%) was able to keep the surfactant activity stable after it had been submitted to neutral and alkaline pH and to reduce the stability in acidic pH (Figure 3(a)). The stability of the

biosurfactant activity did not change after the biosurfactant was exposed to different temperatures (Figure 3(b)) and the activity was not changed after the biosurfactant had been exposed to different concentrations of NaCl of up to 8% (Figure 3(c)). Marchant and Banat [43] affirm that the stability of the biosurfactant is an essential factor for the viability of large-scale production, especially when the production is carried out through a biotechnological process. Therefore, the use of biosurfactants for obtain by-products requires stability in large range of temperature, pH, and salt concentrations.

3.5. Ionic Profile, Critical Micelle Concentration (CMC), and Yield of Biosurfactant. The isolated biosurfactant showed the ionic profile using zeta potential which determines the

FIGURE 3: Stability studies for biosurfactant produced by *Cunninghamella echinulata* in the medium constituted by instant noodle waste 2% (INW), corn steep liquor 2% (CSL), and soybean waste oil 0.5% (SWO), under different conditions: pH (a), temperature (b), and NaCl (c).

function of the surface charge of the particle that serves to predict and control the stability of colloidal suspensions and emulsions and confirmed the higher values obtained, indicating good stability by repulsion between hydrophilic particles. The evaluation of the ionic profile of the biosurfactant showed the presence of a negative charge in molecule with a standard deviation of 1.36 and an average scale of 269.3 uS/cm at 23°C, 100 Volts.

The anionic profile of the biosurfactant produced by *C. echinulata* in medium containing wastes (2% INW, corn steep liquor 2% CSL, and waste postfry oil 0.5%) with C/N 30:1 ratio is the same profile of chemically synthesized surfactant (sodium dodecyl sulfate). Thus, the biosurfactant produced in this study is a possible candidate for using and replacing chemical surfactants. O'Rear [44] affirms that chemical surfactants with an anionic profile are used as the main active compound by many industries.

In addition, in this study was determined the yield of biosurfactant after its extraction from the cell-free metabolic liquid after culturing of *C. echinulata* for 96 h resulting in 6 g·L⁻¹. A similar result of yield of biosurfactant (5.9 g·L⁻¹) was obtained from *Mucor indicus* grown in rice husk as the source of carbon [45] when compared to that of the yield of the biosurfactant (6.0 g·L⁻¹) from *C. echinulata* in this work.

On the contrary, the critical micelle concentration from isolated biosurfactant was determined and resulted in CMC of 10 mg/mL. The CMC (critical micellar concentration) is defined as the minimum concentration of biosurfactants required for the formation of micelles and is directly related to the surface tension [42].

3.6. Compositional Analyses of the Biosurfactant. The molecular parameters obtained by FT-IR, GC-MS, ¹³C NMR, and ¹H NMR gave important information that enabled a possible structure of the partially purified biosurfactant from *C. echinulata* to be determined.

FTIR spectrum of the biosurfactant revealed the presence of sugar observed in bands between 1025 cm⁻¹ and 1152 cm⁻¹ which indicates a complex sequence that is mainly due to the C-O-C stretch vibrations of polysaccharides, whereas the bands at 2057–3100 cm⁻¹ indicate the presence of functional groups CH₃=CH₂ which is a characteristic of fatty acids [26] (Figure 4). Thus, the data

obtained by infrared spectrum (FT-IR) suggest that the biosurfactant from *C. echinulata* comprises sugar in the hydrophilic part and chain fatty acids in the hydrophobic region of the molecule. The infrared spectrum of the biosurfactant of *C. echinulata* was compared with the glycolipid biosurfactant produced by other microorganisms and shows that the biosurfactant is in accordance with the glycolipid reported in the literature [46, 47].

The GC-MS spectra revealed that the main bioactive fatty acid present in the hydrophobic region of the biosurfactant is methyl stearate (stearic acid) with the molecular formula C₁₉H₃₈O₂ and a mass of 298 m/z (Figure 5(a)) followed by methyl 13-methylpentadecanoate (pentadecyl acid) with a mass of 270 m/z and the molecular formula C₁₇H₃₄O₂ (Figure 5(b)) associated with a sugar moiety. The GC-MS spectra revealed that, in this study, the results were similar to the results obtained by Elouzi et al., [48], Ibrahim et al. [49], and Akintunde et al. [50] which detected by GC-MS the presence of stearic acid or octadecanoic acid as the majority fatty acids in the biosurfactant. According to Vecino et al. [51], stearic acid or octadecanoic acid is the main fatty acid chain found in various glycolipid biosurfactants.

On the contrary, the results of ¹H NMR and ¹³C NMR suggest the presence of carbohydrate in the hydrophilic region of the biosurfactant because this reveals a relative signal of 4.38 ppm and 77.0 ppm. Additionally, the signal obtained in this study for ¹H NMR (4.38 ppm) was compatible with those obtained in the sophorolipid of *Candida bombicola* (4.46 ppm) and *Candida* sp. NRRL (4.63 ppm) [20]. The results of ¹H NMR and ¹³C NMR evidence of data was described by Elshafie et al. [52] who characterized the biosurfactant of *Candida bombicola* ATCC 22214 as a sophorolipid due to the presence of signals between 4 and 4.5 ppm in the NMR analysis.

The data obtained by the spectra (¹H NMR and ¹³C NMR) were confirmed after quantitative analysis of the carbohydrate (44.7%) and lipid (46%) content in the partially purified biosurfactant. In addition, information obtained from TLC showed that the biosurfactant reacted with anthrone, thus demonstrating the presence of yellow bands on a silica gel (CCD) plate which indicates that the biosurfactant belongs to the class of glycolipids. Mani et al. [53] affirm that glycolipid biosurfactants are sugar-containing

FIGURE 4: Infrared spectrum (FT-IR) for the structure of the glycolipid biosurfactant produced by *Cunninghamella echinulata*.

FIGURE 5: Spectrum evaluated by gas chromatography and mass (GC-MS) of the biosurfactant of *Cunninghamella echinulata*: predominance of stearic acid (a) with mass of 298 m/z and pentadecyl acid with mass of 270 m/z (b) in the hydrophobic region.

lipids in which a carbohydrate moiety is linked to a fatty acid moiety. Therefore, in conclusion, this study suggests that *C. echinulata* produced is an anionic glycolipid biosurfactant that contains methyl stearate (stearic acid) and methyl 13-methylpentadecanoate (pentadecyl acid) as the major fatty acid in the hydrophobic region which is linked to a sugar moiety in the hydrophilic region.

3.7. Toxicity of the Biosurfactant. The toxicity of the biosurfactant is considered an important factor [18, 53]. The results obtained in the present study indicate that the biosurfactant of *C. echinulata* solubilized in concentrations of 1/2 CMC (0.5 mg/mL), in CMC (10 mg/mL), and 2X the

CMC (20 mg/mL) did not show any inhibitory effect on onion seeds germination/root elongation. Table 4 shows that the values of the seed germination and root elongation of onion seeds were gradually increased in accordance with the concentration of the biosurfactant. The maximum Germination Index (GI) obtained was 122 ± 0.13 with the solution of the biosurfactant at 20 mg/mL. The proportional relation between the concentrations of the biosurfactant and GI also was observed in the study of phytotoxicity of the biosurfactant isolated from *Candida tropicalis* [18].

3.8. Application of the Biosurfactant of *C. echinulata* in Cleaning and Degreasing of Oil in Cotton Fabric. The choice

TABLE 4: Phytotoxicity evaluation of the biosurfactant produced by *C. echinulata*.

Biosurfactant concentration (mg/mL)	Seed germination (%)	Anion seeds (<i>Allium cepa</i> L.)	
		Root elongation (%)	Germination index (%)
5 (1/2 CMC)	100 ± 0.18	108 ± 0.15	114 ± 0.19
10 (CMC)	100 ± 0.15	106 ± 0.28	118 ± 0.17
20 (2X CMC)	100 ± 0.12	121 ± 0.15	122 ± 0.13
Distilled water	100 ± 0.10	125 ± 0.12	130 ± 0.16

FIGURE 6: Cleaning and degreasing of stain of burned engine oil in cotton fabric by biosurfactant of *C. echinulata* comparing with commercial detergent: (a) clean fabric; (b) fabric with burned engine oil; (c) fabric washed with commercial detergent, and (d) fabric washed with biosurfactant of *C. echinulata*.

of sustainable by-products and the widespread support from governments, organizations, and consumers contributes to reduce the environmental impact. In this context, some researchers carry out studies to obtain biosurfactants with potential of use in cleaning and degreasing of cotton fabric in replacement of the chemical surfactants present in commercial detergents.

In this study of application of biosurfactant of *C. echinulata*, the clean cotton fabric analyzed by optical microscopy and scanning electron microscopy (Figure 6(a)) was used as positive control of cleaning, while the fabric impregnated with burned engine oil (Figure 6(b)) was used as negative control. According to the results, the commercial detergent was found to be efficient in cleaning the fabric (Figure 6(c)). However, in various points in the sample, the fibers were damaged and presented rupture. On the contrary, after the use of the biosurfactant of *C. echinulata* in wash of the dirty cotton fabric with burned engine oil (Figure 6(d)), the fabric showed characteristics similar to the clean cotton fabric (Figure 6(a)). Another important observation is that, after use of the biosurfactant, the fibers not were damaged, which is important for maintaining the structural integrity without apparent fiber breakdown.

In addition, the biosurfactant of *C. echinulata* was capable of eliminating 86% of burned engine oil infiltrated in cotton fabric, while the commercial detergent removed 92%. According to the oil content removed, the results indicate that the commercial detergent and the biosurfactant of *C. echinulata* are similar in the cleaning and degreasing potential.

The biosurfactant extracted from *C. echinulata* has an additional advantage over commercial detergent, due to its isolated action without the presence of other compounds that assist the cleaning and degreasing (as the enzymes

present in commercial detergents), low toxicity, and high biodegradability.

In study performed by Bouassida et al. [30], the biosurfactant removed 81% of burned engine oil, while the commercial detergent removed 34%. In this same study was observed that the chemical surfactant isolated exhibited less cleaning efficiency of oil (62%) when compared to the commercial SPB1 biosurfactant (75%).

Bouassida et al. [30] results compared with this study for biosurfactants produced by *C. echinulata* show effective potential for cleaning with burned oil dirtying in cotton fabric. The results obtained suggested replacing the chemical surfactants by biosurfactants considering the preservation of cotton fibers, as well as the conservation and environmental sustainability.

In conclusion, the biosurfactant of *C. echinulata* is an alternative that favors the global surfactant market. The biosurfactant produced by *Cunninghamella echinulata* is attracting much interest due to its potential advantage over their synthetic counterparts, considering the adoption of natural technologies and attend to many fields as environmental, food, biomedical, and other industrial applications.

4. Conclusions

Cunninghamella echinulata isolated from Caatinga soil from Brazil represents a promising source to obtain a biosurfactant bioactive with potential of use as a textile detergent. This present work revealed the capacity of the strain *Cunninghamella echinulata*, filamentous fungus, grown on various industrial wastes in the conversion of biosurfactant production. The GC-MS, FT-IR, and NMR analyses suggest

the glycolipidic nature of the biosurfactant. The stability of the biosurfactant is based on a wide pH range, high temperature, and variable concentrations of salt. The emulsifier and surface tension reduction properties suggest substantial ability to cleaning and degreasing waste motor oil impregnated cotton fabric. Therefore, the biosurfactant showed effective action in the fibers, free of damage, and the property of detergent favors the formulation of products in industries in the future.

Although the biosurfactants are still considered less competitive in the market with purchase value higher than the chemical surfactants, the demand by the biosurfactants is increasing and a number of industries and consumers are willing to pay, because this is an alternative that favors the global market of natural surfactant.

Data Availability

The data used to support the findings of this study are available at <https://www.hindawi.com/research.data/#statement>.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

RFSA and TALS conceived and designed the experiments; RFSA, TALS, DMR, DRR, MABL, RAL, and AFS performed the experiments; RFSA, TALS, MABL, and GMCT analyzed the data; GMCT contributed reagents/materials/analysis tools; RFSA, TALS, DMR, and GMCT wrote the paper.

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