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Abstract
The reutilization of agro-industrial by-products for obtaining of high-value added biosurfactants is a promising approach for minimizing the total production costs. In this context, this study aimed to evaluate the production of biosurfactant by the Mucoralean fungus *Syncephalastrum racemosum* UCP 1302, by bioconversion of renewable substrates: cassava wastewater (CWW), waste soybean oil (WSO) and corn steep liquor (CSL). For this, a 2^3 full-factorial design (FFD) was applied and the results showed the ability of this strain to produce biosurfactant in all conditions of the FFD, standing out the condition 7 due to the greatest reduction of surface tension (from 72 to 30.9 mN/m). Preliminary characterization showed the lipopeptide nature of the biomolecule, as well as its anionic character and critical micellar concentration (CMC) of 1.25 mg/ml. Biotensoactive demonstrated stability to variations of temperature, pH and NaCl concentrations, wettability in polyester textile and it was effective on reduction of viscosity of burned motor oil. Hence, S. *racemosum* showed excellent ability to produce biosurfactant by green bioconversion of low-cost substrates, making the bioprocess economical and enabling its biotechnological applications.
Keywords: Waste bioconversion; Microbial surfactant; Mucoralean fungus; Cassava wastewater; Waste soybean oil; Corn steep liquor.

Resumo
O reaproveitamento de subprodutos agroindustriais para a obtenção de biossurfactantes de alto valor agregado é uma abordagem promissora para minimizar os custos totais de produção. Neste contexto, este trabalho teve como objetivo avaliar a produção de biossurfactante pelo fungo Mucorales *Syncephalastrum racemosum* UCP 1302, por bioconversão de substratos renováveis: manipueira, óleo de soja residual e milhocina. Para isso, foi aplicado um planejamento fatorial

completo de 2^3 e os resultados mostraram a capacidade desta cepa em produzir biossurfactante em todas as condições do planejamento, destacando-se a condição 7 devido à maior redução da tensão superficial (de 72 para 30,9 mN/m). A caracterização preliminar mostrou a natureza lipopeptídica da biomolécula, bem como seu caráter aniônico e concentração micelar crítica (CMC) de 1,25 mg/ml. O biotensoativo demonstrou estabilidade às variações de temperatura, pH e concentrações de NaCl, molhabilidade em tecido de poliéster e foi eficaz na redução da viscosidade do óleo de motor queimado. Assim, *S. racemosum* apresentou excelente capacidade de produzir biossurfactante por bioconversão verde de substratos de baixo custo, tornando o bioprocesso econômico e viabilizando suas aplicações biotecnológicas.
Palavras-chave: Bioconversão de resíduos; Surfactante microbiano; Fungo Mucorales; Manipueira; Óleo de soja residual; Milhocina.

Resumen
La reutilización de subproductos agroindustriales para obtener biosurfactantes de alto valor agregado es un enfoque prometedor para minimizar los costos totales de producción. En este contexto, este estudio tuvo como objetivo evaluar la producción de biosurfactante por el hongo Mucorales *Syncephalastrum racemosum* UCP 1302, por bioconversión de sustratos renovables: aguas residuales de yuca, aceite de soja residual y licor de maceración de maíz. Para ello se aplicó un diseño factorial completo de 2^3 y los resultados mostraron la capacidad de esta cepa para producir biosurfactante en todas las condiciones de diseño, destacándose la condición 7 por la mayor reducción de la tensión superficial (de 72 a 30,9 mN/m). La caracterización preliminar mostró la naturaleza lipopeptídica de la biomolécula, así como su carácter aniónico y concentración micelar crítica (CMC) de 1,25 mg/ml. El biotensoactivo demostró estabilidad a variaciones de temperatura, pH y concentraciones de NaCl, humectabilidad en tejido de poliéster y fue efectivo en la reducción de la viscosidad del aceite de motor quemado. Por lo tanto, *S. racemosum* mostró una excelente capacidad para producir biosurfactante por bioconversión verde de sustratos de bajo costo, haciendo que el bioproceso sea económico y posibilitando sus aplicaciones biotecnológicas.
Palabras clave: Bioconversión de residuos; Tensioactivo microbiano; Hongo Mucorales; Aguas residuales de yuca; Aceite de soja residual; Licor de maceración de maíz.

1. Introduction

Chemical surfactants are amphiphilic compounds, that is, the same molecule has a polar portion, soluble in water, also called hydrophilic portion, and a nonpolar portion, insoluble in water, also called lipophilic or hydrophobic. Due to these characteristics, surfactants reduce the surface tension at the interface of the immiscible phases, thus allowing them to mix (Punniyakotti, 2017; Gaur, et al., 2022).

According to Allied Market Research, the world market for surfactants reached approximately US$41.3 billion in 2019, and it is estimated that world demand for these compounds will reach US$58.5 billion by 2027. Most commercially available surfactants it is synthesized from petroleum derivatives; however, the growing environmental concern among consumers associated with the new legislation to control the environment, has led to the search for natural surfactants, as an alternative to the existing ones (Guidi, et al., 2018; Ismail, et al., 2022).

Biosurfactants (BS) constitute one of the main classes of natural surfactants and have numerous advantages over chemicals, including high surface and interfacial activity, tolerance to temperature, pH and ionic strength, high biodegradability in water and soil, and low toxicity (Banat, et al., 2010). Consequently, there has been a great increase in interest in these products due to their sustainable characteristics (Makkar, et al., 2011; Abreu, et al., 2022).

In the literature, most works report the production of BS by bacteria, mainly from the genera *Bacillus* sp., *Pseudomonas* sp., *Acinetobacter* sp. and *Arthobacter* sp. (Vatsa, et al., 2010; Chebbi, et al., 2017; Faria, et al., 2021), and yeasts, mainly of the *Candida* genus (Garay, et al., 2017; Rubio-Ribeaux, et al., 2020). However, in the last decade several researches have focused on obtaining BS produced by filamentous fungi, especially those belonging to the order Mucorales (Andrade, et al., 2018; Marques, et al., 2019; Pele, et al., 2019; Mendonça, et al., 2021; Montero-Rodríguez, et al., 2022).

Despite the numerous advantages of these BS over chemical compounds, a major problem is still related to the increase in production scale and cheaper production. For example, surfactin produced by the company “Sigma Chemical Company”, costs approximately $USD 153 for a bottle containing 10 mg. This value is still very high when compared to chemical surfactant, which costs approximately $USD 2.0 per kilo (Makkar, et al., 2011; Wang, et al., 2022). According to Kosaric, et al. (1984), cost reduction should be focused on the selection of microorganisms, adapted to the process or genetically modified for this purpose; process adaptations so that it has low application and maintenance costs; selection of low-cost substrates for microbial growth and use of low-cost extraction and purification methodologies (Makkar & Cameotra, 2002; Lima, et al., 2021).

Thus, the biotechnological use of agro-industrial by-products and waste has been distinguished as an attractive alternative for exploitation, as a nutritional source for obtaining high-added value products. In this context, residues of a hydrophilic nature are considered advantageous, considering the possibility of bioconversion by filamentous fungi, which allows for a greater yield of bioproducts (Montero-Rodríguez, et al., 2016; de Souza, et al., 2021). Hence, present study aimed to bioconversion of agro-industrial wastes into BS by *Syncephalastrum racemosum* UCP 1302, as well as the isolation and preliminary characterization of the biomolecule.

2. Methodology

2.1 Microorganism

The Mucoralean fungus *S. racemosum* UCP 1302, isolated from Serra Talhada soil, in the State of Pernambuco, Brazil, was kindly provided by the Culture Collection UCP - Catholic University of Pernambuco (Recife-PE, Brazil), registered to the World Federation for Culture Collections (WFCC) under number 927. This strain was maintained on Sabouraud Dextrose Agar medium at 5°C.

2.2 Agro-industrial by-products

The agro-industrial by-products used in this study were corn steep liquor (CSL), a corn processing by-product provided by Corn Products Ltda industry (Vitória de Santo Antão - PE, Brazil), cassava wastewater (CWW) from a flour house (Pombos-PE, Brazil) and waste soybean oil (WSO), which was kindly provided by the informal trade.

2.3 Biosurfactant production

BS production was carried out in 250 ml Erlenmeyer flasks, containing 100 ml of saline medium (0.1% NH_4NO_3, 0.02 g/L, KH_2PO_4 and 0.02 g/L $MgSO_4$) and the agro-industrial by-products at concentrations established by the 2^3 full-factorial design (FFD) (Section 2.4). The pH of the production media was adjusted to 5.5 by addition of 1 M NaOH or HCl solution and then, they were sterilized by autoclaving at 121°C for 15 min. A 10^8 spores/ml suspension of *S. racemosum* UCP 1302 was used to inoculate each medium and the fermentations were carried out at 150 rpm and 28°C, for 96 h. After this time, the cultures were filtrate using Whatman no.1 filter paper and centrifuged at 8000 *g* and 5ºC for 15 min. The mycelia-free metabolic liquids were used for determination of pH and surface tension, as described later (sections 2.5 and 2.6).

2.4 Full-factorial design (FFD)

In this study, a 2^3 FFD was carried out in order to investigate the effects of each independent variable (concentration of CWW, WSO and CSL in culture medium), as well as the interactions between them, on surface tension as response variable. A set of eight assays with three replicates at the central point was performed, according to levels shown in Table 1. The experimental data were analyzed by Statistica® software, version 12.0 (StatSoft Inc., USA) and the significance of the results was tested (p <0.05).

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Table 1: Variables and levels of the 2^3 full-factorial design applied for biosurfactant production by *S. racemosum* UCP 1302.

Variables	Levels		
	-1	0	+1
Cassava wastewater (%, *v/v*)	3.5	5.0	6.5
Waste soybean oil (%, *v/v*)	3.5	5.0	6.5
Corn steep liquor (%, *v/v*)	3.5	5.0	6.5

Source: Authors.

2.5 Determination of pH

An Orion potentiometer (Model 310) (Orion Research Inc., Cambridge, MA, USA) was used to determine the pH of the aliquots collected from the cell free production media.

2.6 Determination of surface tension

Surface tension was determined on mycelia-free metabolic liquids with a tensiometer model Sigma 70 (KSV Instruments Ltd., Finland) using the Du Noüy ring method at room temperature (±28°C). Measurements of surface tension from distilled water were used as control (Kuyukina, et al., 2001).

2.7 Kinetics of growth, pH and BS production

The condition of FFD with lower value of surface tension was chosen for study the kinetic of production of BS, microbial growth and pH for 96 h at 28°C and 150 rpm. Every 24 h aliquots were collected and subjected to filtration followed by cold centrifugation as described in section 2.3. Then, the mycelia-free metabolic liquids were used for determination of pH and surface tension, as described in sections 2.5 and 2.6, respectively. The biomasses were washed with distilled water, lyophilized and yield was calculated by gravimetry, and the results were expressed in g/L.

2.8 Stability of BS

The stability of the BS was evaluated by determination of surface tension in the mycelia-free metabolic liquids submitted individually to different pH values (2, 4, 6, 8, 10 and 12), NaCl concentrations (0, 2, 4, 6, 8, 10 and 12%) and temperatures (0, 5, 28, 37, 50 and 100°C) (Barros, et al., 2008).

2.9 Effect of BS on the viscosity of burned motor oil

The effect of the BS on the viscosity of burned motor oil was investigated in test tubes containing 6 ml of the hydrophobic compound and 2 ml of mycelia-free metabolic liquids containing BS. Then the tubes were shaken for 1 min and the viscosity was measured at 25°C in an automatic viscometer (Brookfield Middleboro, Middleborough, MA, USA; TC 500). Anionic surfactant sodium dodecyl sulfate (SDS) was used as a control. The viscosity results were expressed in centipoise (cP).

2.10 Isolation of BS

The BS produced by *S. racemosum* was extracted from the mycelia-free metabolic liquid using the ethanol precipitation method, according to Techaoei, et al. (2007). After extraction, crude BS was washed twice with distilled water and subjected to lyophilization; the yield was calculated by gravimetry and expressed in g/L.

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2.11 Preliminary characterization of BS

2.11.1 Ionic charge

The ionic charge of BS was investigated using 100 mg of the biomolecule solubilized in 5 ml of distilled water, using a Zeta ZM3-D-G potentiometer, Zeta Meter System 3.0+, and the direct images were recorded in a Zeta Meter video, San Francisco, CA, USA.

2.11.2 Biochemical composition

The biochemical composition of the BS was investigated. Total proteins were estimated using the total protein test kit from Labtest Diagnostica S.A., Brazil. The total carbohydrates content was estimated by the phenol-sulfuric acid method (Dubois, et al., 1956) and lipids content was determined according to Manocha, et al. (1980).

2.12 Determination of Critical Micellar Concentration (CMC)

The isolated BS was solubilized in distilled water at the different concentrations (0.625, 1.25, 2.5, 5, 10 and 20 mg/ml) and then, surface tension was measured as described in section 2.6. CMC was reached after observing a constant value of the surface tension (Manivasagan, et al., 2014).

2.13 Wettability of BS

The wettability of BS was tested in polyester fabric (2×2 cm), using the cloth wetting test, where the textile was analyzed by gravimetry and optical microscopy, using waster as control (Pradhan & Bhattacharyya, 2017).

3. Results and Discussion

3.1 Production of BS by *S. racemosum* UCP 1302 using agro-industrial by-products

The determination of surface tension has often been used as a rapid method to detect the BS production in the culture medium (Araujo, et al., 2019; Sharma, 2021). Promising BS-producing microorganisms are considered those that reduce surface tension to values less than 35 mN/m (da Silva, et al., 2021). In this context, Table 2 presents the values of surface tension obtained by *S. racemosum* in low-cost media, showing its ability to produce BS in all conditions of the FFD.

Table 2: Values of surface tension obtained according to the 2^3 FFD used for biosurfactant production by *Syncephalastrum racemosum* UCP 1302.

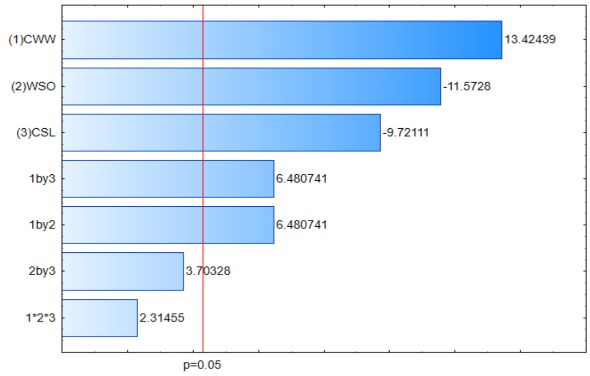
Conditions	Cassava wastewater (%)	Waste soybean oil (%)	Corn steep liquor (%)	Surface tension (mN/m)
1	-1	-1	-1	34.6
2	+1	-1	-1	34.9
3	-1	+1	-1	32.5
4	+1	+1	-1	33.7
5	-1	-1	+1	32.7
6	+1	-1	+1	33.9
7	-1	+1	+1	30.9
8	+1	+1	+1	34.0
9	0	0	0	34.1
10	0	0	0	34.2
11	0	0	0	33.9

Source: Authors.

According to the results, *S. racemosum* demonstrates its great ability to reduce surface tension, when compared with others BS produced by Mucoralean fungi. In current study, the greatest reduction in surface tension (72 to 30.9 mN/m) was verified in condition 7, in medium containing 3.5% CWW, 6.5% WSO and 6.5% CSL. Previously, The BSs produced by *Rhizopus arrhizus* UCP 1607 and *R. microsporus* var. *microsporus* UCP 1304 reached surface tension values of 31.8 mN/m and 34.7 mN/m, respectively (Pele, et al., 2018). Andrade, et al. (2018) isolated the BS produced by *Cunninghamella echinulata* UCP 1299 in medium containing instant noodle waste and WSO, which decreased the surface tension to 32.0 mN/m. Recently, Mendonça, et al. (2021) reported the production of BS by *Absidia cylindrospora* UCP 1301 with ability to reduce de surface tension to 30.2 mN/m, a result similar to that obtained in our study.

In addition, the statistical analysis demonstrated the significant effect of concentrations of the three agro-industrial by-products on surface tension. However, according to the Pareto diagram (Figure 1), only WSO and CSL had a negative influence, from a statistical point of view, on the obtained surface tension values. This means that an increase in WSO or CSL concentration led to lower surface tension, suggesting the production of BS in the culture medium. Previously, several studies reported the effectiveness of WSO and/or CSL as inductors for microbial BS production (Andrade, et al., 2014; Mendonça, et al., 2021; Cândido, et al., 2022).

Figure 1: Pareto diagram obtained from the statistical analysis of the 2^3 full-factorial design applied to the production of biosurfactant by *Syncephalastrum racemosum* UCP 1302. The point at which the effect estimates were statistically significant *(p* = 0.05) is indicated by dashed line.



Source: Authors.

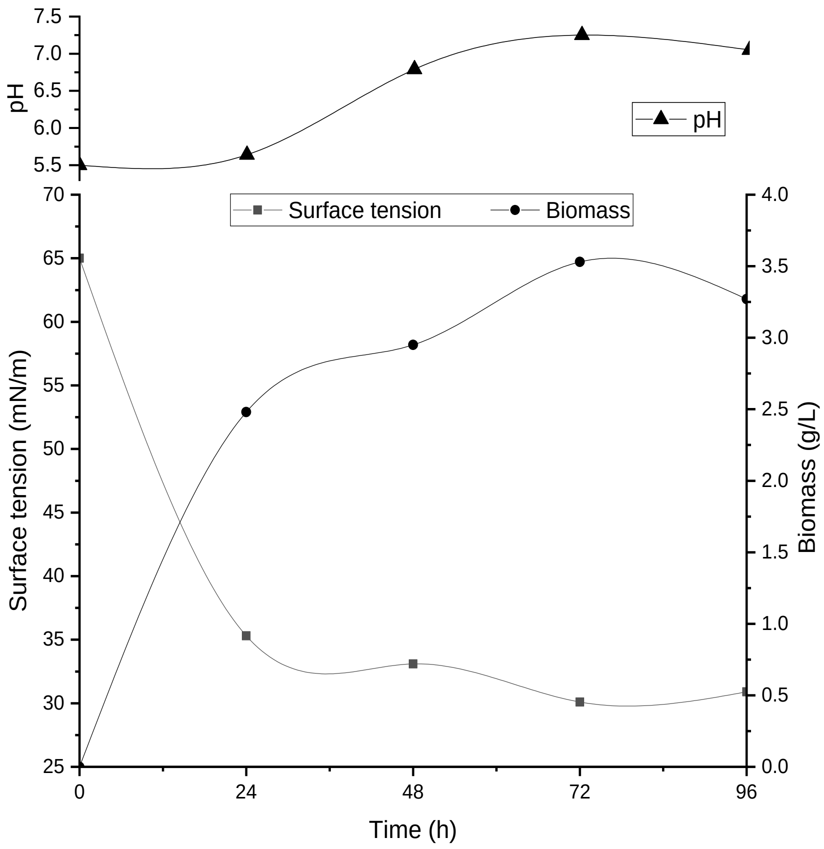
3.2 Growth kinetics and BS production

After the selection of condition 7, the microbial growth kinetic and BS production were monitored during 96 h, at 28ºC and 150 rpm. As can be verified in Figure 2, *S. racemosum* showed a rapid production of BS, associated with growth, since in the first 24 h it reached a biomass yield of 2.5 g/L, while decreasing the tension of the medium to 35.3 mN/m. The greatest reduction in surface tension (30.1 mN/m) was verified at 72 h, and biomass yield achieved 3.5 g/L. Regarding the pH, it can be observed that it increased along with the production of biomass.

3.3 Stability of BS

The use of BSs in various industrial areas depends on their stability under different temperature, pH and salinity conditions (Perfumo, et al., 2018). Figure 3 illustrates the effects of these factors on the BS produced by *S. racemosum* in conditions 7 of FFD. The results showed that the surface tension remained practically unchanged in relation to the different values of pH, temperature and salinity, with only a small increase at 12% NaCl, demonstrating the effectiveness of the BS. Marchant & Banat (2012) argue that stability is an essential factor for the viability of large-scale production, especially when a product is obtained through a biotechnological process. Therefore, BS produced here is a potential candidate for environmental or industrial processes in extreme conditions.

Figure 2: Profile of biosurfactant production with pH, surface tension and biomass yield of *Syncephalastrum racemosum* UCP 1302 grown in alternative medium composed by CWW 3.5%, WSO 6.5% and CSL 6.5%.



Source: Authors.

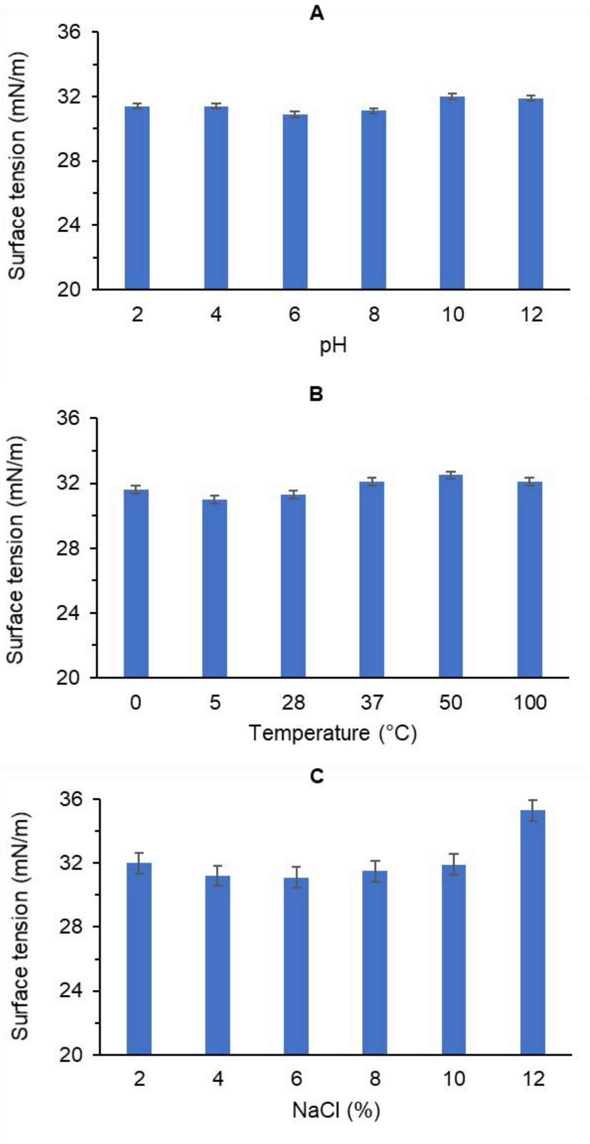
3.4 Effect of BS on viscosity of burned motor oil

The cell-free metabolic liquid containing the BS was able to form a stable system with burned motor oil, reducing its viscosity from 170 to 117.2 cP, result similar to SDS which reduced to 114.7 cP. This test was monitored for 96 h to identify the ability of the BS to maintain the viscosity of the emulsion formed. The result showed small variations, evidencing that the BS of *S. racemosum* maintained the viscosity stability up to 96 h. Previously, Marques et al., (2019) and Ferreira et al. (2020) reported a decrease in the viscosity of motor oil by the BSs produced by *M. circinelloides* and *M. hiemalis*, respectively. This property to viscosity reduction is desirable in BSs with potential application in the oil industry, since it contributes to improve the mobilization of heavy oils from reservoirs or cleaning contaminated sites (Marques, et al., 2019).

3.5 Yield and preliminary characterization of BS

The BS produced by *S. racemosum* UCP 1302 was isolated by precipitation with ethanol, obtaining a yield of 0.9 g/L. The tests performed to determine the biochemical composition of the biomolecule revealed the presence of 53.56% of lipids, 37.88% of proteins and only 8.56% of carbohydrates, suggesting its lipopeptide nature. Similarly, Marques et al. (2019) related the production of a lipopeptide BS by *M. circinelloides* UCP 0001. In addition, the zeta potential showed the anionic character of BS, similar to other BS previously produced by Mucoralean fungi (Andrade, et al., 2014; 2018; Pele, et al., 2019).

Figure 3: Stability of the biosurfactant produced by *Syncephalastrum racemosum* UCP 1302 according to the surface tension in different values of pH (A), temperature (B) and salinity C).



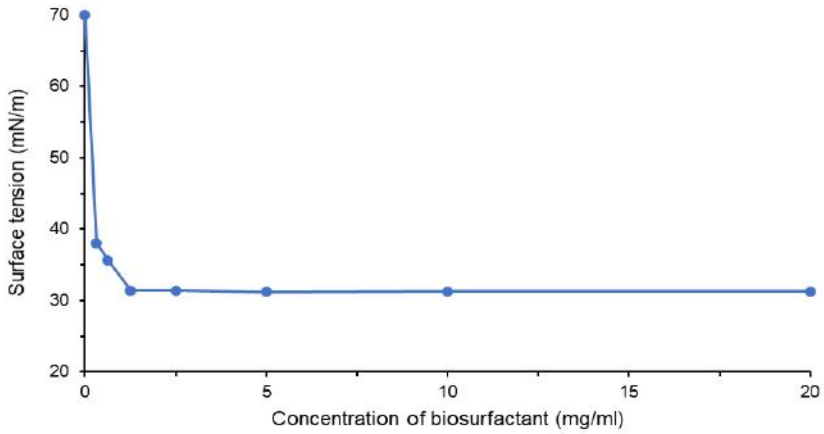
Source: Authors.

3.6 Critical Micellar Concentration of BS

CMC is an important characteristic of any surfactant, which allows making inferences about its efficiency and applicability in various industrial fields (Prado, et al., 2019). It consists of the minimum concentration of surfactant at which clusters of surfactant molecules (micelles) begin to appear in a bulk phase (de França, et al., 2021). That is, above this point, the increase in surfactant concentration does not lead to a further reduction in surface tension (Andrade, et al., 2014).

The BS produced by *S. racemosum* UCP 1302 showed low CMC of 1.25 mg/ml (Figure 4), when compared with biosurfactants produced by other Mucoralean fungi: *C. echinulata* (10 mg/ml) (Andrade, et al., 2018), *M. circinelloides* (15 mg/ml) (Marques, et al., 2019) and *R. arrhizus* (17 mg/ml) (Pele, et al., 2019). Furthermore, it was also inferior to the CMC of chemical-based surfactants, such as sodium lauryl ether sulfate (2.0–2.9 mg/ml) (Bognolo, 1999), showing this biomolecule as a promising candidate to replace synthetic surfactants in industrial applications (de França, et al., 2021). A low CMC also emphasizes the economic advantage of this BS produced by *S. racemosum* in low-cost medium, since a small amount of it is required to obtain a high efficiency in a process (de França, et al., 2015; Prado, et al., 2019).

Figure 4: Critical micellar concentration of the biosurfactant produced by *Syncephalastrum racemosum* UCP 1302.

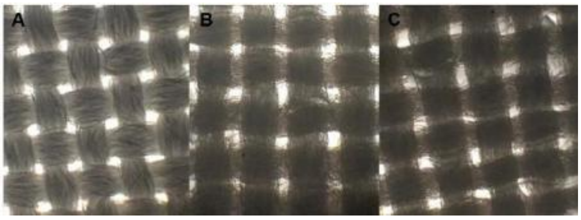


Source: Authors.

3.7 Wettability properties of BS

The gravimetric analysis allowed to prove the wettability properties of BS, since the polyester absorbed 0.16245 g of the BS, similar to the result with distilled water (0.16740 g). This finding was verified microscopically, where the dry polyester fiber (Figure 5A) has a light gray color and the fiber with absorbed BS (Figure 5C) has a dark gray color, similar to the fiber with water (Figure 5B).

Figure 5: Optic microscopy of dry polyester fiber (A), polyester fiber with absorbed water (B) and polyester fiber with biosurfactant produced by *Syncephalastrum racemosum* UCP 1302 (C).



Source: Authors.

4. Conclusion

S. racemosum UCP 1302 produced a biosurfactant in an agro-based medium with great potential for industrial applications, considering its excellent surfactant properties and stability under adverse conditions, including wide ranges of pH, temperature and salinity. The lipopeptide biosurfactant showed anionic properties, confirming its biotechnological potential, as anionic surfactants are on the rise in the global market. Moreover, this bioprocess proved to be sustainable from the valorization of agro-industrial substrates.

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