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Research article

Conversion of renewable substrates for biosurfactant production by *Rhizopus arrhizus* UCP 1607 and enhancing the removal of diesel oil from marine soil

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ABSTRACT

Background: The use of agro-industrial wastes to produce high value-added biomolecules such as biosurfactants is a promising approach for lowering the total costs of production. This study aimed to produce biosurfactants using *Rhizopus arrhizus* UCP 1607, with crude glycerol (CG) and corn steep liquor (CSL) as substrates. In addition, the biomolecule was characterized, and its efficiency in removing petroderivatives from marine soil was investigated.
Results: A 2^2 factorial design was applied, and the best condition for producing the biosurfactant was determined in assay 4 (3% CG and 5% CSL). The biosurfactant reduced the surface tension of water from 72 to 28.8 mN/m and produced a yield of 1.74 g/L. The preliminary biochemical characterization showed that the biosurfactant consisted of proteins (38.0%), carbohydrates (35.4%), and lipids (5.5%). The compounds presented an anionic character, nontoxicity, and great stability for all conditions tested. The biomolecule displayed great ability in dispersing hydrophobic substrates in water, thereby resulting in 53.4 cm^2 ODA. The best efficiency of the biosurfactant in removing the pollutant diesel oil from marine soil was 79.4%.
Conclusions: This study demonstrated the ability of *R. arrhizus* UCP1607 to produce a low-cost biosurfactant characterized as a glycoprotein and its potential use in the bioremediation of the hydrophobic diesel oil pollutant in marine soil.
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1. Introduction

In recent years, microbial surface-active compounds, commonly known as biosurfactants, have gained the special attention of researchers. Despite the large diversity of biosurfactants with regard to their compositional structure, they are recognized universally as amphiphilic compounds, which have both hydrophilic and hydrophobic domains [1]. Owing to this particular organization, these compounds may concentrate at interfaces between immiscible phases such as air/

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water and oil/water, thus leading to surface and interfacial tensions being reduced [2].

The abilities of biosurfactants to reduce surface and interfacial tension define their different physicochemical properties such as emulsifying capability, mobilizing and solubilizing agents, detergency, foaming, wetting ability, and phase dispersion [2,3]. These properties make them attractive molecules with potential applications in many industrial sectors including oil and food industries, domestic activities, and environmental remediation of contaminated sites and make them available as more environmentally friendly and low-cost techniques [3,4].

Biosurfactants possess many advantages compared with their chemical counterparts, e.g., ecological acceptability, biocompatibility, and digestibility as well as efficiency under extreme conditions of pH, temperature, and salinity, in addition to their functional specificity and the possibility of being synthesized from renewable and less costly substrates [1,5].

A huge amount of research throughout the world has been directed to microbial tensioactive agents ever since they were discovered [6]. However, most studies have used the ability of bacteria and yeasts to synthesize biosurfactants using low-cost substrates [5,7,8]. The potential of filamentous fungi in producing biosurfactants has also been reported by a few investigations [9,10,11]. In the present study, the biotechnological potential of the new strain *Rhizopus arrhizus* UCP 1607 isolated from Caatinga soil (PE, Brazil) [12] was investigated by using crude glycerol (CG) and corn steep liquor (CSL) as renewable substrates for biosurfactant production.

2. Materials and methods

2.1. Microorganism

R. arrhizus UCP 1607 was isolated from soils of the semi-arid region of Caatinga biome in the state of Rio Grande do Norte, northeast of Brazil. The strain was morphologically and molecularly identified as a new strain of *R. arrhizus* by our research group [12] and subcultured from the stock culture on Sabouraud dextrose agar (SDA) slants and stored at 4°C in a refrigerator. They were transferred to fresh SDA slants each three months to ensure the fungus remained viable.

2.2. Agro-industrial substrates

The agro-industrial substrates used were kindly provided by agro-processing industries: CSL from corn wet-processing industry (Cabo-PE, Brazil), and CG from biodiesel production from cotton oil (CETENE-PE, MCT, Brazil).

2.3. Culture condition and biosurfactant production

The experiments were carried out in 250-mL Erlenmeyer flasks containing 100 mL of the medium for biosurfactant production consisting of salt solution (g/L) (KH_2PO_4, 0.2; $MgSO_4 \cdot 7H_2O$, 0.2; distilled water — 1000 mL), added with 1 mL/L of trace element solution ($FeSO_4 \cdot 7H_2O$, 0.63 mg; $MnSO_4$, 0.01 mg; $ZnSO_4$, 0.62 mg) [9]. CG and CSL were used as carbon and nitrogen sources, respectively [13,14], in varying concentrations according to the 2^2 factorial design. The pH of the production medium in the flasks was adjusted to 5.5 ± 0.03 and sterilized by autoclaving at 121°C for 15 min. A sporangiospore suspension of *R. arrhizus* UCP 1607 grown on potato dextrose agar (PDA) medium (g/L) (peeled potato 200, dextrose 20, and agar 15) at 28°C for 96 h was prepared in distilled water and adjusted to a concentration of 10^7 spores/mL. Then, 5% (v/v) of the spore suspension was inoculated into each flask containing the production medium, and the flask was incubated in an orbital shaker at 150 rpm and 28°C for 96 h. Mycelia-free broths were obtained by filtration followed by cold centrifugation at 10,000× *g* at 5°C for 15 min, and then, they were used in screening analyses for biosurfactant production.

Table 1
Levels and variables applied of the factorial design for biosurfactant production by *Rhizopus arrhizus* UCP 1607.

Variables	Low (−1)	Levels central (0)	High (+1)
Crude glycerol (% v/v)	2	2.5	3
Corn steep liquor (% v/v)	3	4	5

2.4. Factorial design for biosurfactant production

To analyze the effects of CG and CSL and the interactions between them on biosurfactant production by *R. arrhizus* UCP 1607, a 2^2 factorial design was developed, with surface tension as the variable response. A set of eight assays with four replicates in the central points were carried out (Table 1). Statistical analysis of the data obtained from the experiments was performed using STATISTICA software package version 7.0 (StatSoft Inc., Tulsa, OK, USA), and the significance of the results was tested at $P \leq 0.05$.

2.5. Surface tension determination

Surface tension was determined on mycelia-free metabolic liquid using a tensiometer model Sigma 70 (KSV Instruments Ltd., Finland) by the Du Nouy ring method at room temperature (±28°C). Measurements of surface tension from distilled water were used as control [15].

2.6. Kinetics of growth, pH, and biosurfactant production

The kinetics of growth, pH, and biosurfactant production by *R. arrhizus* were established for 96 h. Every 24 h, aliquots were collected and subjected to filtration followed by cold centrifugation at 10,000× *g* and 5°C for 15 min. Then, mycelia-free metabolic liquids were used for the determination of surface tension using a tensiometer model Sigma 70 (KSV Instruments Ltd., Finland) and pH using an Orion potentiometer (Model 310) (Orion Research Inc., Cambridge, MA, USA). The biomass yield was calculated by gravimetry, and results were expressed in g/L.

2.7. Extraction of biosurfactant

The biosurfactant produced by *R. arrhizus* UCP 1607 was extracted from the supernatant by the precipitation method with acetone 1:1 (v/v). The mixture was allowed to stand for 24 h at 4°C. Afterwards, the precipitated materials were centrifuged at 5000× *g* for 15 min at 5°C. The supernatant was discarded, and the isolated material was submitted to dialysis against deionized water, which was changed every 3 h, for 96 h at 5°C.

2.8. Critical micelle concentration (CMC)

To determine the critical micelle concentration (CMC), different concentrations of the partially purified biosurfactant were diluted in distilled water. Using an automatic tensiometer (model Sigma 70 KSV Ltd., Helsinki, Finland) and by the Du Nouy ring method, surface tensions of the aqueous solutions of the compound were measured up to a value at which the surface tension remained constant. A graph of the reduction in surface tension in percentages (%) as a result of biosurfactant concentration was plotted [16].

2.9. Ionic charge

The ionic charge of the biomolecule was determined using a Zeta potentiometer model ZM3-D-G, Zeta Meter System 3.0 +, with direct images to the video of the Zeta Meter, San Francisco, CA, USA [7].

2.10. Biochemical characterization

The protein concentration in the isolated biosurfactant was determined using the total protein test kit from Labtest Diagnóstica S.A., Brazil. The total carbohydrate content was estimated by the phenol-sulfuric acid method [17], whereas the lipid content was determined in accordance with the method by Manocha et al. [18].

2.11. Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared (FTIR) spectroscopy was carried out as described by Kiran et al. [19]. One milligram of the partially purified freeze-dried biosurfactant sample was ground with 100 mg of potassium bromine (KBr) and pressed with 7500 kg for 30 s to obtain translucent pellets. The infrared spectra were recorded on a Varian 640-R spectrometer in the spectral region of 4000–400 cm^{-1}, and the KBr pellet was used as the background reference.

2.12. Phytotoxicity assay

The phytotoxicity of the biosurfactant produced by *R. arrhizus* UCP 1607 was assessed on the basis of the seed germination and root elongation of lettuce (*Lactuca sativa* L.) and cabbage (*Brassica oleracea*), following the methodology described by Tiquia et al. [20]. Solutions of the isolated compound were prepared with distilled water at concentrations of 1.0, 1.7, and 2.15 g/L of the biosurfactant. The phytotoxicity of the biomolecule against ten seeds of each plant was determined in sterilized Petri dishes containing Whatman no. 1 filter paper. After 5 d of incubation in the dark, seed germination, root elongation (≥5 mm), and germination index (GI, a factor of relative seed germination and relative root elongation) were determined as follows:

- Relative seed germination (%) = (number of seeds germinated in the extract / number of seeds germinated in the control) × 100
- Relative root length (%) = (mean root length in the extract / mean root length in the control) × 100
- Germination index = (% of seed germination) × (% of root growth) / 100%

Controls were prepared with distilled water. The mean and standard deviation of triplicate experiments from each concentration were calculated.

2.13. Stability analysis

The metabolic liquid without mycelia, which was obtained by filtration followed by centrifuging the culture broth at 10,000× *g* for 15 min, was used to assess the stability of the biosurfactant in relation to surface tension. Forty milliliters of the supernatant culture was heated at 0, 5, 70, 100, and 120°C for 15 min and cooled to room temperature, and the surface tension was determined. The stability of the compound to pH was tested by adjusting the pH of the mycelia-free broths to different values (pH 2–12) by adding 1.0 M HCl or 1.0 M NaOH to solutions, and the surface tension was measured. The effect of NaCl concentrations (2.0%–14%) was also determined. All experiments were performed in triplicate [5].

2.14. Oil displacement area (ODA)

The oil spreading test was performed by placing 40 mL of distilled water in a Petri dish (9 cm in diameter), followed by adding 1 mL of burnt motor oil onto the surface of the water layer. Afterwards, 0.5 mL of the cell-free metabolic liquid was gently dropped onto the thin surface of the oil layer. The positive control for oil displacement was the anionic synthetic surfactant sodium dodecyl sulfate (SDS) and negative control was water. The average values of the diameters of the clear zones of experiments in triplicate were measured and calculated as an oil displacement area (ODA) according to Morikawa et al. [21], using the following equation: ODA = 22 / 7 $(radius)^2$ cm^2.

2.15. Emulsification index

The emulsification capacity of the biosurfactant was assessed by adding 2 mL of the mycelia-free broth to an equal volume of hydrocarbon mixture (diesel, kerosene, hexadecane, gasoline, motor oil, and burnt motor oil) and shaking rigorously in a vortex for 2 min. The mixture was left standing at room temperature. The emulsification index ($\%EI_{24}$) was calculated after 24 h as the height of the emulsion layer divided by the total mixture height, and the results were expressed in percentages [22]. The measurements were taken in triplicate.

2.16. Removal of diesel oil from contaminated marine soil

The ability of the biosurfactant to remove the hydrophobic compounds of petroderivatives from marine soil was assessed using 20 g of marine soil impregnated with 2 g of diesel oil, and then, the mixtures were left to stand for 7 d at room temperature to simulate the pollution of marine soil with petroleum hydrocarbons. Afterwards, the contaminated marine soil was transferred to 250-mL Erlenmeyer flasks, followed by the addition of 40 mL of the mycelia-free broth produced by *R. arrhizus* UCP 1607 and 40 mL of distilled water as the negative control. The samples were incubated on a rotary shaker (150 rpm) for 24 h at 28°C and then were centrifuged at 5000× *g* for 10 min to separate the washing solution and the sand. The amount of oil residing in the sand after the impact of the biosurfactant was gravimetrically determined as the amount of material extracted from the sand by hexane [23].

3. Results and discussion

3.1. Production of biosurfactant by R. arrhizus UCP 1607 using CG and CSL

In this study, the two agro-industrial byproducts CG and CSL were used as inexpensive culture medium components to produce biosurfactants using *R. arrhizus* UCP 1607. Results in Table 2 show that this mucoralean fungus had the ability to reduce surface tension and to form consistent emulsions. A lower surface tension of 28.8 mN/m was achieved in condition 4 corresponding to the medium consisting of 3% CG and 5% CSL, in agreement with the result obtained by the

Table 2
Surface tension values for the 2^2 factorial design applied for biosurfactant production by *Rhizopus arrhizus* UCP 1607.

Runs	Crude glycerol (%)	Corn steep liquor (%)	Surface tension (mN/m)
1	−1	−1	32.1
2	+1	−1	30.0
3	−1	+1	33.0
4	+1	+1	28.8
5	0	0	34.5
6	0	0	34.1
7	0	0	34.6
8	0	0	34.4

factorial design developed (Table 1). Therefore, this condition was selected as the best one for formulating growth to produce biosurfactant by *R. arrhizus* UCP 1607.

According to Mnif and Ghribi [3], the most powerful biosurfactants are those that are able to reduce the surface tension of water to values equal or below 30 mN/m. They are referred to in the literature as being mostly produced by bacteria such as *Pseudomonas aeruginosa* and *Bacillus subtilis*. However, the biosurfactant produced by this new fungal strain of *R. arrhizus* in condition 4 demonstrated a greater effectiveness in reducing the surface tension of water. Similar results were reported for a biosurfactant obtained from the filamentous fungus *Aspergillus* sp. MSF that showed a reduction in the surface tension of water from 66 to 28mN/m [19]. Recently, Silva et al. [24] observed that the biosurfactant produced by *Mucor circinelloides* could reduce the surface tension of water to 26 mN/m. The biosurfactant produced by *R. arrhizus* reduced the surface tension of water to 26.5 mN/m [25], which corroborates to the ability of the genus *Rhizopus* to synthesize surface-active compounds.

The effects of concentrations of CG and CSL as well as their interaction on surface tension as dependent variables are shown in Fig. 1. The statistical analysis of the results from factorial designs using a Pareto chart indicates that CG was the independent variable, which had the greatest influence in reducing surface tension, followed by interaction between the byproducts. The results suggest that CG and CSL are alternative substrates suitable for biosurfactant production from *R. arrhizus* UCP 1607.

According to Helmy et al. [26], the fact that CG and CSL consist of an appreciable diversity of organic materials and minerals that can work as essential supplements for microbial metabolism renders them attractive byproducts for the microbial production of tensoactive agents. Moreover, they are plentifully generated in the respective industries [27]. With regard to this, the role of both byproducts in producing surface-active compounds has been broadly studied, particularly with bacteria [13,28,29]. However, the potential of these agro-industrial wastes in biosurfactant production has not yet been sufficiently studied for filamentous fungi. Thus far, reports on producing surface-active compounds by means of the bioconversion of CG among fungi [30] remain more scarce than those regarding CSL in this group [7,9,24].

3.2. Growth kinetics

Different parameters related to the growth profile and production of biosurfactant by *R. arrhizus* UCP 1607 cultivated at temperature of 28°C during 96 h of incubation under orbital shaking at 150 rpm are summarized in Fig. 2. According to Sarubbo et al. [31], the growth of a microorganism results from its capacity to use the essential nutrients available in the culture medium, and this has the consequence of releasing microbial metabolites. With regard to this, *R. arrhizus* UCP 1607 showed great ability in using CG and CSL as alternative substrates and bioconverting them to different essential materials for its growth, but this also resulted in biosurfactant production [30]. According to the data, the fungus demonstrated its most active profile of growth in the first 48 h, with slight changes up to the end of cultivation time.

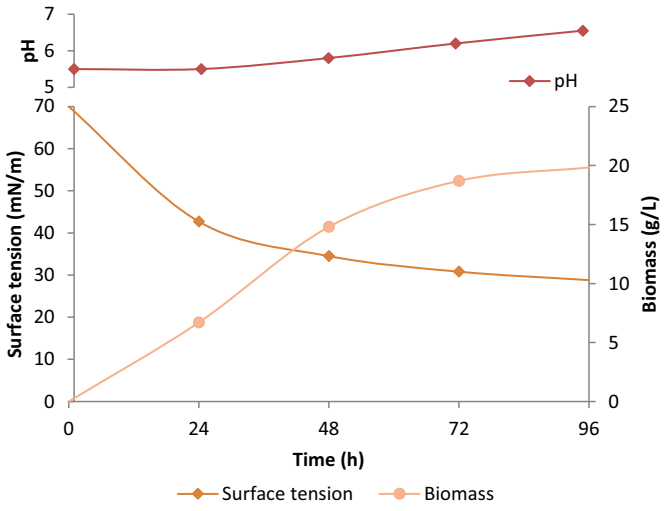

Fig. 2. Parameters of growth and production of biosurfactant by *Rhizopus arrhizus* UCP 1607 at 28°C, during 96 h of incubation in the culture medium supplemented with 3% crude glycerol and 5% corn steep liquor.

Thus, the growth of *R. arrhizus* UCP 1607 in the medium consisting of 3% CG and 5% CSL showed a rapid increase in biomass (14.8 g/L) in the first 48 h and then continuously increased, thereby reaching a biomass of 19.84 g/L after 96 h. In parallel, the surface tension decreased clearly in the first 48 h of growth and reached 34.5 mN/m, thus suggesting strong surface properties of the biomolecule, which then gradually decreased to approximately 28.8 mN/m at the end of 96 h of cultivation. The biosurfactant showed a growth-associated production that was similar to those produced by *Cunninghamella echinulata* grown in soybean oil residue and CSL [9] and by *Candida lipolytica* cultivated in glucose and canola oil as substrates [31].

The pH of neither culture media underwent significant changes throughout the growth of *R. arrhizus* UCP 1607. Several studies have shown that the acidity of the medium is related to the production of a minor surfactant [30,32]. Thus, as the pH remained almost unchanged

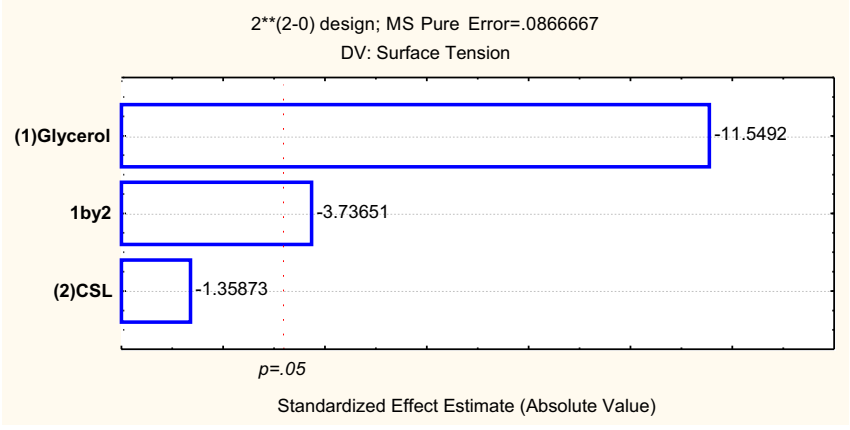

Fig. 1. Pareto chart for the 2^2 factorial design of standardized effects for (1) crude glycerol — CG and (2) corn steep liquor — CSL — with surface tension as the dependent variable. The point at which the effect estimates were statistically significant (at $P = 0.05$) is indicated by the vertical broken line.

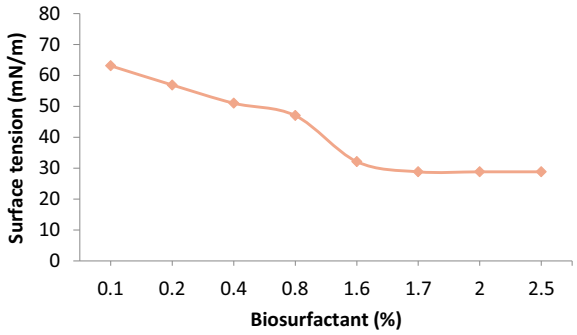

Fig. 3. Surface tension versus concentration of the isolated biosurfactant produced by *Rhizopus arrhizus* UCP 1607 using crude glycerol and corn steep liquor.

during the cultivation, it may have not exerted negative influence on the performance of the synthesis of the biosurfactant by *R. arrhizus* UCP 1607.

3.3. Biosurfactant yield

The yield of the isolated biosurfactant produced by *R. arrhizus* UCP 1607 grown in CG and CSL during 96 h of incubation was 1.74 g/L. Biosurfactants from *Ustilago maydis* FBD12 [33] showed a production yield of 0.183 g/L and 0.096 g/L using fish oil and soy oil as substrates, respectively, during 9 d of cultivation. Although the yield of biosurfactant from water-soluble substrates is referred to as yielding less biosurfactant in comparison with that from hydrophobic materials [1], this result suggested the potential of using the CG and CSL as suitable alternative sources for producing tensioactive agents. Additionally, the results showed that the biosurfactant yield is dependent not only on the nature of the composition of the medium but also on the ability of the microorganism to produce biosurfactants, as shown by the new strain of *R. arrhizus* UCP 1607.

3.4. Critical micelle concentration (CMC)

From a practical point of view, CMC is the minimum concentration of a biosurfactant required to attain the maximum diminution in the surface tension of water, and it measures the efficiency of a biosurfactant [34]. In this context, when the concentration of the isolated biosurfactant was increased, the surface tension of water gradually decreased from 72 to 28.8 mN/m with a CMC of 1.7%, and from this critical point onward, no further decrease in the surface tension was observed when the biosurfactant concentration was increased, thus indicating that the CMC had been attained (Fig. 3).

Table 3
Phytotoxicity of the biosurfactant isolated from *Rhizopus arrhizus* UCP 1607 grown in 3% crude glycerol and 5% corn steep liquor against *Brassica oleracea* and *Lactuca sativa* L. The results represent mean values of the experiments undertaken in duplicate.

Biosurfactant (g/L)	Seed germination (%)		Root elongation (%)		Germination index (%)	
	B. oleracea	*L. sativa*	*B. oleracea*	*L. sativa*	*B. oleracea*	*L. sativa*
1.0	100	100	100	104	100	104
1.7	100	100	96	102	96	102
2.5	99	97	102	106	101	103

The result reveals that the biosurfactant produced by *R. arrhizus* UCP 1607 has a greater capacity to reduce tension when compared with those from other fungi, e.g., *Candida glabrata* UCP 1002 (32 mN/m) [35] and *Fusarium* sp. BS-8 (32 mN/m) [10]. Furthermore, the biosurfactant synthesized by the new strain of *R. arrhizus* UCP 1607 exhibited lower CMC values than those observed with other tensioactive agents of fungi, when considering a CMC of 2% and 2.5% for biosurfactants from *C. echinulata* [9] and *C. glabrata* UCP 1002 [35], respectively.

3.5. Ionic charge and biochemical composition of biosurfactant

A − 31.28 ± 0.29 ZPmv Zeta meter was used to show that the biosurfactant produced by *R. arrhizus* UCP 1607 has an anionic character, which is 147 μS/cm at 27.4°C, full scale, thus indicating that its polar hydrophilic heads are negatively charged [9]. Biosurfactants from other fungi showed an anionic profile when subjected to Zeta meter analysis [7,9].

Preliminary biochemical characterizations demonstrated that the biosurfactant consisted of proteins (38%), carbohydrates (35.4%), and

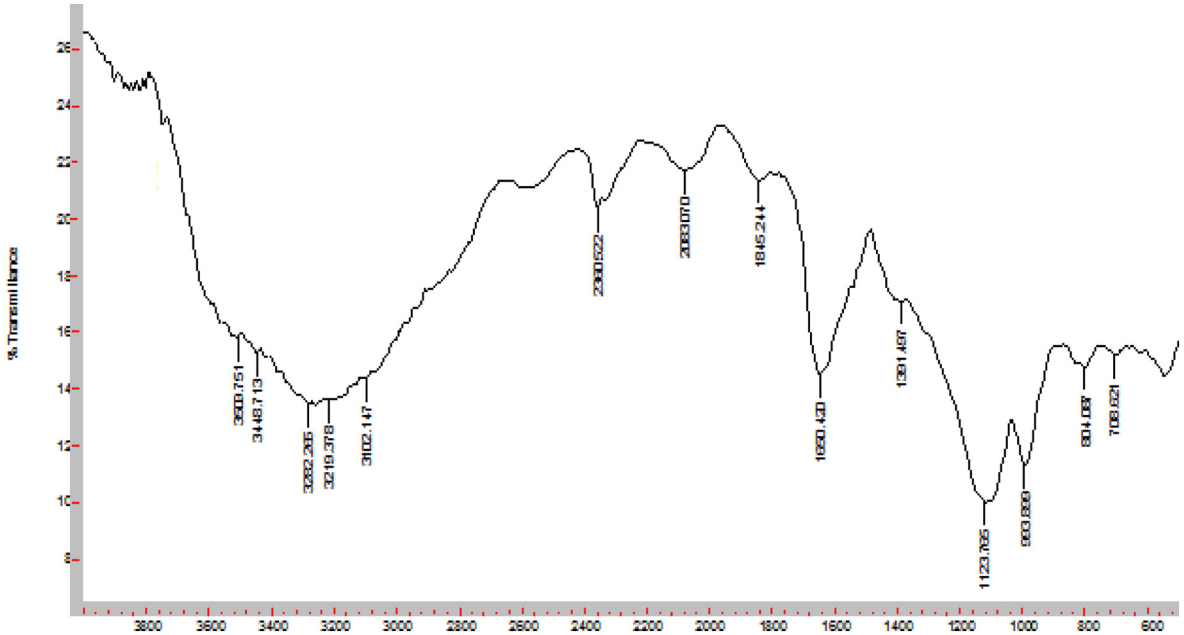

Fig. 4. FTIR spectra of the biosurfactant produced by *Rhizopus arrhizus* UCP 1607 using crude glycerol and corn steep liquor.

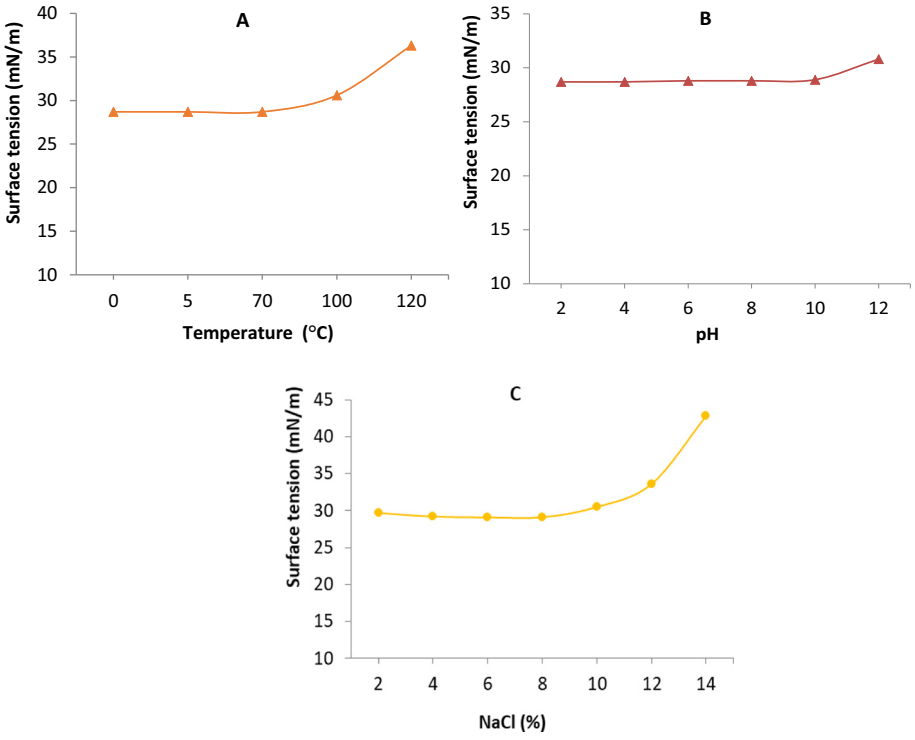

Fig. 5. Effects of temperature (A), pH (B) and sodium chloride (NaCl) concentrations (C) on surface tension of biosurfactant produced by *Rhizopus arrhizus* UCP 1607 using crude glycerol (3%) and corn steep liquor (5%) as substrates.

lipids (5.5%), thus suggesting that it is a type of biosurfactant. A marine endosymbiotic fungus, *Aspergillus ustus* MSF3 isolated from the marine sponge *Fasciospongia cavernosa*, produced a biosurfactant in Sabouraud dextrose broth, which was partially characterized as a glycoprotein [36].

The molecular composition of the partially purified freeze-dried biosurfactant produced by the new strain of *R. arrhizus* UCP 1607 was investigated by FTIR spectroscopy (Fig. 4). It is a quick and simple method and the one most frequently used to identify functional groups (alkyls, carbonyls, and esters) in biosurfactant samples [37]. The most important bands were located at 3282.265 cm^{-1} (for O—H bonds, typical of polysaccharides), 1650.420 cm^{-1} (C—O ester bond), 1123.765 cm^{-1} (CH_2—CH_2, polysaccharides), and 1391.497 cm^{-1} (C—N amide groups). As the infrared spectra of the biosurfactant sample at 1650.420 cm^{-1} suggested the presence of C—O ester bonds, indicating the ester linkage between the polysaccharide-forming monomers, and at 1391.497 cm^{-1} band for the C—N bond corresponding to the amide group of proteins, the compound was confirmed as consisting of a structure of glycoprotein moieties. These results are in agreement with those reported in Andrade et al. [7], because of the presence of the ester functional group, which absorbs energy in the infrared region. When biosurfactant molecules are subjected to FTIR evaluation, it results in a strong peak in the infrared spectrum of a compound.

3.6. Biosurfactant toxicity

Plant bioassays play a crucial role, as they allow predicting the effect of chemicals in ecosystems. Therefore, assessing the toxicity of a new biosurfactant in the context of its applications in diverse environmental bioremediation processes is of great importance to safeguard so that its potential application does not trigger harmful effects for the terrestrial environment [38,39]. In the current study, the evaluation of the biosurfactant for phytotoxicity against cabbage (*B. oleracea*) and lettuce (*L. sativa* L.) showed a noninhibitory effect of the tensioactive solutions toward seed germination or root elongation in the vegetables tested, as the GI values attained during the growth of the plants were above the value of 80%, which is considered the indicator of the absence of phytotoxicity [20]. These results indicate that the biosurfactant obtained from *R. arrhizus* UCP 1607 is a nontoxic compound. In addition, the elongation of secondary roots and leaf growth occurred under all conditions tested for both biomolecules (Table 3).

3.7. Stability analysis

Fig. 5 shows the influence of different levels of pH, temperature, and salinity monitored on the surface tension of biosurfactant produced

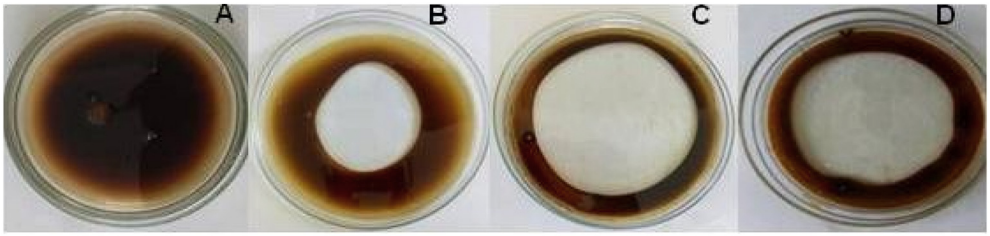

Fig. 6. Burnt motor oil displacement areas (ODAs) obtained using the dispersants: (A) water; (B) commercial detergent; (C) chemical surfactant (SDS), and (D) crude biosurfactant produced by *R. arrhizus* UCP 1607.

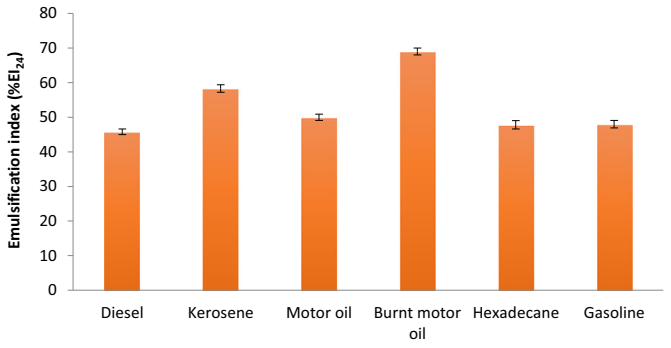

Fig. 7. Emulsifying capacity of the biosurfactant produced by *Rhizopus arrhizus* UCP 1607 toward various hydrocarbons.

by *R. arrhizus* UCP 1607. According to the data, the biosurfactant maintained its capacity to reduce surface tension over a wide range of temperatures (0–100°C), pH (2–12), and NaCl concentrations (2–10%). The biosurfactant activity was slightly affected when the temperature was increased to 120°C (34.4 mN/m) and salinity to 14% NaCl (42.8 mN/m). These results suggest that it is feasible for the biomolecule to be applied in industries that work under extreme conditions of salinity, pH, and temperature, and these results are comparable with those reported for other biosurfactants from fungi [9,40].

According to Khopade et al. [41] and Santos et al. [42], biosurfactants are able to tolerate wide ranges of temperature, pH, and salt concentration when compared with synthetic surfactants, which, at 70°C and 2% NaCl, can get inactivated with significant loss of their surfactant stability.

3.8. Oil displacement area (ODA)

Dispersion is one of the characteristics of biosurfactants that aids in enhancing the trapped oil from oil wells [43]. Fig. 6 illustrates the capacity of the biosurfactant from *R. arrhizus* UCP 1607 to disperse the layer of burnt motor oil, as indicated, because clear zones are formed on the surface of water. According to the data, no dispersion activity was observed with distilled water (Fig. 6A); when commercial detergent (1%) was used, it displaced the oil in 44.2 cm^2 ODA (Fig. 6B), and with SDS (1%), it resulted in 72.7 cm^2 ODA (Fig. 6C). The use of biosurfactant crude extract induced the formation of a clear zone of 53.4 cm^2 ODA (Fig. 6D).

Andrade Silva et al. [9] reported a biosurfactant produced by the mucoralean fungus *C. echinulata* that showed dispersing action of 37.36 cm^2 ODA. Poomtien et al. [44] also reported a dispersion rate of 44.5 cm^2 ODA for the biosurfactant from *Cyberlindnera samutprakarnensis* JP52. We observed superior results with the biosurfactant produced by *R. arrhizus* UCP 1607 and suggest that it has the potential for being applied in enhanced oil recovery and oil spill bioremediation [3,5].

3.9. Emulsification index ($\%EI_{24}$)

Emulsification capacity has been frequently used as a suitable technique in the detection of surface-active compounds that produce strains, as it correlates to the biosurfactant concentration [45]. However, this functional property also has been used as a powerful measuring tool to determine the ability of the biosurfactant to form and stabilize emulsions of different hydrophobic substrates, when considering their potential practical applications in many interesting areas, particularly in enhanced processes to protect the environment and in the petroleum processing industry [43].

The emulsion-stabilizing capacity of a surface-active compound is assessed by its ability to maintain at least 50% of the original emulsion volume for 24 h after it has formed. Considering this criterion, the biosurfactant produced by *R. arrhizus* UCP 1607 was able to emulsify a variety of hydrocarbons, as shown in Fig. 7. With regard to this, the biosurfactant demonstrated great ability in forming stable emulsions for kerosene (58.3%), motor oil (50%), and burnt motor oil (79.4%) at the best emulsification values ($\%EI_{24}$).

Similar results for biosurfactants produced by *R. arrhizus* UCP 1607 were reported by Elshafie et al. [43], who tested the efficacy of a biosurfactant from *Candida bombicola* ATCC 22214, which showed emulsification values ranging from 23.8% to 68.75% with various petroderivatives. Additionally, Rubio-Ribeaux et al. [46] reported a novel biosurfactant from *Candida* sp. strain, which formed significantly stable emulsions with diesel (79%) and motor oil (67%). The promising $\%E_{24}$ values shown by the biosurfactant produced by *R. arrhizus* UCP 1607 suggest its potential application in enhanced oil recovery and in the bioremediation of hydrophobic organic carbon-contaminated environments [3,5].

3.10. Study of applying biosurfactant to remove diesel oil

The application of biosurfactant solutions to hydrocarbon-contaminated soils can be effective in enhancing the solubilization and mobilization of hydrophobic organic compounds entrapped in soil particles, thereby facilitating desorption and the removal of the pollutants from the soil [5,47]. Considering this fact, the crude extracts of the biosurfactant produced by the new strain of *R. arrhizus* UCP 1607 were evaluated for their efficiency in removing hydrophobic contaminants adsorbed onto marine soil. The results obtained showed that the biosurfactant removed 79.4% of the diesel impregnated in marine soil (Fig. 8).

Andrade et al. [7] investigated the effectiveness of cell-free broth containing biosurfactants from *C. glabrata* that showed a removal

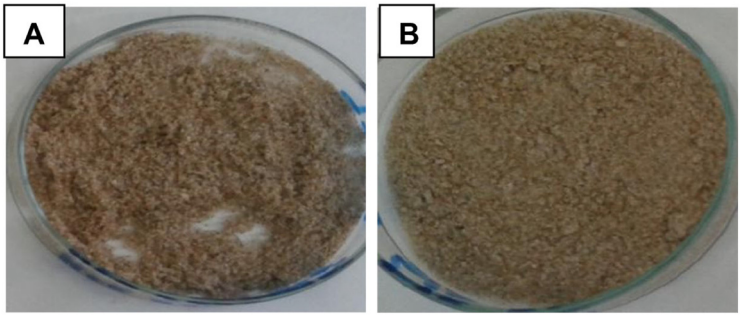

Fig. 8. Removal of diesel oil impregnated in marine soil sand using biosurfactant and bioemulsifier crude extracts produced by *Rhizopus arrhizus* UCP 1607. (A) Marine soil with remaining diesel oil after contact with distilled water and (B) marine soil with remaining diesel oil after contact with crude biosurfactant.

capacity of 95.7% of burnt motor oil impregnated in sand. As reported in literature, the washing efficiency of crude biosurfactant solutions from *Candida sphaerica* removes approximately 50% of the oil adsorbed on the sand [13,48]. Our results demonstrate that the biosurfactant from *R. arrhizus* UCP 1607 is a promising candidate for the application of the enhanced removal of hydrophobic contaminants from polluted soil.

According to Silva et al. [24], the crude biosurfactants and the isolated biosurfactants are almost equally effective at removing the motor oil pollutant. One of the potential advantages of using crude extracts containing biosurfactants is that they can be directly used without purification steps, which would further reduce 30–50% of the production cost of biosurfactants.

4. Conclusions

The new strain *R. arrhizus* UCP 1607 demonstrated a great potential in the synthesis of biosurfactants using low-cost substrates. The biosurfactant produced in this study, in addition to possessing a desirable emulsifying capacity toward different hydrophobic substrates, also has excellent tensioactive properties and was characterized as a glycoprotein. This biosurfactant constitutes a promising candidate for application in the enhanced oil recovery and bioremediation of site of hydrocarbon contamination.

Declarations of interest

All authors declare no conflict of interest.

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Supplementary material

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