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Innovative Production of Biosurfactant by *Candida Tropicalis* UCP 1613 through Solid-State Fermentation

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Microbial surfactants are amphipathic molecules mainly produced by submerged fermentation, and capable of decreasing the surface and interfacial tension between two immiscible phases. However, their obtention by solid state fermentation (SSF) has gained attention due to the less complex equipment involved, less energy demand, low volumes of water and requirement of less solvent for extraction. Hence, the aim of this study was to evaluate the ability of *Candida tropicalis* UCP 1613 to produce biosurfactant through solid-state fermentation and determine its stability under different environmental conditions. A factorial design 2^4 was performed to determine the influence of four variables, which were inoculum size, temperature, and particle size and inductor volume on the surface tension. In addition, the stability of the biomolecule was analysed in different pH, temperature and salinity ranges. The results showed that yeast synthesized biosurfactant under all conditions tested. From the statistical analysis, it was bserved that all variables except the inductor concentration had a significant influence on the surface tension decrease. The lowest value 25.8 mN/m was detected at condition 8 (10^{-6} cells/mL; 31 °C; 32 mesh and 0 mL of the inductor). The biosurfactant displayed a good performance when analysing its activity in different temperature, salinity and pH ranges. The study showed the feasibility of the solid-state fermentation to obtain a biosurfactant with excellent activity produced by a yeast. At the same time this is the first report of its kind in the literature.

1. Introduction

Microbial surfactants are an important group of molecules synthetized by bacteria, filamentous fungi and yeast. They are amphiphilic molecules that decrease the surface and interfacial tension by accumulating at immiscible interfaces (Souza et al., 2016). In addition, these compounds are produced in stationary phase of microbial growth. According to their chemical structure they are classified as glycolipids, lipopeptides, phospholipids, and polymeric or particulate compounds (Varjani and Upasani, 2017; Karlapudi et al., 2018). The hydrophobic moiety is usually made up of long-chain fatty acids, hydroxyl fatty acids or a-alkyl-b-hydroxyl fatty acids. In the case of the hydrophilic portion can be a carbohydrate, amino acid, cyclic peptide, phosphate, carbox- ylic acid or alcohol.

The interest in biosurfactants is based on their low toxicity, high biodegradability and effectiveness at extreme temperatures, pH and salinity. In addition, their functional properties such as emulsification/de-emulsification, dispersion, foaming and wetting determine its application in bioremediation, agriculture, food production, cosmetic and other industrial sectors. (Banat et al., 2014; Fenibo et al., 2019).

However, the low yields, the carbon source and high cost input required for downstream processing are among the main challenges to make most of them marketable. Hence, to overcome this scenario one of the strategies have been the use of inexpensive and easily available raw materials (Banat et al., 2014; Satpute et al., 2017). In this context, the increasing amounts of organic solid waste because of the human activity is a challenge that needs to be handled at every level. Thus, solid-state fermentation (SSF) is a technology that allows the biological transformation of this kind of residues without a previous pre-treatment to obtain products with added value (Lourenço et al., 2018; Sadh et al., 2018).

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Moreover, the adequate selection of residual substrates is essential in the process to ensure the right balance of necessary nutrients for the microbial growth and the production of biosurfactants. Renewable substrates from different industrial activities such as, food processing and biodiesel production usually possess a high content of carbohydrates, lipids, and proteins that are attractive to be used as precursors for synthesis of microbial surfactants (Konishi et al., 2017; Satpute et al., 2017). Bearing this in mind, this study reports for the first time, the use of instant noddle waste as substrate for biosurfactant production from *Candida tropicalis* UCP 1613 through solid-state fermentation as well as the assessment of its stability in different conditions of pH, temperature and salinity.

2. Materials and methods

2.1 Microorganism

Candida tropicalis UCP 1613 was kindly provided by the Nucleus of Resources in Environmental Sciences, Catholic University of Pernambuco, Recife, Brazil. The yeast was kept at 4 °C in Yeast Mold Agar (YMA) slant containing (g/L): yeast extract, 3; malt extract, 3; peptone, 5; glucose, 10; agar, 20 at final pH 6.5.

2.2 Inoculum preparation

To obtain the inoculum a loop full of biomass was inoculated into Erlenmeyer flask of 250 mL containing 50 mL of YM broth and was incubated at for 24 h, at 28 °C and 150 rpm. After that period different concentrations (10^{-4}, 10^{-5}, 10^{-6}) were prepared to use as inoculum for the experimental design.

2.3 Culture medium for solid-state cultivation (SSF)

The raw glycerol (RG) was kindly provided by Cetene (Centro de Tecnologias Estratégicas do Nordeste, Recife, Pernambuco; Brazil) and its elementary composition was determined using a Carlo-Erba NA-111 Elemental Analyzer. The instant noddle waste (INW) was obtained from a food industry and the Table 1 shown the chemical composition of the substrates: raw glycerol and instant noddle waste (Yang et al., 2014, Andrade et a., 2018). The assays were performed in 250 mL Erlenmeyer flasks containing 5 g of instant noddle waste (which was used as support). Then 25 mL of impregnating solution contained the following composition (g/L): 3.0 g KH_2PO_4, 7.0 g K_2HPO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 1 g $(NH_4)_2SO_4$ were added. The volume of impregnating solution used was defined as the amount of medium (mL) added to the instant noddle waste without the appearance of free liquid. The aliquots raw glycerol and inoculum were determined as 10 % of the volume of the impregnating solution.

Table 1: Chemical compositions of raw glycerol and instant noodle waste used in SSF for biosurfactant production by C. tropicalis UCP 1613

Raw glycerol	Quantity (%)
Carbon	65.18
Hydrogen	10.69
Nitrogen	0
Instant noddle waste	Quantity (mg)
Carbohydrates	51000
Proteins	9.400
Total fat	16.000
Others	1370.66

2.4 Experimental strategy to biosurfactant production by SSF

The effects of the temperature (25, 28 and 31 °C), particle size (16, 32 and 60 mesh), inoculum (10^{-4},10^{-5} and 10^{-6}) and concentration of raw glycerol (0,2 and 4 %) on the production of biosurfactant were assessed by a full experimental design consisting of 20 runs (16 experiments plus 4 replicates at the central point) (Table 1). The flasks were previously sterilized at 121 °C for 15 min, and subsequently, incubated for 96 h. After that period, the samples were collected by adding 50 mL of deionized water per flask and incubated in an incubator shaker (Tecnal, TE 421) 150 rpm at 28 °C for 1 h. The liquid obtained was filtered and centrifuged at 5000 rpm for 20 min to separate the cells.

Table 2: Full factorial design to biosurfactant production by C. tropicalis UCP 1613 through SSF

Runs	Temperature (°C)	Size particle	Inoculum (cell/mL^{-1})	Raw glycerol (%)	Surface tension (mN/m)
1	25	3	10^{-5}	0	43.6
2	31	3	10^{-5}	0	27.6
3	25	1	10^{-5}	0	37.0
4	31	1	10^{-5}	0	27.1
5	25	3	10^{-6}	0	45.7
6	31	3	10^{-6}	0	38.9
7	25	1	10^{-6}	0	36.7
8	31	1	10^{-6}	0	25.8
9	25	3	10^{-5}	4	45.0
10	31	3	10^{-5}	4	27.3
11	25	1	10^{-5}	4	41.7
12	31	1	10^{-5}	4	26.9
13	25	3	10^{-6}	4	36.9
14	31	3	10^{-6}	4	27.3
15	28	1	10^{-6}	4	53.3
16	28	1	10^{-6}	4	26.3
17	28	2	10^{-4}	2	28.9
18	28	2	10^{-4}	2	29.5
19	28	2	10^{-4}	2	28.3
20	28	2	10^{-4}	2	28.5

2.5 Measurement of surface tension

Surface tension was estimated using the Du-Nuoy ring method performed in a Tensiometer Sigma 70 (KSV Instruments LTD, Finland) at room temperature (25 °C) (Kuyukina et al., 2005).

2.6 Stability assays

The thermal stability of the biosurfactant was determined from the cell-free broth submitted at temperature range from 20 to 75 °C. To evaluate the effect of the pH the samples were adjusted to different values of pH (2-12) by adding 1 M HCL or 1 M NaOH. The influence of the salinity was investigated from different concentrations of NaCl (2-12 %) (Pinto et al., 2018).

3. Results and Discussion

The overall statistical approach consisted in the assessment of four variables on the biosurfactant production by *C. tropicalis* UCP 1613 through solid state fermentation. The results obtained showed that the yeast was able to produce biosurfactant under all conditions tested, with surface tension variations between 45 and 25.8 mN/m (Table 1). Even though surface tensions around 25 mN/m are more frequent for bacteria, this result is in good agreement with previous studies which demonstrate the potential of *Candida* spp. for biosurfactant production (Rubio et al., 2017; Almeida 2018). By other side, the use of SSF as fermentative process confirmed the feasibility of this technology for the bioconversion of organic substrates used as either nutrient or inert support for the biosurfactant production. In addition, another point that highlights the potential of SSF is the search for sustainable processes to transform traditional chemical processes (Abu et al., 2017).

Figure 1 shows the Pareto chart with the main effects of the input variables on the surface tension. As can be seen only temperature, inoculum and particle size had a significant from the statistic point of view. In the case of the temperature and particle size, their increased produced an adverse effect on the reduction of surface tension. Similar effect was observed for inoculum but with positive influence. In the case of glycerol, even when exhibited a positive influence as inductor for the biosurfactant production, its influence was no significant. In this sense, the lower value of surface tension was detected in the condition 8 with 31 °C, 10^{-6} cells/mL, 32 mesh and 0 mL of glycerol. Regarding the use of instant noddle waste for the biosynthesis of biosurfactant, a few studies have displayed its potential as precursor for this purpose. However, this substrate was not explored until now for SSF applications. The present study showed that its influence was relevant to triggered the production of a microbial surfactant from the yeast. This fact is probably due to its rich nutritional composition.

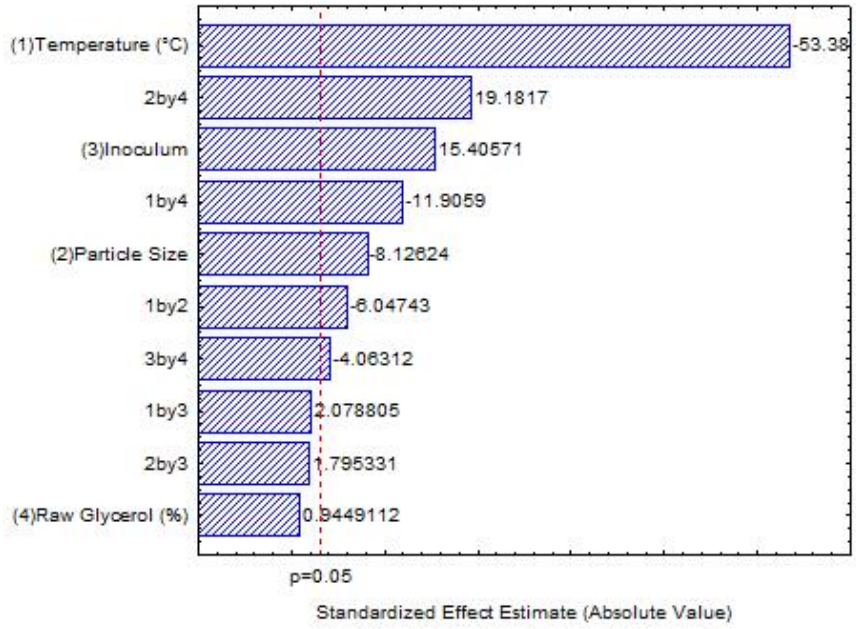


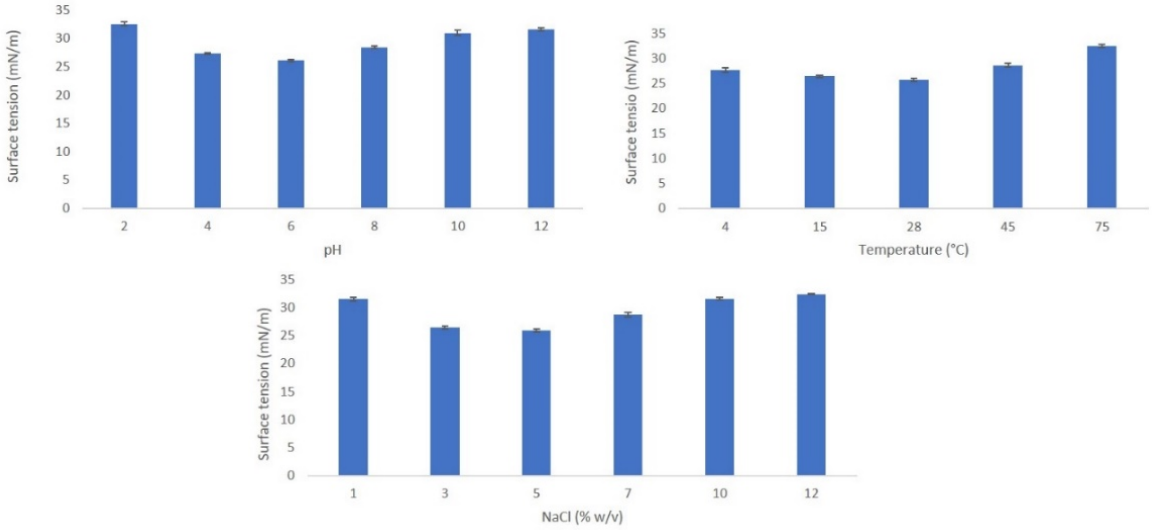
Figure 1: Pareto diagram of standardized effects of temperature, particle size, inoculum, and concentration of raw glycerol using surface tension as output variable. The dashed line specifies the statistically significant (p = 0.050

By other side, majority of researches on biosurfactant production by *Candida* spp. have been based on utilize submerged fermentation (Elshafie et al., 2015; Almeida et al., 2017). In this sense, several reports have informed the synthesis of surface-active compound from yeast using inexpensive raw material which are residues various industries (Santos, et al., 2013, Luna et al., 2013; Chaprão et al., 2015). These wastes are rich in nutrients and its reuse allows the decreasing of the production cost and as well as the reduction of environmental pollution. In addition, the bioconversion of these substrates has demonstrated to be feasible to obtain efficient biosurfactants. Likewise, the current study confirmed the potential of *C. tropicalis* UCP 1613 to use a renewable substrate from food industry by SSF as beneficial approach (Table 2).

Table 2: Production of biosurfactant by Candida spp.

Yeast	Renewable substrate	Fermentation process	Surface tension (mN/m)	Reference
C. sphaerica UCP 995	Refinery residue and corn steep liquor	SmF	27	Luna et al., 2008
Candida lipolytica UCP0988	Animal fat and corn steep liquor	SmF	28	Santos et al., 2013
Candida glabrata UCP/WFCC1556	Cassava wastewater, whey and corn steep liquor	SmF	25	Andrade et al., 2015
C. tropicalis UCP 1613	Instant noddle waste	SSF	25.8	This study

The assessment of environmental factors it is essential, since they affect the characteristics of the produced biosurfactant and consequently determine its future applications. In general, when the biosurfactant was submitted to different environmental variations the values of surface tension were around 30 mN/m which indicates a good performance.



4. Conclusions

The current study demonstrated the ability of *C. tropicalis* UCP 1613 to produce biosurfactant by SSF. It is important to highlight the statistical analysis showed the potential of instant noddle waste as substrate to initiate the synthesis of the biomolecule, which represents an economical advantage for the process. The biosurfactant maintained a good activity when analyzing its performance at variable conditions of pH, temperatures and salinity which suggest its application in different industrial sectors.

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