Eco-friendly production of biosurfactant by *Mucor circinelloides* UCP 0018 using agro-industrial substrates

Produção ecológica de biossurfactante por *Mucor circinelloides* UCP 0018 usando substratos agroindustriais

Producción ecológica de biosurfactante por *Mucor circinelloides* UCP 0018 utilizando sustratos agroindustriales

Received: 07/15/2022 | Reviewed: 07/29/2022 | Accept: 08/07/2022 | Published: 08/21/2022

Ana Paula Bione
ORCID: https://orcid.org/0000-0003-4614-5312
Federal Rural University of Pernambuco, Brazil
E-mail: anapaulabione@outlook.com
Amanda Barbosa Lins
ORCID: https://orcid.org/0000-0002-0132-6390
Federal Rural University of Pernambuco, Brazil
E-mail: amanlins@hotmail.com
Camila Freire de Melo
ORCID: https://orcid.org/0000-0001-8601-5489
Federal Rural University of Pernambuco, Brazil
E-mail: camila_melo84@hotmail.com
Rafael de Souza Mendonça
ORCID: https://orcid.org/0000-0001-9226-1627
Catholic University of Pernambuco, Brazil
E-mail: rafa.13souza@hotmail.com
Dayana Montero Rodríguez
ORCID: https://orcid.org/0000-0001-8954-7309
Catholic University of Pernambuco, Brazil
E-mail: dayanamontero87@gmail.com
Galba Maria de Campos-Takaki
ORCID: https://orcid.org/0000-0002-0519-0849
Catholic University of Pernambuco, Brazil
E-mail: galba.takaki@unicap.br

Abstract

Green bioconversion of low-cost agro-industrial substrates into high-value-added products becomes a biotechnological strategy to enable the production on an industrial scale. In this context, two agro-industrial by-products were used in this study in order to formulate an economic and sustainable media for biosurfactant (BS) production by *Mucor circinelloides* UCP 0018. For this, a 2^2 full-factorial design was used to investigate the influence of concentrations of corn steep liquor (CSL) and crude glycerol (CG) on surface tension as response variable. The results showed the ability of this Mucoralean fungus to produce BS under all design conditions, highlighting condition 4 (9% of both substrates) due to the greater reduction in surface tension (from 72 to 29.7 mN/m). Statistical analysis confirmed significant influence of both by-products on surface tension. This BS exhibited excellent emulsifying properties (EI_{24} 98.9%), as well as ODA of 28.26 cm², with burnt motor oil. Preliminary characterization showed the polymeric nature of the biomolecule, with a content of 55.7% of carbohydrates, 28.2% of lipids and 16.1% of proteins, as well as its anionic character and critical micellar dilution (CMD) at 50%. The stability in the range of pH 2-6, temperature 20-80°C and salinity 5-25% evidenced a stable BS, with promising potential of application in several industrial activities or environmental processes in adverse conditions.

Keywords: Microbial surfactant; Mucorales fungus; Waste bioconversion; Emulsifying properties.

Resumo

A bioconversão verde de substratos agroindustriais de baixo custo em produtos de alto valor agregado torna-se uma estratégia biotecnológica para viabilizar a produção em escala industrial. Nesse contexto, dois subprodutos agroindustriais foram utilizados neste estudo com o objetivo de formular um meio econômico e sustentável para a produção de biossurfactante (BS) por *Mucor circinelloides* UCP 0018. Para isso, foi utilizado um planejamento fatorial completo 2^2 para investigar a influência das concentrações de milhocina e glicerol bruto na tensão superficial como variável de resposta. Os resultados mostraram a capacidade deste fungo Mucorales em produzir BS em todas as

condições de planejamento, destacando a condição 4 (9% de ambos substratos) devido à maior redução da tensão superficial (de 72 para 29,7 mN/m). A análise estatística confirmou a influência significativa de ambos os subprodutos na tensão superficial. Este BS apresentou excelentes propriedades emulsificantes (IE_{24} 98,9%), assim como ADO de 28,26 cm², com óleo de motor queimado. A caracterização preliminar mostrou a natureza polimérica da biomolécula, com teor de 55,7% de carboidratos, 28,2% de lipídios e 16,1% de proteínas, bem como seu caráter aniônico e diluição micelar crítica (DMC) a 50%. A estabilidade na faixa de pH 2-6, temperatura 20-80°C e salinidade 5-25% evidenciou um BS estável, com potencial promissor de aplicação em diversas atividades industriais ou processos ambientais em condições adversas.
Palavras-chave: Surfactante microbiano; Fungo Mucorales; Bioconversão de resíduos; Propriedades emulsificantes.

Resumen

La bioconversión verde de sustratos agroindustriales de bajo costo en productos de alto valor agregado se convierte en una estrategia biotecnológica para permitir la producción a escala industrial. En este contexto, en este estudio se utilizaron dos subproductos agroindustriales con el fin de formular un medio económico y sostenible para la producción de biosurfactante (BS) por *Mucor circinelloides* UCP 0018. Para ello, se utilizó un diseño factorial completo 2^2 para investigar la influencia de las concentraciones de licor de maceración de maíz y glicerol crudo sobre la tensión superficial como variable de respuesta. Los resultados mostraron la capacidad de este hongo Mucorales para producir BS en todas las condiciones del diseño, destacando la condición 4 (9% de ambos substratos) debido a la mayor reducción de la tensión superficial (de 72 a 29,7 mN/m). El análisis estadístico confirmó la influencia significativa de ambos subproductos en la tensión superficial. Este BS exhibió excelentes propiedades emulsionantes (IE_{24} 98,9%), así como una ADO de 28,26 cm², con aceite de motor quemado. La caracterización preliminar mostró la naturaleza polimérica de la biomolécula, con un contenido de 55,7% de carbohidratos, 28,2% de lípidos y 16,1% de proteínas, así como su carácter aniónico y dilución micelar crítica (DMC) al 50%. La estabilidad en el rango de pH 2-6, temperatura 20-80°C y salinidad 5-25% evidenció un BS estable, con potencial prometedor de aplicación en diversas actividades industriales o procesos ambientales en condiciones adversas.
Palabras clave: Surfactante microbiano; Hongo Mucorales; Bioconversión de residuos; Propiedades emulsionantes.

1. Introduction

Surfactants are a versatile group of chemicals with various applications such as household detergents, personal care products, pharmaceutical, chemical, agricultural agents, oilfield chemicals, food processing agents, industrial additives, environmental remediation agents, among others (Varjani & Upasani, 2017). They are amphiphilic compounds with hydrophilic and hydrophobic moieties that align according to various interfaces of air, water, oil and solid phases and affect the properties of these phases (Lamichhane, et al., 2017; Ofon, et al., 2022). However, chemically synthesized surfactants emerge as organic contaminants of great concern, due to their high toxicity, low biodegradability, causing severe environmental contamination to soil and groundwater, being increasingly recognized as causing serious environmental problems. In addition, as surfactants are derived from fossil fuels, production is not sustainable and production costs are subject to the price variation of fossil fuels (Otzen, 2017; Ofon, et al., 2022).

In this context, biosurfactants (BSs) emerge as an efficient and advantageous alternative over their synthetic counterparts, considering that they are produced microbially, from renewable sources by fermentation, presenting environmental compatibility, biodegradability, low toxicity, foaming properties and stability in a wide range of conditions (pH, salinity and temperature). However, they are still not competitive with synthetic ones, due to the high production costs and the low yield obtained through fermentation processes (Jiménez-Peñalver, et al., 2019; Jahan, et al., 2020; Gaur, et al., 2022).

Filamentous fungi have been proven to produce BSs, especially *Mucor circinelloides*, *Absidia cylindrospora*, *Rhizopus arrhizus* and several species of the genus *Cunninghamella* (Bione, et al., 2016; Andrade, et al., 2018; Mendonça, et al., 2021; Cândido, et al., 2022;). In many respects, filamentous fungi are superior to bacteria in the production of bioactives. Fungi do not produce toxic by-products; pathogenic strains are rarely (if any) among the fungal species used for biotechnological processes. In addition, filamentous fungi can produce BSs from low-cost substrates rich in nutritional value, such as crude glycerol (CG) and corn steep liquor (CSL) (Silva, et al., 2018; Pinto, et al., 2022). Thus, this study focused on the formulation of BS production medium by *M. circinelloides* UCP 0018 using CG and CSL, and the preliminary characterization of the BS.

2. Methodology

2.1 Microorganism

The Mucoralean fungus *M. circinelloides* UCP 0018, isolated from Caatinga 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). 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) and crude glycerol (CG), from biodiesel production, kindly provided by CETENE.

2.3 Biosurfactant production

BS production was carried out in 250 ml Erlenmeyer flasks, containing 100 ml of saline medium (L-asparagine 2 g, thiamine chlorhydrate 0,5 mg, potassium phosphate 0,5 g, magnesium sulphate 0,25 g per liter of distilled water) and the agro-industrial by-products at concentrations established by the 2^2 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 *M. circinelloides* UCP 0018 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 surface tension, as described in sections 2.5.

2.4 Full-factorial design (FFD)

In this study, a 2^2 FFD was carried out in order to investigate the effects of each independent variable (concentration of CSL and CG), 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).

Table 1: Variables and levels of the 2^2 full-factorial design applied for biosurfactant production by *M. circinelloides* UCP 0018.

Variables	**Levels**		
	-1	**0**	**+1**
Corn steep liquor (%, *v/v*)	3.0	6.0	9.0
Crude glycerol (%, *v/v*)	3.0	6.0	9.0

Source: Authors.

2.5 Determination of surface tension

Surface tension was determined on mycelia-free metabolic liquids, using an automatic tensiometer model Sigma 701 (Biolin Scientific, São Paulo, Brazil), and 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).

2.6 Determination of emulsification index

The emulsification index was analyzed after mixing the cell-free metabolic liquid and the hydrophobic compounds (soybean, corn, diesel and burnt motor oil), according to the methodology described by Cooper & Goldenberg (1987). The emulsification index (EI_{24}) was calculated after 24 h, by dividing the height of the emulsion formed by the total height of the mixture and multiplying by 100%.

2.7 Determination of dispersing properties

Following the method established by Morikawa, it was possible to identify the occurrence of BS production by its specificity to disperse hydrophobic compounds. Briefly, 40 ml of distilled water and subsequently, 0.5 ml of burnt motor oil, were added In Petri dishes of 10 cm in diameter. In the center of the Petri dish containing water and oil, 1 ml of the cell-free metabolic liquid containing the crude BS was added. The formation of halo (cm) was characterized by the appearance of a clear zone indicating the presence of surface-active compounds and their ability to disperse hydrophobic components (Morikawa, et al., 2000).

2.8 Isolation of BS

The BS produced by *M. circinelloides* 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, subjected to lyophilization and then, BS yield was expressed in g/L.

2.9 Preliminary characterization of BS

2.9.1 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 (Bione, et al., 2022).

2.9.2 Biochemical composition

The biochemical composition of the isolated BS was determined using specific commercial kits for quantification of total proteins and enzymatic glucose (In Vitro Diagnóstica Ltda; Itabira-MG, Brazil). The total lipid content was obtained after extraction with chloroform and methanol, according to Manocha, et al. (1980).

2.9.3 Analysis of the functional groups

The functional groups present in the BS molecule were identified by Fourier transform infrared (FTIR) spectroscopy in the Shimadzu equipment (model IR-TRACER 100), using attenuated total reflectance (ATR) accessory consisting of a mixed crystal “diamond/ZnSe”.

2.10 Determination of critical micelle dilution (CMD)

The critical micelle concentration (CMC) of BS was indirectly determined through the critical micelle dilution (CMD), using the mycelia-free metabolic liquid. For this, samples of cell-free metabolic liquid were diluted in distilled water in different proportions and surface tension values were determined for each corresponding dilution in an automatic tensiometer model Sigma 701 (Biolin Scientific, São Paulo, Brazil), using the Du Noüy ring method (Manivasagan, et al., 2014).

2.11 Stability of BS

The stability of the crude BS was evaluated by determination of surface tension in the mycelia-free metabolic liquid submitted individually to different pH values (2, 4, 6, 8, 10 and 12), NaCl concentrations (5, 10, 15, 20 and 25%) and temperatures (5, 10, 20, 40, 80 and 100°C) (Fonseca, et al., 2022).

3. Results and Discussion

3.1 Production of BS by *M. circinelloides* using agro-industrial by-products

In this study, renewable substrates CSL and CG were used as carbon and nitrogen sources, respectively, on the formulation of culture medium for the production of BS by *M. circinelloides*. According to the results showed in Table 2, this Mucoralean fungus demonstrated great potential for BS production, highlighting the reduction in surface tension exhibited in condition 4 (72.1 to 29.7 mN/m), in a medium containing 9% CSL and 9% CG. Similar result was recently reported for *M. circinelloides* UCP 0005, in medium composed by 1% instant noodle waste, 4% CSL and 1% post-frying soybean oil (Cândido, et al., 2022). Previously, Santiago et al. (2021) related this strain as BS producer, because of the reduction of surface tension to 34.0 mN/m in medium formulated with Jatobá (*Hymenaea stilbocarpa*) husks and CSL.

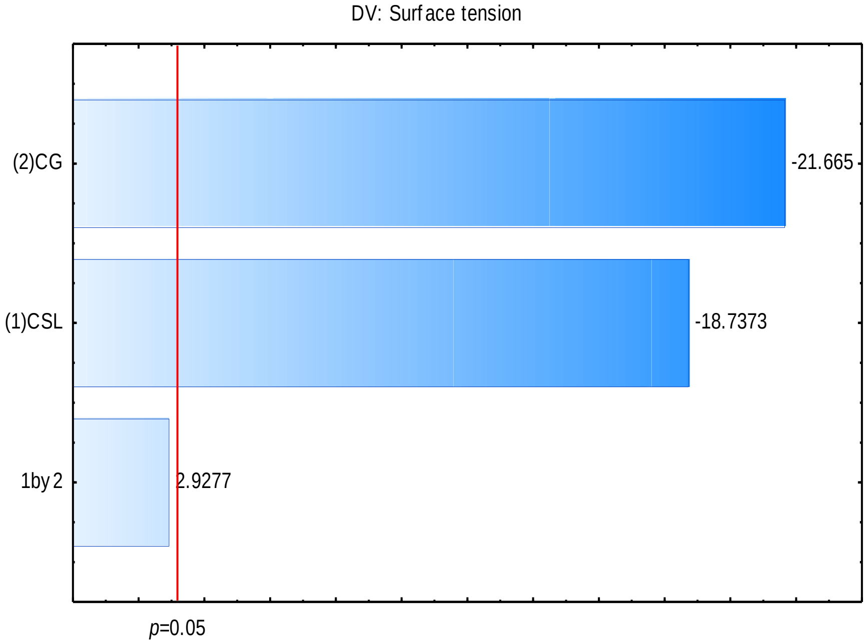
Table 2: Values of surface tension obtained according to the 2^2 FFD used for biosurfactant production by *Mucor circinelloides* UCP 0018.

Conditions	Corn steep liquor (%)	Crude glycerol (%)	Surface tension (mN/m)
1	-1	-1	36.6
2	+1	-1	32.9
3	-1	+1	32.4
4	+1	+1	29.7
5	0	0	33.9
6	0	0	34.1
7	0	0	33.7
8	0	0	34.0

Source: Authors.

Several researches reported the effectiveness of CSL and CG as inductors for microbial BS production (Pele, et al., 2019; Mendonça, et al., 2021; Bione, et al., 2022). In this context, the statistical analysis verified the significant effect of the concentrations of both agro-industrial by-products on surface tension. According to the Pareto diagram (Figure 1), both CSL and CG had a negative influence, from a statistical point of view, on the surface tension values obtained. This means that an increase in the concentration of them led to a decrease on surface tension, suggesting the production of BS in the culture medium.

Figure 1: Pareto diagram obtained from the statistical analysis of the 2^2 full-factorial design applied to the production of biosurfactant by *Mucor circinelloides* UCP 0018. The point at which the effect estimates were statistically significant *(p* = 0.05) is indicated by dashed line.



Source: Authors.

3.2 Emulsifying capacity of BS

The ability of a surface-active compound to form an emulsion is evaluated based on its potential to keep the emulsion stable with values above 50% of the emulsification index (EI) after 24 hours of its formation (Pele, et al., 2018; Nogueira, et al., 2020). Based on this principle, the mycelia-free metabolic liquids from the conditions of the FFD were used for investigating the emulsification capacity using soybean, corn, diesel and burnt motor oil, as hydrophobic compounds. Results exhibited on Table 3 demonstrated the excellent ability of *M. circinelloides* UCP 0018 to emulsify burnt motor oil, standing out the EI_{24} achieved in condition 4 of the FFD (98.9%). Good results were also verified for soybean oil > corn oil > diesel oil (Table 3), confirming that the ability to form stable emulsions depends not only on the intrinsic properties of the BS, but also on the type of hydrophobic component used in the test (Uzoigwe, et al., 2015; Rahman, et al., 2019).

Table 3: Values of emulsification index obtained according to the 2^2 FFD used for biosurfactant production by *Mucor circinelloides circinelloides* UCP 0018.

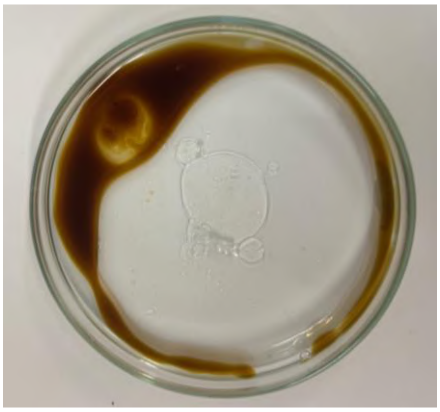
Conditions	Soybean oil (%)	Corn oil (%)	Diesel (%)	Burnt motor oil (%)
1	69.5	66.5	52.4	97.3
2	63.6	59.0	54.7	96.6
3	68.1	69.5	52.1	96.2
4	59.1	71.5	53.5	98.9
5	70.0	71.7	52.1	95.4
6	71.2	68.1	51.1	95.2
7	70.7	70.9	51.2	95.2
8	71.3	71.5	53.1	95.9

Source: Authors.

3.3 Dispersing properties of BS

Oil dispersion is one of the most promising characteristics of BSs, which enable their use in various industrial applications, mainly in oil spill bioremediation in aquatic ecosystems (Pele, et al., 2019; dos Santos, et al., 2021). In this sense, dispersing properties were investigated using mycelia-free metabolic liquid from condition 4 of the FFD. A halo of 6.0 cm was visualized (Figure 2), corresponding to ODA of 28.26 cm^2. This result was consistent and comparable to other reported BSs (Andrade, et al., 2018; Pele, et al., 2018). Dispersants have the property of breaking an oil slick into small droplets and diffusing them into water where their concentrations are reduced to below-toxic limits (Cai, et al., 2021).

Figure 2. Dispersion test of burnt motor oil using biosurfactant produced by *Mucor circinelloides* UCP 0018 in condition 4 of FFD.



Source: Authors.

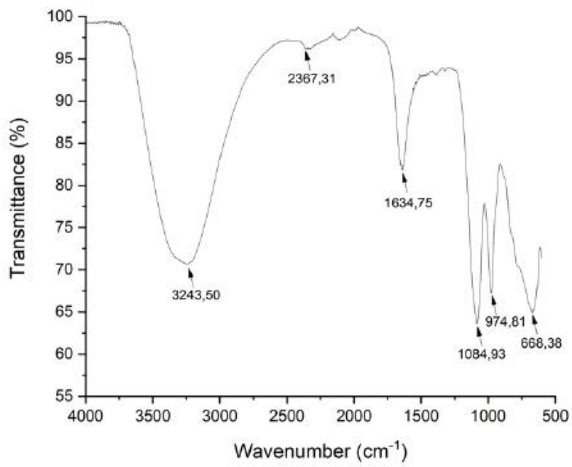
3.4 Yield and preliminary characterization of BS

The BS produced by *M. circinelloides* UCP 0018 was isolated by ethanol precipitation, reaching a yield of 2.174 g/L. Previously, lower yields were reported for BS produced by Mucoralean fungi *Rhizopus arrhizus* UCP 1607 (1.74 g/L) (Pele, et al., 2019) and *Syncephalastrum racemosum* UCP 1302 (0.9 g/L) (Bione, et al., 2022).

According to the analysis done by Potential Zeta Meta 3.0 +, the BS showed anionic character (-17.4 mV), similar to others BS produced by Mucoralean fungi (Andrade, et al., 2018; Pele, et al., 2019; Bione, et al., 2022). Anionic surfactants have a negative charge in their polar part, as well as strong foaming, detergent and wetting power. Therefore, they are used in the formulation of cosmetic products such as creamy soaps and cleansing lotions (Chua, et al., 2019; Yorke, et al., 2021).

In addition, the biochemical composition of the isolated BS indicated its polymeric nature (55.7% carbohydrates, 28.2% lipids and 16.1% proteins). The analysis of the infrared spectrum shown in Figure 3 revealed absorption peak at 3243 cm^{-1}, indicating the presence of N-H bond, while the peak at 1634 cm^{-1} was associated with the CO-N stretching (Javed, et al., 2022). Other significant peak observed at 1084 cm^{-1} suggest the presence of the CO-C stretching vibration belonging to the ester moieties (Gautam, et al., 2014). The last peaks at the region around 974 and 668 cm^{-1} represented -CH- bending of alkenes (Gautam, et al., 2014; Parthipan, et al., 2018). Despite this preliminary characterization suggested that the BS produced by *M. circinelloides* belonged to the polymers family, further characterization using more powerful techniques such as LC-MS, GC-MS, and/or NMR should be carried out.

Figure 3: FTIR spectrum of biosurfactant produced by *M. circinelloides* UCP 0018.



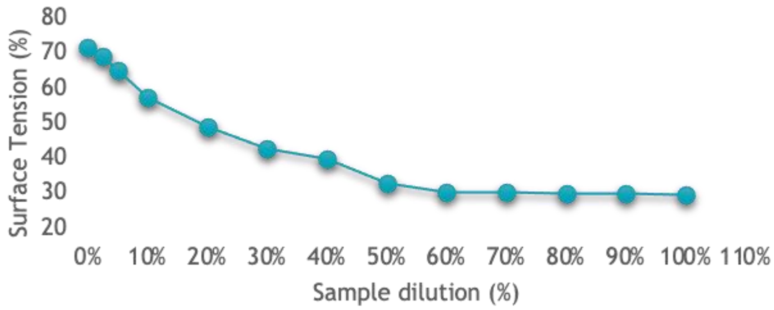
Source: Authors.

3.5 Determination of critical micellar dilution (CMD) of BS

Since the isolated BS did not show good solubility in water, it was only possible to determine the critical micellar concentration (CMC) of the biomolecule indirectly, by the critical micelar dilution (CMD) technique, using the crude BS (cell-free metabolic liquid) (Rocha & Silva, et al., 2014; Campos, et al., 2019). As shown in Figure 4, the surface tension was gradually reducing from 72 to 29.7 ± 0.2 mN/m, with increasing concentration of the metabolic liquid (0-100%). However, from at 50% the surface tension reached 31.7 mN/m, and thereafter, no significant reduction in surface tension was lead, indicating that the CMD was reached.

(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i11.33025

Figure 4: Critical micellar dilution of biosurfactant produced by *Mucor circinelloides* UCP 0018.

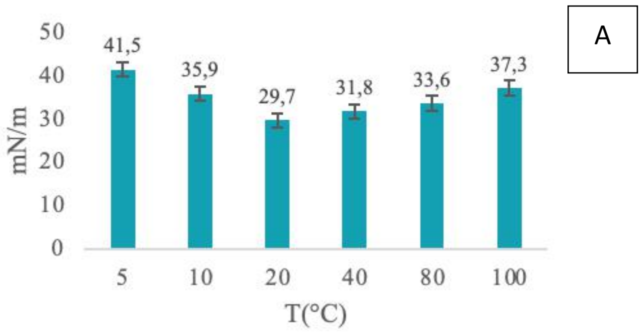


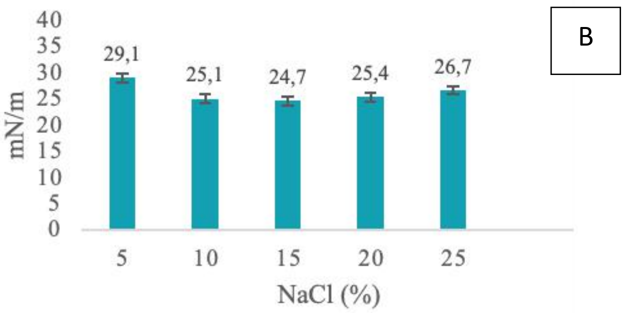
Source: Authors.

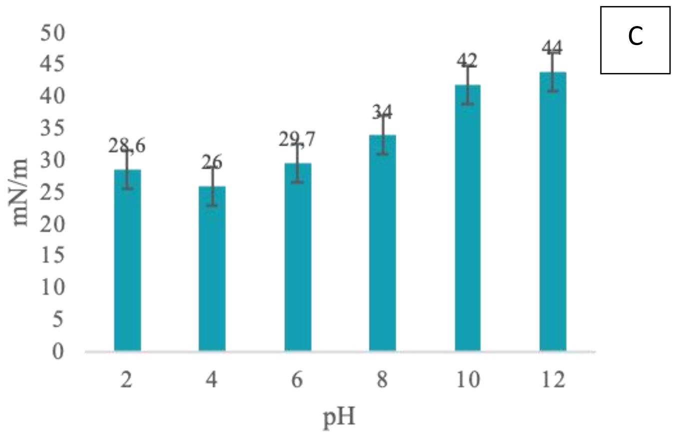
3.6 Stability of BS

The action of BSs can be influenced by the variation of physical and chemical factors, such as pH, temperature and salinity (Khopade, et al., 2012; Negin, et al., 2017). Therefore, it is essential to assess their stability in order to suggest the type of application (Purwasena, et al., 2019). In this sense, stability studies of the crude BS produced by *M. circinelloides* UCP 0018 were carried out, and the results are shown in Figure 5.

Figure 5: Stability of the biosurfactant produced by *Mucor circinelloides* UCP 0018 according to the surface tension in different values of pH (A), temperature (B) and salinity C).







Source: Authors.

Despite the BS showed a slight increase in surface tension when it was subjected to pH 2 and 4, compared to pH 6 (Figure 5A), it was considered that its surfactant activity remained stable, since the variation did not exceed 5 mN/m (Fai, et al., 2015). However, in alkaline conditions, it showed a more accentuated increase in surface tension, reaching 44 mN/m at pH 12, proving to be sensitive to alkaline environments, similar to BSs previously produced by *M. circinelloides* strains (Hasanizadeh, et al., 2018; Marques, et al., 2019).

On the other hand, BS showed thermostability at 20-80°C, although the surface tension increased at 5, 10 and 100°C (Figure 5B), showing susceptibility to extreme temperatures. Yet, the biomolecule proved to be halotolerant, evidencing efficiency in the reduction of surface tension up to 25% of NaCl (Figure 5C). According to the literature, BSs commonly tolerate saline concentrations of up to 10% (*w/v*), while synthetic surfactants are inactivated with ≥ 2% NaCl (Santos, et al., 2016; Pele, et al., 2019). Therefore, the BS produced here is a potential candidate for environmental, agricultural and industrial processes under high ionic strength.

4. Conclusion

This study demonstrated the biotechnological potential of *Mucor circinelloides* UCP 0018 for bioconversion of agro-industrial by-products into polymeric and anionic BS. The biomolecule showed a yield of 2.17 g/L and was efficient on reduction of surface tension to 29.7 mN/m, remaining stable at different concentrations of salinity, pH and temperature. The BS also exhibited excellent emulsifying and dispersing properties against burnt motor oil, suggesting potential application in environmental processes. Future studies should be carried out addressing further characterization of BS as well as assessment of its toxicity.

Acknowledgments

This work was financially supported by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) Process Nr. 563382/2010-4, G.M.C.T. Process Nr. 314422/2018-8, and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) Edital Pró-Equipamentos CAPES nº 11/2014. We also thanks to NPCIAMB (Núcleo de Pesquisas em Ciências Ambientais e Biotecnologia), at Catholic University of Pernambuco – Brazil

References

Andrade, R. F. S., Silva, T. A. L., Lima, R. A., Santos, E. R., França, E. S., Silva, K. J. C., & Campos-Takaki, G. M. (2018). Simultaneous production of surface active agent and lipids by *Rhodotorula glutinis* UCP/WFCC 1556. *Exploring Microorganisms: Recent Advances in Applied Microbiology*, 144.

Andrade, R. F., Silva, T. A., Ribeaux, D. R., Rodriguez, D. M., Souza, A. F., Lima, M. A., & Campos-Takaki, G. M. (2018). Promising biosurfactant produced by *Cunninghamella echinulata* UCP 1299 using renewable resources and its application in cotton fabric cleaning process. *Advances in Materials Science and Engineering*, 2018.

Bione, A. P., Lins, A. B., Rodríguez, D. M., de Souza, A. F., de Souza Mendonça, R., de Lima Filho, H. J., & Campos-Takaki, G. M. (2022). Valorization of agro-industrial by-products for sustainable production of biosurfactant by *Syncephalastrum racemosum* UCP 1302. *Research, Society and Development*, *11*(9), e58011932372-e58011932372.

Bione, A. M., Lins, A. B., Silva, T. C., & Montero-Rodríguez, D. (2016). Production of biosurfactant by *Cunninghamella phaeospora* in submerged fermentation using water soluble substrates. *Microbes in the Spotlight: Recent Progress in the Understanding of Beneficial and Harmful Microorganisms*, 361.

Cai, Q., Zhu, Z., Chen, B., Lee, K., Nedwed, T. J., Greer, C., & Zhang, B. (2021). A cross-comparison of biosurfactants as marine oil spill dispersants: Governing factors, synergetic effects and fates. *Journal of Hazardous Materials*, *416*, 126122.

Cândido, T. R., de Souza Mendonça, R., Lins, U. M. D. B. L., de Souza, A. F., Rodriguez, D. M., de Campos-Takaki, G. M., & da Silva Andrade, R. F. (2022). Production of biosurfactants by Mucoralean fungi isolated from Caatinga bioma soil using industrial waste as renewable substrates. *Research, Society and Development*, *11*(2), e13411225332-e13411225332.

Chotard, M., Mounier, J., Meye, R., Padel, C., Claude, B., Nehmé, R., & Lucchesi, M. E. (2022). Biosurfactant-producing *Mucor* strains: selection, screening, and chemical characterization. *Applied Microbiology*, *2*(1), 248-259.

Cooper, D. G., & Goldenberg, B. G. (1987). Surface-active agents from two *Bacillus* species. *Applied and environmental microbiology*, *53*(2), 224-229.

dos Santos, R. A., Rodríguez, D. M., Ferreira, I. N. D. S., de Almeida, S. M., Takaki, G. M. D. C., & de Lima, M. A. B. (2021). Novel production of biodispersant by *Serratia marcescens* UCP 1549 in solid-state fermentation and application for oil spill bioremediation. *Environmental Technology*, 1-12.

Fai, A. E. C., Simiqueli, A. P. R., de Andrade, C. J., Ghiselli, G., & Pastore, G. M. (2015). Optimized production of biosurfactant from *Pseudozyma tsukubaensis* using cassava wastewater and consecutive production of galactooligosaccharides: an integrated process. *Biocatalysis and Agricultural Biotechnology*, *4*(4), 535-542. https://doi.org/10.1016/j.bcab.2015.10.001

Fonseca, T. C. S., Rodríguez, D. M., Mendonça, R. S., Ferreira, I.N.S., Costa, L. O., & Campos-Takaki, G. M. (2022). Eco-friendly production of thermostable, halotolerant and pH wide range biosurfactant by *Issatchenkia orientalis* UCP 1603. *Research, Society and Development*, *11*(10).

Gaur, V. K., Sharma, P., Sirohi, R., Varjani, S., Taherzadeh, M. J., Chang, J. S., & Kim, S. H. (2022). Production of biosurfactants from agro-industrial waste and waste cooking oil in a circular bioeconomy: An overview. *Bioresource technology*, *343*, 126059.

Gautam, G., Mishra, V., Verma, P., Pandey, A. K., & Negi, S. (2014). A cost effective strategy for production of bio-surfactant from locally isolated *Penicillium chrysogenum* SNP5 and its applications. *Journal of Bioprocessing & Biotechniques*, *4*(6), 1.

Hasanizadeh, P., Moghimi, H., & Hamedi, J. (2018). Biosurfactant production by *Mucor circinelloides*: Environmental applications and surface‐active properties. *Engineering in Life Sciences*, *18*(5), 317-325.

Jahan, R., Bodratti, A. M., Tsianou, M., & Alexandridis, P. (2020). Biosurfactants, natural alternatives to synthetic surfactants: Physicochemical properties and applications. *Advances in colloid and interface science*, *275*, 102061.

Javed, S., Faisal, M., Raza, Z. A., Rehman, A., & Shahid, M. (2022). Isolation and characterization of indigenous biosurfactant producing *Bacillus* and *Staphylococcus* spp. during motor oil degradation. *Applied Ecology and Environmental Research*, *20*(1), 79-102.

Jiménez-Peñalver, P., Rodríguez, A., Daverey, A., Font, X., & Gea, T. (2019). Use of wastes for sophorolipids production as a transition to circular economy: state of the art and perspectives. *Reviews in Environmental Science and Bio/Technology*, *18*(3), 413-435.

Khopade, A., Biao, R., Liu, X., Mahadik, K., Zhang, L., & Kokare, C. (2012). Production and stability studies of the biosurfactant isolated from marine *Nocardiopsis* sp. B4. *Desalination*, *285*, 198-204.

Kuyukina, M. S., Ivshina, I. B., Philp, J. C., Christofi, N., Dunbar, S. A., & Ritchkova, M. I. (2001). *Recovery of Rhodococcus biosurfactants using methyl tertiary-butyl ether extraction. Journal of Microbiological Methods*, 46(2), 149-156.

Lamichhane, S., Krishna, K. B., & Sarukkalige, R. (2017). Surfactant-enhanced remediation of polycyclic aromatic hydrocarbons: a review. *Journal of Environmental Management*, *199*, 46-61.

Marques, N. S. A. A., de Lima, T. A., da Silva Andrade, R. F., Júnior, J. F. B., Okada, K., & Takaki, G. M. C. (2019). Lipopeptide biosurfactant produced by *Mucor circinelloides* UCP/WFCC 0001 applied in the removal of crude oil and engine oil from soil. *Acta Scientiarum. Technology*, *41*, e38986-e38986.

Manocha, M. S., San-Blas, G., & Centeno, S. (1980). Lipid composition of *Paracoccidioides brasiliensis*: possible correlation with virulence of different strains. *Microbiology*, *117*(1), 147-154.

Mendonça, R. S., Sá, A. V. P., Rosendo, L. A., Santos, R. A., Marques, N. S. A. A., Souza, A. F., Rodriguez, D. M., & Campos-Takaki, G. M. (2021). Production of biosurfactant and lipids by a novel strain of *Absidia cylindrospora* UCP 1301 isolated from Caatinga soil using low-cost agro-industrial byproducts. *Brazilian Journal of Development*, *7*(1), 8300-8313. 10.34117/bjdv7n1-564

Morikawa, M., Hirata, Y., & Imanaka, T. (2000). A study on the structure–function relationship of lipopeptide biosurfactants. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, *1488*(3), 211-218.

Negin, C., Ali, S., & Xie, Q. (2017). Most common surfactants employed in chemical enhanced oil recovery. *Petroleum*, *3*(2), 197-211.

Ofon, U. A., Shaibu, S. E., Ndubuisi-Nnaji, U. U., Inam, E. J., Okop, I. J., Enin, G. N., & Ibuotenang, N. D. (2022). Bio-and chemical surfactants for remediation of emerging organic contaminants. In *Emerging Contaminants in the Terrestrial-Aquatic-Atmosphere Continuum:* (pp. 367-380). Elsevier.

Otzen, T., & Manterola, C. (2017). Técnicas de Muestreo sobre una Población a Estudio. *International journal of morphology*, *35*(1), 227-232.

Parthipan, P., Elumalai, P., Ting, Y. P., Rahman, P. K., & Rajasekar, A. (2018). Characterization of hydrocarbon degrading bacteria isolated from Indian crude oil reservoir and their influence on biocorrosion of carbon steel API 5LX. *International Biodeterioration & Biodegradation*, *129*, 67-80.

Pele, M. A., Montero-Rodriguez, D., Rubio-Ribeaux, D., Souza, A. F., Luna, M. A., Santiago, M. F., & Campos-Takaki, G. M. (2018). Development and improved selected markers to biosurfactant and bioemulsifier production by Rhizopus strains isolated from Caatinga soil. *African Journal of Biotechnology*, *17*(6), 150-157.

Pele, M. A., Ribeaux, D. R., Vieira, E. R., Souza, A. F., Luna, M. A., Rodríguez, D. M., & Campos-Takaki, G. M. (2019). Conversion of renewable substrates for biosurfactant production by Rhizopus arrhizus UCP 1607 and enhancing the removal of diesel oil from marine soil. *Electronic Journal of Biotechnology*, *38*, 40-48.

Pinto, M. I. S., Campos Guerra, J. M., Meira, H. M., Sarubbo, L. A., & de Luna, J. M. (2022). A Biosurfactant from *Candida bombicola*: Its Synthesis, Characterization, and its Application as a Food Emulsions. *Foods*, *11*(4), 561.

Purwasena, I. A., Astuti, D. I., Syukron, M., Amaniyah, M., & Sugai, Y. (2019). Stability test of biosurfactant produced by *Bacillus licheniformis* DS1 using experimental design and its application for MEOR. *Journal of Petroleum Science and Engineering*, *183*, 106383.

Rahman, P. K., Mayat, A., Harvey, J. G. H., Randhawa, K. S., Relph, L. E., & Armstrong, M. C. (2019). Biosurfactants and bioemulsifiers from marine algae. In *The Role of Microalgae in Wastewater Treatment* (pp. 169-188). Springer, Singapore. 10.1007/978-981-13-1586-2_13

Santiago, M. G., Lins, U. M. D. B. L., de Campos Takaki, G. M., da Costa Filho, L. O., & da Silva Andrade, R. F. (2021). Produção de biossurfactante por *Mucor circinelloides* UCP 0005 usando novo meio de cultura formulado com cascas de jatobá (Hymenaea courbaril L.) e milhocina. *Brazilian Journal of Development*, *7*(5), 51292-51304.

Silva, A. C. S. D., Santos, P. N. D., Silva, T. A. L., Andrade, R. F. S., & Campos-Takaki, G. M. (2018). Biosurfactant production by fungi as a sustainable alternative. *Arquivos do Instituto Biológico*, *85*.

Techaoei, S., Leelapornpisid, P., Santiarwarn, D., & Lumyong, S. (2007). Preliminary screening of biosurfactant-producing microorganisms isolated from hot spring and garages in Northern Thailand. *Current Applied Science and Technology*, *7*(1-1), 38-43.

Uzoigwe, C., Burgess, J. G., Ennis, C. J., & Rahman, P. K. (2015). Bioemulsifiers are not biosurfactants and require different screening approaches. *Frontiers in microbiology*, 6, 245.

Varjani, S. J., & Upasani, V. N. (2017). Critical review on biosurfactant analysis, purification and characterization using rhamnolipid as a model biosurfactant. *Bioresource technology*, *232*, 389-397.