Strategy for sustainable biosurfactant production by mucor circinelloides UCP0017

Estratégia para produção sustentável de biossurfactante por *mucor circinelloides* UCP0017

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INTRODUCTION

Surfactants are molecules with amphipathic structures, that is, one polar extreme (hydrophilic) and the other nonpolar (hydrophobic), possessing a high capacity to reduce surface tension, emulsification production and wetting (PELE et al., 2019; BIONE, 2019). Its origin can be chemical usually derived from petroleum, being compounds that easily harm the environment making it difficult to remove it (BIONE, 2019; GAYATHIRI et al., 2022). As they can also be natural, through microorganisms such as bacteria, filamentous fungi and yeasts or are biodegradable and of low toxicity, not harming the environment or human health. (RULLI et al., 2019; CÂNDIDO et al., 2022). Therefore, the present study aims to seek the optimization of the production of biosurfactant becoming a more advantageous alternative because they use renewable substrates in their composition, and because their "green" properties do not harm the environment, besides being biodegradable, thus improving their cost-benefit, aiming at new opportunities for applications in the food, agricultural, cosmetic and pharmaceutical industries (MARQUES et al., 2020; GAYATHIRI et al., 2022; MULLIGAN, 2023).

The main responsible for the production of biosurfactants and bioemulsifiers are bacteria, followed by yeasts and finally filamentous fungi because they have a potential for the production of secondary metabolites and a high value of biomass, however, studies with filamentous fungi are little explored (SAŁEK & EUSTON, 2019; DERGUINE-MECHERI; KEBBOUCHE-GANA; DJENANE, 2021). According to Geethanjali et al., (2020) and Marques et al., (2020) the species *Mucor circinelloides, of the* phylum Mucoromycota, order Mucorales, presents a high biotechnological potential in the production of biomolecules of industrial interest, as well as biosurfactant. In this sense, the research proposed to study the performance of biosurfactant production through the filamentous fungus *Mucor circinelloides* UCP 0017 using alternative substrates (MARQUES et al., 2020; RADHA et al., 2020).

GOAL

Produce biosurfactant efficiently through a medium formulated by by-product (corn) and agro-industrial residues (post-frying soybean oil and English potato peel), as economically viable substrates. In addition to evaluating its dispersing activity and its stability in different environmental conditions.

METHODOLOGY

Microorganism: The fungus used in the project was *Mucor circinelloides* UCP 0017, isolated from sediments of the Rio Formoso mangrove swamp - PE and kept in the Cultures Collection bank of the Catholic University of Pernambuco, registered in the World Federation of Culture Collection (WFCC), which is located in the NPCIAMB. The maintenance of the microorganism during the experiment was by continuous repique in Sabouraud Agar medium at 5 °C.

Agro-industrial residues: In the formulation of the means of production were used the agro-industrial residues, Milhocina (from Cabo-PE), English potato peels and residual soybean oil, from the informal trade.

Experimental Design: A simple factorial design was designed to investigate the influence of the concentrations of the independent variables of milhocine and residual oil on the production of biosurfactant and milhocine with English potato peels for the production of bioemulsifiers, aiming to obtain the efficiency of the bioprocess and the productivity of the results, where the statistical analysis of the planning data was analyzed in the software STATISTICA version 10.0 (StatSoft Inc., USA). Table 1 presents the levels for the independent variables corn with residual oil and corn with English potato peels.

Table 1. Matrix containing the levels for the independent variables

Factors	-1	0	+1	Factors	-1	0	+1
Milhocina (%v/v)	2	6	4	Milhocina (%v/v)	0,2	0,5	0,8
Soybean oil (%v/v)	6	9	12	Potato peel (%v/v)	0,2	0,4	0,6

Biosurfactant production: The production of the biosurfactant was performed by submerged fermentation in 250 mL Erlenmeyers vials containing 100 mL of basal medium [KH2PO4 (2 g/L), MgSO4 (1 g/L) distilled water 1000 ml/L] plus agro-industrial residues at concentrations established in the factorial design, with inocula of 5% and 10% of a suspension of 10^7 spores/mL of *the isolate of Mucor circinelloides* UCP 0017. The vials were incubated at 28°C for 96 h under 150 rpm orbital agitation. The biomass was separated from the metabolic fluid by filtration (nylon silkscreen 120 mesh). From the cell-free metabolic fluid, surface and interfacial tension, emulsification index, and dispersion in hydrophobic substrate were analyzed.

Determination of pH: The Orion potentiometer (model 310) will be used to determine the pH of the aliquots collected from the cell-free production media.

Surface and interfacial tension: The surface tension was determined in the cell-free metabolic fluid by means of an automatic tensiometer (model Sigma 70 KSV Ltd., Finland) using the DU NUOY ring according to the methodology of Kuyukina (2001). The species that present the best values of surface tension will be selected for the experiments of production and characterization of the biosurfactant.

Emulsification index: The cell-free metabolic fluid will be used to estimate the emulsification index of the biosurfactant produced, using the method described by Cooper and Goldenberg (1987). To determine the emulsification index, 2 mL of metabolic fluid and 1 mL of hydrophobic compounds (soybean oil, motor oil, corn oil and burnt motor oil) were used. The material was homogenized in a vortex for 2 min. After 24 h, the readings were performed through the emulsion index equation: IE24 (%) = emulsion height/total height x 100

Parafilm M test: 10 µl of cell-free metabolic fluid was added to the hydrophobic surface of Parafilm M. The shape of the droplet on the surface was observed after 1 min. The diameter of the drops was evaluated. Distilled water was used as a negative control.

Dispersing Activity: The oil dispersion technique was performed according to the method of Morikava et al., (1993) with modifications. 1 ml of crude motor oil was added in 40 ml of distilled water in a petri dish. 500 µl of the metabolic fluid was added in the center of the petri dish and the clear zone of dispersion was observed. The oil displacement was calculated according to the equation: π x r2

Stability: The stability of the biosurfactant will be evaluated from the selected condition of the experimental design, according to the determination of the surface tension and emulsification index of the cell-free metabolic fluid at different pHs (3, 5, 7, 9 and 11), different salinity concentrations (5%, 10%, 15%, 20% and 25%) and different temperatures (4 ºC, 20 ºC, 37 ºC, 60 ºC, 90 ºC and 120 °C), for 20 min (MARQUES et al. 2020).

Critical Micellar Dilution (CMD): The CMD will be determined in the cell-free metabolic fluid according to Kuyukina et al. (2001), in an automatic tensiometer, using the NUOY ring.

FINDINGS

In this study, at first, two agro-industrial residues – residual soybean oil and cornocin – were used as carbon and nitrogen sources for biosurfactant production by *M. circinelloides* UCP 0017 using factorial design 22 with the inoculum of 5% of a suspension of 107 spores/mL.

Table 2. Results of surface tension, parafilm M test and metabolic fluid dispersion of M. *circinelloides* UCP 0017 grown in corncine and residual oil according to complete factorial design 2 ^{2}

Conditions	Milhocina	Soybean oil	Surface tension	Parafilm M (cm)	Dispersion (cm2)
1	2	6	36,7	0,8	39,59
2	2	12	35,7	0,7	39,59
3	6	6	35,0	0,7	24,63
4	6	12	35,5	0,9	34,21
5	4	9	35,9	0,8	31,31
6	4	9	35,2	0,8	31,17
7	4	9	35,7	0,8	30,19
8	4	9	35,5	0,8	30,27
Water			72,0	0,4	

The results demonstrate the best results of surface tension between 35.0 and 35.9 mN/m in the conditions of the central points and in condition 2. According to (SHAH et al., 2019) a biosurfactant-producing microorganism reduces surface tension to 40mN/m or lower values. Therefore, this study indicates that *M. circinelloides* UCP 0017 is a good producer of biosurfactant, being similar to the results obtained in other studies with Mucorales fungi grown in agro-industrial residues (Table 3).

Table 3. Results of surface tension in Mucorales fungi grown in different residues agro-industrial as substrates.

Mucorales	Substrates	Surface Tension (mN/m)	References
M. circinelloides UCP 0017	Milhocine and residual oil	35,0	Present study
M. circinelloides UCP 0005	Jatobá bark and milhocina	34,0	Santiago *et al.* 2021
Rhizopus arrizhus UCP 1609	Instant noodle residue, milhocine and post-frying oil	31,0	Cândido *et al.* 2022
M. circinelloides UCP 0006	Instant noodle residue, milhocine and post-frying oil	27,0	Cândido *et al.* 2022
Absidia cylindrospora UCP 1301	Crude glycerol, milhocin and whey	30,2	Mendonça *et al.* 2020
M. hiemalis	Sodium Glutamate and Post-Frying Soybean Oil	32,0	Ferreira INS *et al.* 2020

Source: Prepared by the authors.

The results obtained in the M parafilm test confirm that all conditions reached a level higher than the diameter of the negative control droplet which was 0.4 cm, however, in condition 4 it reached the highest value with 0.9 cm. For the dispersion test, the displacement diameter of the engine oil reached by the biosurfactant produced under conditions 1 and 2 of the factorial design by Mucorales *M. circinelloides* UCP 0017, was 39.59cm2, being similar to other strains of Mucorales as *M. circinelloides* UCP 0005 (37-38 cm2) (SANTIAGO et al. 2021), *Cunninghamella echinulata* UCP 1299 (32.15 cm2) (ANDRADE et al., 2018). Indicating that the *M. circinelloides* UCP 0017 in addition to being a good biosurfactant, it is a good biodispersant.

The results of the emulsification indices in the hydrophobic substrates (soybean oil, motor oil, corn oil and burnt motor oil) were proven only with the engine oil in condition 2 corresponding to IE 24 of 51.66%. According to Kebbouche-Gana et al., (2009) emulsification index values higher than 50% indicate emulsifying activity, not having been observed in other statistically represented results.

The results obtained through the agro-industrial residues, milhocina and English potato peel, as carbon and nitrogen sources, respectively, for biosurfactant production by *M. circinelloides* UCP 0017 using factorial design 22, with the inoculum of 10% of a suspension of 107 spores/mL, did not obtain results below 40 mN/m according to Shah et al., (2019) to be considered good biosurfactants. However, the studies were favorable for the production of a good bioemulsifier according to Kebbouche-Gana et al., (2009), where it was observed in condition 2 an emulsification index value higher than 50% indicating emulsifying activity with the motor oil acquiring 58.61% of emulsification, similar to other strains of Mucorales such as: *Rhizopus arrizhus* UCP 1609 (60.0%) (CÂNDIDO et al. 2022), *M. circinelloides UCP 0006 (55.2%) (Cândido et al. 2022), M. circinelloides* UCP 0005 (70.0%) (CÂNDIDO et al. 2022).

The stability of the biosurfactant was evaluated with condition 3 (6% corn and 6% soybean oil), because it is the best condition of the factorial design of the results obtained from the surface tension of the variables milhocina and soybean oil. Tests were performed with different levels of pHs (3, 5, 7, 9 and 11), salinity (5%, 10%, 15%, 20% and 25%) and temperature (4ºC, 20ºC, 37ºC, 60ºC, 90ºC and 120°C) to analyze the stability of the biosurfactant that will be observed in the following table.

Table 4. Stability results with different levels of pHs, salinity and temperature

pH	Surface tension	Salinity	Surface tension	Temperature	Surface tension
3	37,4	**5%**	31,5	**4°C**	30,1
5	35,9	**10%**	32,3	**20°C**	29,4
7	32,6	**15%**	32,5	**37°C**	29,2
9	33,7	**20%**	33,2	**60°C**	29,3
11	42,2	**25%**	32,7	**90°C**	30,5
				120°C	31,0

The results regarding the pH levels showed that the biosurfactant remains in approximate points of each other, demonstrating that for better results it should be used at neutral pH, but it can withstand even at more acidic levels such as 3 and alkaline levels such as 9, with so much that it does not reach alkalinity 11. With the salinity results, the metabolic fluid remained stable in the different amounts of salinity, being able to tolerate up to 25% of salinity and the tests with different temperatures remained stable, being able to maintain levels from 4°C to 120°C without changing its tension.

The critical micellar dilution (CMD) of the cell-free metabolic fluid obtained good results from its pure concentration of 100% to a dilution of 1% where they will be demonstrated in the following table.

Table 5. Results of metabolic fluid CMD

DMC	Surface tension	DMC	Surface tension
100%	31,0	9%	35,4
90%	30,5	8%	36,5
80%	31,0	7%	37,8
70%	30,8	6%	37,2
60%	30,8	5%	38,4
50%	31,0	4%	38,4
40%	30,0	3%	41,0
30%	30,0	2%	48,0
20%	30,3	1%	45,0
10%	30,5		

The results obtained demonstrate that the metabolic fluid remained constant in its tension at a dilution of up to 10%, proving to be a good biosurfactant, which can bring greater profitability in its production. We also saw that it can be used even at a dilution of 4% by maintaining a voltage below 40 mN/m according to (SHAH et al., 2019) to be a good producer of biosurfactant.

FINAL CONSIDERATIONS

In this study we evidenced the efficiency in the production of biosurfactants and bioemulsifiers through *M. circinelloides* UCP 0017 by renewable substrates, of great industrial interest, for its ability to remain stable to variables of base, neutral and alkaline pHs, as well as its stability in the midst of high concentrations of salinity serving for bioremediation through the salinity of seawater. The same also serves for several applicability by the fact that it remains regular at different temperatures, facilitating its production both for its high level of stability, as well as for having an excellent constancy in its dilution, bringing a good cost-benefit in its production.

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