Research, Society and Development, v. 11, n. 11, e87111132995, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i11.32995

Production and functional stability of the biourfactant isolated from *Stenotrophomonas maltophilia* UCP 1601

Produção e estabilidade funcional de biosurfactante isolado de *Stenotrophomonas maltophilia* UCP 1601

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Received: 07/15/2022 | Reviewed: 07/29/2022 | Accept: 08/07/2022 | Published: 08/16/2022

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Abstract
The present study investigated the production of biosurfactant by *Stenotrophomonas maltophilia* UCP 1601under submerse fermentation using renewable substrates as carbon and nitrogen sources applying two statistical tools as Plakket-Burman experimental design and full factorial design to optimize the bioprocess. A partial characterization was performed by measuring the surface tension, emulsification index, determination of CMC, ionic charge. The biosurfactant was tested to different conditions of temperature, pH and salinity, in order to evaluate the stability of the biomolecule. The surface tension of the biosurfactant produced using corn steep liquor and glycerol was 27 mN/m, whose yield was 12.6 g/L, and its character is anionic. The critical micelle concentration (CMC) was 70% using the cell-free metabolic liquid. The biosurfactant was able to emulsify 56% of the burned engine oil. The biomolecule showed high functional stability and performance under extreme conditions of thermal, pH and saline concentrations stability. All determinations carried out confirm the generation of a stable, efficient bioproduct that contributes to the circular bioeconomy and the great potential for remediation under harsh environmental conditions.
Keywords: Tensoactive; Bacteria; Agro-industrial wastes; Higher stability; Circular bioeconomy.

Resumo
O presente estudo investigou a produção de biossurfactante por *Stenot*como *rophomonas m*altophilia UCP 1601 sob fermentação submersa utilizando substratos renováveis fontes de carbono e nitrogênio aplicando duas ferramentas estatísticas como planejamento experimental Plakket-Burman e planejamento fatorial completo para otimizar o bioprocesso. Uma caracterização parcial foi realizada medindo-se a tensão superficial, índice de emulsificação, determinação de CMC, carga iônica. O biossurfactante foi testado para diferentes condições de temperatura, pH e salinidade, a fim de avaliar a estabilidade da biomolécula. A tensão superficial do biossurfactante produzido com milhocina e glicerol foi de 27 mN/m, cujo rendimento foi de 12,6 g/L, e seu caráter é aniônico. A concentração micelar crítica (CMC) foi de 70% usando o líquido metabólico livre de células. O biossurfactante foi capaz de emulsionar 56% do óleo de motor queimado. A biomolécula apresentou alta estabilidade funcional e desempenho sob condições extremas de estabilidade térmica, pH e concentração salina. Todas as determinações realizadas confirmam a

geração de um bioproduto estável e eficiente que contribui para a bioeconomia circular e o grande potencial de remediação em condições ambientais adversas.
Palavras-chave: Tensoativo; Bactérias; Resíduos agroindustriais; Elevada estabilidade; Bioeconomia circular.

Resumen
El presente estudio investigó la producción de biosurfactantes por *Stenotrophomonas maltophilia* UCP 1601 bajo fermentación sumergida utilizando sustratos renovables como fuentes de carbono y nitrógeno aplicando dos herramientas estadísticas como el diseño experimental de Plakket-Burman y el diseño factorial completo para optimizar el bioproceso. Se realizó una caracterización parcial midiendo tensión superficial, índice de emulsificación, determinación de CMC, carga iónica. El biosurfactante fue probado para diferentes condiciones de temperatura, pH y salinidad, con el fin de evaluar la estabilidad de la biomolécula. La tensión superficial del biosurfactante elaborado con licor de maceración de maíz y glicerol fue de 27 mN/m, cuyo rendimiento fue de 12,6 g/L, y su carácter es aniónico. La concentración micelar crítica (CMC) fue del 70 % utilizando el fluido metabólico libre de células. El biosurfactante pudo emulsionar el 56% del aceite de motor quemado. La biomolécula mostró alta estabilidad funcional y desempeño bajo condiciones extremas de estabilidad térmica, pH y concentración de sal. Todas las determinaciones realizadas confirman la generación de un bioproducto estable y eficiente que contribuye a la bioeconomía circular y al gran potencial de remediación en condiciones ambientales adversas.
Palabras clave: Tensoactivos; Bacterias; Residuos agroindustriales; Mayor estabilidad; Bioeconomía circular.

1. Introduction

Biosurfactants are one of the most recent biomolecules produced/synthesized by exploited microbes and are frequently used in various agricultural, environmental, food, and other industries. (Singh et al., 2021; Kumar et al., 2021; Cruz-Hernández et al., 2022). The term biosurfactant has been referred to as surfactants that can improve surface-surface interactions through the formation of micelles produced by the natural source of origin, such as plants, microbes, and animals. (Varjani; Upasani, 2017; Ferreira et al, 2020). In other aspects, the addition of surfactants in an oil/water or water/air system causes a reduction in surface tension to a point where the surfactants form structures such as micelles, vesicles, and bilayers; This critical point is generally known as the critical micelle concentration. (CMC) (Vieira et al., 2021).

Among the main advantages of biosurfactants, stability under different environmental conditions can be highlighted. This characteristic keeps its surface-active properties stable in the face of wide variations in pH, temperature, and salinity. This ability allows this biomolecule to be applied in various industrial and environmental fields with the certainty that its efficiencies will be maintained. (Kumar et al., 2021).

Different from chemically synthesized surfactants, biosurfactants have low toxicity, biodegradability, and ecological acceptability, since they are produced by microorganisms. (Gayathiri et al., 2022; Santos et al., 2019). Scholars have made efforts to use renewable, natural, and low-cost substrates in the production of biosurfactants, such as post-frying soybean oil, sugarcane residues, fruit and vegetable peels, among others. This process favors the circular bioeconomy, which consists of the use of waste that would be discarded in the environment, generating environmental contamination and also reducing the cost of production of these biomolecules. The use of waste as a raw material for the production of value-added products has opened new paths contributing to environmental sustainability (Gaur et al., 2022).

Bacteria are microorganisms of wide biotechnological interest and are widely described in the literature as producing biosurfactants. (Carrilho et al., 1996; Jaysree et al., 2011; Putra; Hakiki, 2019; Lamilla et al., 2021; Singh; Sachan, 2022; Parthipan et al., 202). *Stenotrophomonas maltophilia* is a gram-negative bacterium of high biotechnological interest, already described in the literature in the production of biomolecules (Nogueira et al., 2020; Semai et al.., 2022).

In this sense, investigations were carried out to produce a biosurfactant from *Stenotrophomonas maltophilia* UCP 1601, using renewable substrates in its composition as sources of carbon and nitrogen, to evaluate its stability under different environmental conditions and to offer a biomolecule of relevant biotechnological interest and that contributes to the circular bioeconomy. In addition, the high cost is associated with organic carbon sources is a major bottleneck for the

commercialization of fatty acid bioprocesses. The use of non-edible materials and industrial waste as glycerol sources from biodiesel production could reduce overall production costs, thereby aiding the transition to large-scale lipid and chitosan production. To the best of our knowledge, this is the first report to lipid accumulation biomass produced by culture media using crude glycerol from biodiesel manufacturing and corn steep liquor as agroindustrial substrates for the production of nutraceutical fatty acids from *Stenotrophomonas maltophilia* UCP 1601.

2. Methodology

2.1 Microorganism and pre-inoculum

The bacterium *Stenotrophomonas maltophilia* UCP 1601, was isolated from clayey soil on the banks of the Capibaribe River, Pernambuco, Brazil. The bacterium is deposited in the Culture Bank of the Catholic University of Pernambuco (UCP), registered with the World Federation for Culture Collection (WFCC). The bacteria were maintained in nutrient agar and in 30% glycerol, at a temperature of 4ºC, with repetitions performed every 4 months to maintain the culture.

2.2 Agro-industrial substrates

The substrates used were corn steep liquor kindly provided by Corn Products Ltd. and residual glycerol from the biodiesel manufacturing using cotton oil, kindly supplied by the Experimental Plant of Biodiesel Production of Northeastern Center of Strategic Technologies (CETENE – Centro de Tecnologias Estratégicas do Nordeste, Recife-PE, Brazil). Aliquots of corn steep liquor and crude glycerol were subjected to elementary analysis to determine the amounts of carbon, hydrogen, and nitrogen (%) using the model EA 1110 analyzer (Carlo Erba Instruments).

2.3 Inoculum preparation

Stenotrophomonas maltophilia UCP 1601 was grown on nutrient agar and transferred to 50 ml of nutrient broth, incubated at 37°C, 150 rpm under orbital shaking, for 24 h. Then, the growth was determined by optical density (O.D.600) in a UV/Vis Libra S32 spectrophotometer (Biochrom Ltd.), corresponding to an O.D. of 0.8, being used as inoculum.

2.4 Biosurfactant production

The production of surfactants was carried out in Erlenmeyers flasks containing 100 ml of saline mineral medium (MMS) with the following composition in: $(NH^4)2SO^4$ (1,0 g^{-1}), K_2HPO^4 (2,0 g^{-1}), KH2PO4 (0,5 g^{-1}), $MgSO^4.7H2O$ (10,0 g/l), NaCl (5,0 g/l), FeSO4 (0,2 g/l) e CaCl2 (0,5 g/l) (Hemlata et al., 2015). 5% of the carbon and/or nitrogen source (selected in the next section) was added to the saline mineral medium. The media were adjusted to pH 7 and sterilized in an autoclave for 15 min at 121ºC.

2.5 Screening of substrates for biosurfactant production

Five different sources of carbon and nitrogen were tested, namely, sugarcane molasses, cassava wastewater, corn steep liquor, crude glycerol, and glucose, in the proportion of 5% (v/v). Each substrate was added to the properly sterilized saline mineral medium, the pH was adjusted to 7 and the Erlenmeyer containing the productive medium was incubated (150 rpm under orbital agitation, for 96 h, 37°C).

2.6 Plackett Burman's experimental design

In order to select the most relevant variables for the production of *Stenotrophomonas maltophilia* UCP 1601 biosurfactant, the PBD was applied using the following independent variables: agitation, temperature, time, pH, glucose concentration, peptone concentration, glutamic acid concentration, glycerol, corn steep liquor, NaCl and post-frying soybean

oil. The response variable (dependent variable) was surface tension. Each independent variable will have two levels, maximum (+) and minimum (-), according to Table 1.

Table 1: Matrix of Plackett Burman's experimental design to produce biosurfactant by *Stenotrophomonas maltophilia* UCP 1601.

Assay	Glucose (g)	Peptone (g)	Corn Steep liquor (g)	NaCl (g)	Glutamic acid (g)	Glycerol (ml)	Post frying soybean oil (ml)	Temperature (°C)	pH	Time (h)	Rotation (rpm)
1	2	1	5	2.5	0	2.5	5	30	7	72	150
2	2	2	2.5	5	0	2.5	5	30	7	96	100
3	0	2	5	2.5	1	2.5	2.5	27	7	96	150
4	2	1	5	5	0	5	2.5	27	5	96	150
5	2	2	2.5	5	1	2.5	5	27	5	72	150
6	2	2	5	2.5	1	5	2.5	30	5	72	100
7	0	2	5	5	0	5	5	27	7	72	100
8	0	1	5	5	1	2.5	5	30	5	96	100
9	0	1	2.5	5	1	5	2.5	30	7	72	150
10	2	1	2.5	2.5	1	5	5	27	7	96	100
11	0	2	2.5	2.5	0	5	5	27	5	96	150
12	0	1	2.5	2.5	0	2.5	2.5	30	5	72	100

Source: Authors.

2.7 Optimization of biosurfactant production

After performing the PBD and selecting the most relevant variables to produce *Stenotrophomonas maltophilia* UCP 1601 biosurfactant, the process optimization was performed using a 24-factor statistical design using the most representative variables in the PBD.

2.8 Biosurfactant extraction

The biosurfactant produced by *S. maltophilia* UCP 1601 was extracted through precipitation of metabolic liquid / 70% ethanol in different proportions and treatments, as shown in Table 2. The mixture was left overnight at 4 °C, the precipitate was centrifuged at 5000g for 15 min, at 5°C (Andrade Silva et al., 2014).

Table 2: Treatments for the extraction of the biosurfactant produced by *Stenotrophomonas maltophilia* UCP 1601.

Treatment of metabolic fluid	Metabolic liquid/ethanol ratio 70%(v/v)
-	2:1
pH 2	2:1
pH 12	1:1
pH 12	2:1

Source: Authors.

2.9 Biosurfactant characterization: Determination of surface tension and CMC

Surface tension was determined in the cell-free metabolic fluid using an automatic tensiometer (model Sigma 70 KSV Ltd, Finland) by the Du Nouy ring method, as described by Kuyukina et al, (2001) and measurements were performed in triplicate. The critical micelle concentration (CMC) of the crude and purified biosurfactant was determined by plotting the surface tension as a function of the biosurfactant concentration. The biosurfactant was solubilized in Milli-Q water at a concentration ranging from 0 to 500 mg/L (crude biosurfactant) and 0-100 mg/L for purified biosurfactant (Hentati et al., 2019). For each concentration, the surface tension measurement was determined until reaching a constant value.

2.10 Determination of the emulsification index (IE24)

The emulsification index was determined in test tubes added with oil and cell-free metabolic liquid in a 1:1 ratio. Unused and burnt engine oil was used. The tubes were vortexed vigorously for 2 minutes at room temperature, the emulsification index was determined after 24 hours, according to equation (1), where: IE - emulsification index in percentage, Ae is the height of the formed emulsion and At - total height of the liquid column (Liu et al., 2013).

Equation 1: Emulsification index

$$IE = Ae/At \times 100$$

2.11 Determination of ionic charge

The ionic charge of the biomolecule was determined using a Zeta potential model ZM3-D-G, Zeta Meter System 3.0+, with direct-to-video images from the Zeta Meter, San Francisco, CA, USA (Andrade et al., 2015).

2. 12 Study of the thermal, ionic and saline stability of the produced biosurfactant

The cell-free metabolic fluid was subjected to different temperatures (5, 10,20,40,80 and 100 °C), pH's (2, 4, 6, 8, 10 and 12) and NacL concentrations (0, 5, 10, 15, 20%). After 30 min the surface tension was measured in triplicate.

3. Results and Discussion

3.1 Screening of the selection of agro-industrial substrates in the production of biosurfactant

Five nutritional sources were selected, according to Table 3, to determine which would be the most relevant substrate to produce biosurfactant by *Stenotrophomonas maltophilia* UCP 1601. Considering that the use of renewable substrates to produce biosurfactants contributes to circular bioeconomy. The surface tension was the parameter to determine if there was the production of biosurfactants.

The results indicate that corn steep liquor and crude glycerol favored the reduction of the surface tension of the metabolic liquid from 72 mN/m to 47 and 45 mN/m, respectively. In this sense, these substrates were selected for the continuation of the experiments, where new parameters were evaluated to enhance the new results.

Table 3: Selection of nutritional sources for biosurfactant production.

Substrate	Superficial tension (mN/m)
Corn steep liquor	47
Cassava wastewater	52
Crude Glycerol	45
Glucose	55
Sugar Cane Molasses	58

Source: Authors.

3.2 Experimental design by Plackett Burman

This statistical tool allows you to evaluate 2 to 31 factors where each factor is varied at two levels. The PBD design is useful for testing robustness where little or no effect on response due to factors is expected. Thus, the first necessary step to optimize the production of microbial metabolites can be done by tracking the factors that affect the response of interest. (Bertrand et al., 2018). Ten factors of high importance to produce biosurfactants were evaluated, with surface tension as the response variable.

In test 8, it was possible to observe that the surface tension of 33.8 mN/m (Table 4). According to the literature, surface tension values below 35mN/m indicate that the microorganism is an efficient biosurfactant producer. (Lima et al., 2017; HentatI et al., 2019; Ferreira et al., 2020). In this assay, the increase in cornflour favored the reduction of surface tension, in agreement with the results obtained in the initial screening.

Câmara et al. (2019), carried out a study of the production of biosurfactant by a bacterium using the experimental design of Plackett Burman. The surface tension of the biomolecule obtained was 35.26 mN/m. Mouafo et al. (2018), evaluated the production of biosurfactant by *Lactobacillus paracasei* using the same statistical tool and obtained a surface tension of 37.85 mN/m**.**

Table 4: Results of Plackett Burman's experimental design used for biosurfactant production.

Assay	Glucose (g)	Peptone (g)	Corn steep liquor (mL)	NaCl (g)	Glutamic Acid (g)	Glycerol (mL)	Post frying soybean oil (mL)	pH	Time (h)	Rotation (rpm)	ST
1	2	1	5	2.5	0	2.5	5	7	72	150	38
2	2	2	2.5	5	0	2.5	5	7	96	100	39.3
3	0	2	5	2.5	1	2.5	2.5	7	96	150	38.7
4	2	1	5	5	0	5	2,5	5	96	150	42,8
5	2	2	2.5	5	1	2.5	5	5	72	150	38.5
6	2	2	5	2.5	1	5	2.5	5	72	100	42.1
7	0	2	5	5	0	5	5	7	72	100	38.3
8	**0**	**1**	**5**	**5**	**1**	**2.5**	**5**	**5**	**96**	**100**	**33.8**
9	0	1	2.5	5	1	5	2.5	7	72	150	37.3
10	2	1	2.5	2.5	1	5	5	7	96	100	42.5
11	0	2	2.5	2.5	0	5	5	5	96	150	36.2
12	0	1	2.5	2.5	0	2.5	2.5	5	72	100	45.2

Source: Authors.

3.3 Optimization of biosurfactant production through Full Factorial Design 2^4

This step consists of consolidating all the results obtained so far and providing all possible conditions for the highest performance in the production of the biosurfactant. We evaluated the effect of 4 substrates in the reduction of the surface tension of the produced biosurfactant, being them renewable substrates (post-frying soybean oil, corn, and glycerol (crude) and a synthetic substrate (Glutamic acid), which is an amino acid. These independent variables were evaluated, as well as their interactions, and the response variable were the surface tension. Table 4 presents the results obtained. In test 13, the surface

tension of the biosurfactant was 27.6 mN/m. This result is excellent since the increase in the variables maize, glycerol and OSPF favored the reduction of surface tension, these being the renewable substrates used in the experiment, which implies a reduction in the use of the synthetic substrates. Recent work by Aboelkhair et al. (2022) presents a biosurfactant produced to be applied in the recovery of environments contaminated with oil, whose surface tension was 25.7 mN/m. Another study carried out by Uyar and Saglam (2021) presents the production of biosurfactant produced by bacteria isolated from soil, where the surface tension obtained was 30.5 mN/m.

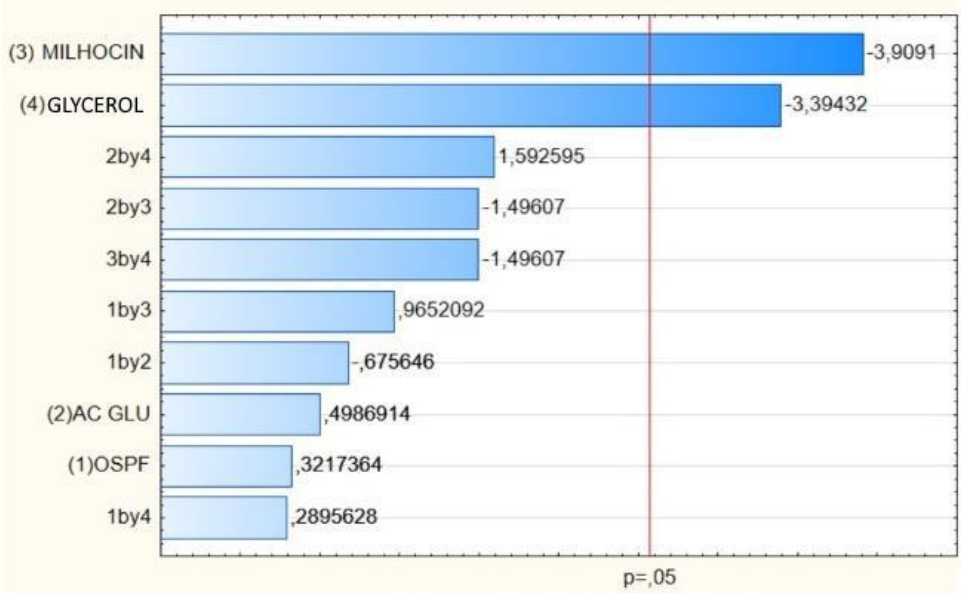
Table 5: Surface tension results of 2^4 factorial design for biosurfactant production by *Stenotrophomonas maltophilia* UCP1601.

Assay	OSPF (mL)	Glutamic acid (g)	Corn steep liquor (mL)	Glycerol (mL)	Superficial Tension (mN/m)
1	7.5	0.5	2.5	2.5	38.9
2	10	0.5	2.5	2.5	40.7
3	7.5	1.5	2,5	2.5	44.6
4	10	1.5	2.5	2.5	38.3
5	7.5	0.5	7.5	2.5	38.8
6	10	0.5	7.5	2,.5	40.0
7	10	1.5	7.5	2.5	36.1
8	7.5	1.5	7.5	2.5	32.6
9	10	0.5	2.5	7.5	37.7
10	7.5	0.5	2.5	7.5	33.1
11	10	1.5	2.5	7.5	37.9
12	7.5	1.5	2.5	7.5	42.0
13	**10**	**0.5**	**7.5**	**7.5**	**27.6**
14	7.5	0.5	7.5	7.5	29
15	10	1.5	7.5	7.5	32.6
16	7.5	1.5	7.5	7,5	27,9

Source: Authors.

The factorial design performed generated a Pareto diagram for the standardized effects, with surface tension as the response variable. This image (Figure 1) represents the most representative variables of the process, as well as the interactions between them. The line that cuts these vertical bars represents the “p” value, which indicates the variables that showed a statistical significance of 95% confidence.

Figure 1: Pareto diagram for standardized effects, with surface tension as the response variable.

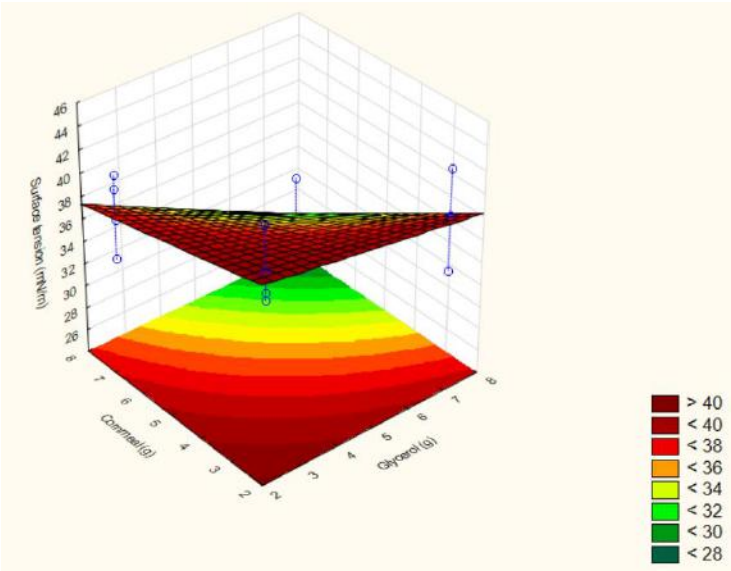


Source: Authors.

The variables corn steep liquor and glycerol were the most representative of the biosurfactant production process. The negative value before the number in front of the horizontal bars indicates that the increase of this variable contributes to the decrease of the response variable, which confirms the surface tension results obtained since the reduction of the response variable is the objective of our study.

Response surface plots are another resource that aid in the statistical interpretation of experimental data. The area of the graph, in 3 dimensions, reflects the interaction of two variables in relation to the dependent variable (surface tension). The lighter areas (closer to the green color) represent smaller values of the dependent variable, while the darker areas (closer to the red color) represent higher values of the dependent variable. In Figure 2 we can see how the interaction of corn steep liquor and glycerol interferes with surface tension. As the objective of our study is to reduce the surface tension was evaluated the lighter areas of the graph, which represent lower values of surface tension. In this image, it is possible to observe that the higher values of corn steep liquor and glycerol favored the reduction of surface tension. This data agrees with the results obtained in Table 5, as well as in Figure 1, which represents the Pareto chart.

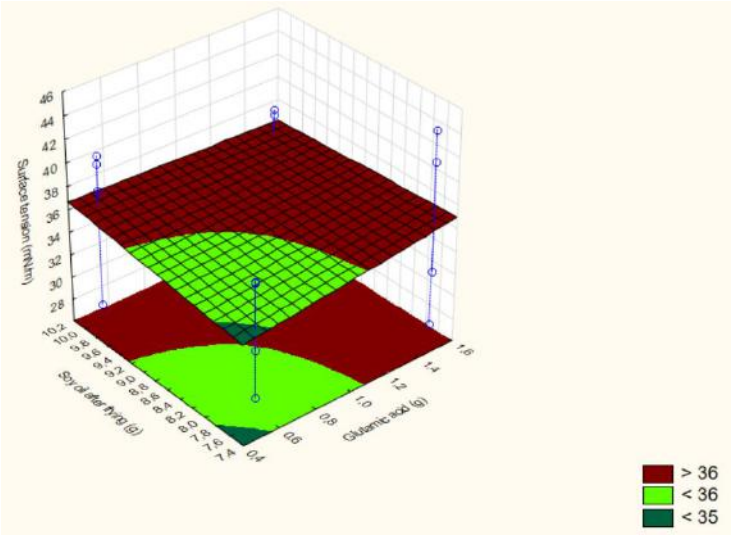
Figure 2: Surface response graph of the interaction between corn steep liquor and glycerol as a function of surface tension of the biosurfactant produced by *Stenotrophomonas maltophilia* UCP1601.



Source: Authors.

The evaluation of the relationship between post-frying soybean oil and glutamic acid in relation to surface tension, and was observed that the reduction of these two variables favors the reduction of surface tension. These data agree with the results obtained in the Pareto chart (Figure 3).

Figure 3: Response surface graph demonstrating the interaction between post-frying oil and glutamic acid in the reduction of surface tension by the biosurfactant produced by *Stenotrophomonas maltophilia* UCP1601.



Source: Authors.

3.4 Extraction of produced biosurfactant

The different treatments of the metabolic liquid for the isolation of the produced biomolecule showed very satisfactory results, as described in Table 6. When the metabolic liquid had its pH raised to 12, and the proportion of the metabolic liquid/ethanol mixture, the yield was 2:1 obtained (12.64 g/L) was excellent. The solvent used for the extraction is

easily accessible, low cost and low toxicity and the proportion that used less solvent was the most efficient. All these facts reaffirm the effectiveness of the applied method and the excellent performance of the biomolecule produced.

The literature describes lower isolation results than those obtained in the present study. She et al. (2022) studied the production of a biomolecule produced by *Pseudomonas aeruginosa* and isolated 3.03 g/L. Goyal and Singh carried out a recent study about the extraction of biosurfactant from *Lactobacillus delbrueckii*, where they used chloroform, methanol, and butanol, 1:2:1 (v/v) and obtained 5 g/L of isolated biomolecule. Zargar et al. (2022) obtained 6.9 g/L of the biosurfactant produced by *Bacillus* sp.

Table 6: Results of biosurfactant extractions from *Stenotrophomonas maltophilia* UCP 1601.

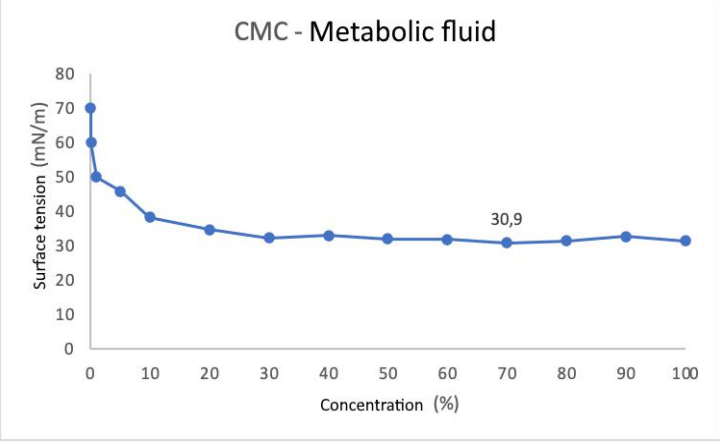
Treatment of metabolic fluid	Metabolic liquid/ethanol ratio 70% (v/v)	Productivity (g/L)
-	2:1	1.9
pH 2	2:1	1.99
pH 12	1:1	6.76
pH 12	2:1	12.64

Source: Authors.

3.5 Critical micellar concentration CMC of metabolic fluid

The critical micellar concentration - CMC value means that the adsorption of the molecules across the interface is complete and the surface properties are at their optimum. (Vieira et al., 2021). And therefore, at the moment when the entire surface is full of biosurfactant monomers, micelles are formed through the union of biosurfactant molecules. This assay was performed using cell-free metabolic fluid. The lowest concentration was obtained, thus expressing the lowest surface tension at a concentration of 70%, with a surface tension of 30.5 mN/m (Figure 4). This result demonstrates that it was not necessary to add solvents for extraction and to obtain an efficient, economical, and promising biomolecule. However, we can observe that from 30% a stability in the surface tension values begins, varying approximately 2 mN/m for more and for less. Which means that we can use 30% of the metabolic liquid and we will already be able to obtain a satisfactory surfactant response.

Figure 4: Concentration Micelar Critical of biosurfactant produced by *Stenotrophomas maltophilia* UCP 1601 present in metabolic fluid (CMC = 70%).



Source: Authors.

3.6 Emulsification Index (IE24)

This technique is known to determine the emulsifying action of a molecule (Uzoigue et al., 2015). Typically, bioemulsifiers are best known for emulsifying liquids without significant changes in the surface tension of the growth medium, whereas biosurfactants have excellent surface tension reduction ability and low emulsification index (Alizadeh-Sani et al., 2018; Ferreira et al., 2020). Two different hydrophobic substrates were tested to evaluate the emulsifying ability of the biomolecule produced. Burnt motor oil is a synthetic substrate and corn oil is a vegetable substrate. The results show an emulsification index of 56 and 43% for burnt motor oil and corn oil, respectively. The results confirm that the biomolecule produced is a biosurfactant, since there is a good reduction in surface tension, but it does not present high IE24 values.

3.7 Ionic charge and biochemical composition of the biosurfactant

The -37.57±9.7 ZPmv Zeta meter was used to show that the biosurfactant produced by *Stenotrophomas maltophilia* UCP 1601 has an anionic character, which is 122 μS/cm at 23.3 °C, full scale, thus indicating that their polar hydrophilic heads are negatively charged (Nogueira et al., 2020).

3.8 Stability in the face of different environmental conditions

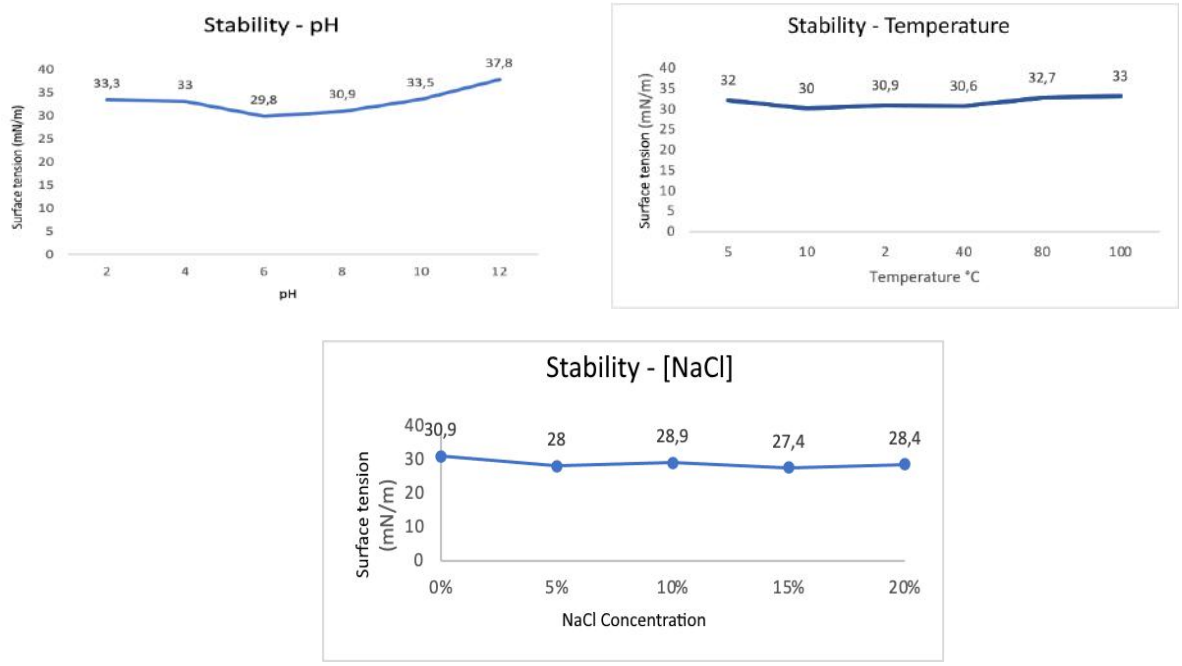
Many biosurfactants and their surface activities remain unaffected by environmental conditions such as pH and temperature and different salinity concentrations (Purwasena et al., 2019; Sanchita; Pritisnigdha, 2019; Ventriglio et al., 2021). This characteristic favors the application of this biomolecule in several areas.

All variations tested were evaluated for surface tension. In this case, we compared the variations with the surface tension of the metabolic fluid without change (30.5 mN/m). Regarding the different pH variations, it is possible to observe a greater stability between pH 2 and 10 (± 3mN/m), with the best result at pH 6. When the metabolic liquid is subjected to pH 12, the surface tension showed an increase to 37.8mN/m, indicating that this condition favors an increase in surface tension (Figure 5).

A recent study by Jumpathong et al. (2022), where the authors evaluated the production of a biosurfactant by *Bacillus velezensis* for application as a biocontrol agent, the stability of the biomolecule produced also presented its best result at pH . Figure 5 shows the stability results against different saline conditions of the biosurfactant produced by *Stenotrophomonas maltophila* UCP 1601. We can observe an excellent stability of the produced biomolecule, with the persistence of its excellent surfactant properties in a range of 0 to 20% of NaCl addition (Figure 5). The most interesting is that the increase in the saline concentration favored the reduction of the surface tension of the biosurfactant, having its best performance in the concentration of 15% of NaCl, with surface tension of 27.4 mN/m.

The recent study by Guo et al. (2022) presents a *Planococcus* sp biosurfactant applied in marine recovery, whose stability test showed a subtle increase in surface tension proportional to the increase in NaCl concentration. This behavior is very common in the literature, which emphasizes the valorization of the biomolecule produced in the present study, due to this result of potentiation of the surfactant action in saline medium.

Figure 5: Evaluation of the stability of the biosurfactant produced by *Stenotrophomas maltophilia* UCP 1601 in relation to different conditions of pH 6, temperature and salinity.



Source: Authors.

4. Conclusion

The present study with *Stenotrophomonas maltophilia* UCP 1601 showed excellent performance in producing a biomolecule of high biotechnological interest. The biosurfactant obtained through the present study has relevant surface-active properties, satisfactory stability and can be applied in its raw form with great efficiency, without the need to use organic solvents with high monetary value and which are also highly toxic. The use of renewable substrates makes the production of the biomolecule less expensive and contributes to the circular bioeconomy, meeting the initial objectives of the work. In this sense, the authors recommend the intensification of studies on this bioproduct of relevant environmental and technological interest in special to remediation under harsh environmental conditions.

Acknowledgments

The authors are grateful for the financial support of FACEPE (IBPG-1156-306-19), CAPES and CNPq (Process nº 314422/2018-8), and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) Edital Pró-Equipamentos CAPES nº 11/2014.

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