

Multivariate selection of nutrient parameters in submerged culture of *kluyveromyces marxianus* for biosurfactant production**Seleção multivariada de parâmetros nutricionais em cultivo submerso de *kluyveromyces marxianus* para produção de bio surfactante**

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ABSTRACT

Surfactants are compounds that decrease surface tension and are produced by chemical or biological synthesis. Biosurfactants are produced by the metabolism of microorganisms and have advantages regarding the use of chemicals, mainly due to the low toxicity and biodegradability. Initially a fractional factorial design $2^{(6-2)}$ was applied to study the effect and interactions of concentrations of different nutrients on the production of biosurfactants by yeast *Kluyveromyces marxianus* (CCT-3172). Aliquots of 1% (v/v) of microbial inoculum were transferred to the culture media prepared according to the experimental design and incubated on a shaker table at 200 RPM and with a temperature control at $28^{\circ}\text{C} \pm 2$ for 144 hours. The biosurfactant production was verified by the emulsification index (E_{24}) following the methodology of Cooper and Goldenberg (1987). Based on the experimental results, the yeast *K. marxianus* (CCT-3172) demonstrated potential for biosurfactant production, evidenced by the emulsifying index in fermentation medium. With the application of factorial design, it was concluded that to maximize the biosurfactant production process, the ammonium sulfate and glycerol must be absent from the fermentation medium to eliminate its negative effects in the process. In a next step, the objective is to apply the response surface methodology to optimize biosurfactant production.

Keywords: Environmental microbiology, Culture médium, Emulsifying index, Vegetable oils, Yeast.

RESUMO

Surfactantes são compostos tensoativos que diminuem a tensão superficial e são produzidos por síntese química ou biológica. Os surfactantes biológicos ou biossurfactantes são produzidos pelo metabolismo de microrganismos e apresentam vantagens em relação ao uso dos químicos, principalmente pela baixa toxicidade e biodegradabilidade. Inicialmente um planejamento fatorial fracionado $2^{(6-2)}$ foi aplicado para estudar o efeito e interações das concentrações de diferentes nutrientes na produção de biossurfactantes pela levedura *Kluyveromyces marxianus* (CCT-3172). Aliquotas de 1% (v/v) de inóculo microbiano foram transferidas para os meios de cultura preparados de acordo com o planejamento experimental e incubados em mesa agitadora a 200 R.P.M. e com controle de temperatura a $28^{\circ}\text{C} \pm 2$ por 144 horas. A produção de biossurfactante foi verificada pelo índice de emulsificação (E_{24}) seguindo a metodologia de Cooper e Goldenberg (1987). Com base nos resultados experimentais, a levedura *K. marxianus* (CCT-3172) demonstrou potencial para produção de biossurfactante, evidenciadas pelo índice emulsificante em meio de fermentação. Com a aplicação do planejamento fatorial, concluiu-se que para maximizar o processo de produção de biossurfactante, o sulfato de amônio e glicerol devem estar ausentes do meio fermentativo para eliminar seus efeitos negativos no processo. Em uma próxima etapa, têm-se como objetivo aplicar a metodologia de superfície de resposta para otimização da produção do biossurfactante.

Palavras-chave: Microbiologia Ambiental, Meio de Cultura, Índice Emulsificante, Óleos vegetais, Leveduras.

1 INTRODUCTION

Surfactants are compounds with surfactant properties, which can be produced by chemical synthesis (synthetic surfactants/chemical) or biological (biosurfactants). Its molecular structure is composed of a hydrophilic and a hydrophobic part, which guarantee unique properties of great industrial interest such as: detergency, emulsification, lubrication, foaming capacity, wetting capacity, solubilization and phase dispersion (GOUVEIA et al., 2003). Most commercially available surfactants are synthesized from petroleum derivatives and are toxic to different organisms, and are not readily biodegradable. When compared to chemical surfactants, biologicals have advantages such as: biodegradability; low toxicity; reduction of surface tension; solubility; pH and temperature tolerance; production from alternative waste and environmental acceptability (COLLA and COSTA, 2003).

Biosurfactants are a natural class of surface active molecules with diverse structures that are produced by different microorganisms, including bacteria, yeasts and filamentous fungi (SOUZA et al., 2017). In literature consulted (BICCA et al, 1999; NITSCHKE and PASTORE, 2003; BARROS et al., 2008; ABDEL-MAWGOUD et al., 2008; GASPARIN et al., 2012; DOS SANTOS and JÚNIOR, 2011; DECESARO et al., 2013; AL-WAHAIBI et al., 2014; DE FRANÇA et al., 2014; ALMEIDA et al., 2015; MORAIS et al., 2015; AGUIAR et al., 2015, ZHANG, 2016) a high incidence of studies reporting the production of microbial biosurfactants of bacterial origin was found, and the pathogenic nature of some producers restricts the wide application of these compounds.

The evaluation of biosurfactant production by yeasts has increased in recent years and several lineages have been reported as promising biosurfactant producers due to their high production yield and high substrate conversion rates. Among them are species belonging to genera *Candida*, *Starmerella*, *Pseudozyma* ou *Yarrowia*, *Wickerhamomyces* (SARUBBO et al., 2001; LUKONDEH et al, 2003; AMARAL et al., 2006; AMÉZCUA-VEGA et al., 2007; FONTES et al., 2008; DAVEREY and PAKSHIRAJAN, 2010; ACCORSINI et al, 2012; FONTES et al., 2012; ALMEIDA et al., 2014; FREITAS et al., 2015; JIMÉNEZ-PEÑALVER et al., 2016; CLAUS and VAN BOGAERT, 2017; SOUZA et al., 2017; JIMÉNEZ-PEÑALVER et al., 2018; LIU et al., 2018).

A great advantage of the use of yeasts lies in the status GRAS (*generally regarded as safe*), that is, many of them, such as *Yarrowia lipolytica*, *Saccharomyces cerevisiae* and *Kluyveromyces lactis*, pose no risk of toxicity and pathogenicity (BARTH and GAILLARDIN, 1997; MEDEIROS et al., 2001; LUKONDEH et al, 2003) which allows its use for applications in food and pharmaceutical industries.

The influence of the carbon source on the biosurfactant production by different microorganisms strains has been studied and the scientific literature indicates the use of a wide diversity among carbon sources, such as soybean oil, sunflower oil, corn oil, glycerol, glucose, hexadecane and others (CASAS and GARCIA-OCHOA, 1999; CAVALERO and COOPER, 2003; FONTES, et al., 2008; KURTZMAN et al., 2010; RUFINO et al., 2014; KONISHI et al., 2015; SHAH et al., 2017; SOUZA et al., 2017; LIU et al., 2018). These compounds play an important role in the survival of the producing microorganisms, since they increase the solubility of the water insoluble compounds, facilitating their transport to the cell, participate in processes such as adhesion and cell aggregation, *quorum sensum* detection, biofilm formation and defense against other microorganisms (VAN BOGAERT et al., 2007; GUDIÑA et al., 2013).

Although the scientific work clearly demonstrates the influence of different nutritional variables on biosurfactant production, there is no agreement on the optimum concentration values for some nutrients used in the culture medium.

The carbon and nitrogen source choice based on the classic optimization method by changing an independent variable and keeping all others at a fixed level is extremely time consuming and expensive in the case of a large number of variables. In addition, the observation of the effects of variables and their interactions is extremely important to understand the processes that are being monitored in a certain system (PEREIRA-FILHO et al., 2002). However, the limitations of the single-factor optimization process can be overcome through the use of multifactor statistical experimental designs, such as the experimental design the factorial and surface response (JOSHI et al., 2008). According to PERALTA-ZAMORA et al. (2005), factorial experimental planning allows us to simultaneously evaluate the effect of a large number of variables from a small number of experimental trials.

Thus, the objective of this work was to study the effect and interactions of the concentrations of different nutrients on biosurfactants production by the yeast *Kluyveromyces marxianus* applying a factorial design.

2 MATERIALS AND METHODS

2. 1 EXPERIMENTAL DESIGN - PROCESS VARIABLES

The test production followed a fractional experimental design of Resolution IV $2^{(6-2)}$ with a total of 16 tests obtained with the aid of the statistical software *Statistica 8.0*. The process independent variables studied were: soybean oil (X_1), corn oil (X_2), glycerol (X_3), glucose (X_4), ammonium sulfate (X_5), and yeast extract (X_6) and the responses to the experimental planning trials were the emulsifying index.

2. 2 INOCULUM PREPARATION AND YEAST INOCULATION

The inoculum of the yeast *Kluyveromyces marxianus* (CCT-3172) preparation occurred by means of yeast growth in yeast culture medium (Malt Extract – 0.3 g%; Yeast Extract – 0.3 g%; Bacterial Peptone - 0.5 g%; Glucose – 0.1g% Agar – 2.0g%), incubated in BOD for 48 hours at 28°C. After this period, with the aid of a platinum loop, the inoculum was transferred to erlenmeyer flask containing 150 mL of previously sterilized yeast culture medium broth. The incubation was carried out in a shaker at 200 RPM and with temperature control at 28°C for 24 hours (RUFINO et al., 2007). After this period, aliquots of 1% (v/v) of the inoculum were transferred to the sixteen experiments containing the base medium (0.1% ammonium nitrate, 0.02% monopotassium phosphate, 0.02% magnesium sulfate heptahydrate) and the independent variables soybean oil (X^1), corn oil (X_2), glycerol (X^3), glucose (X^4), ammonium sulfate (X^5) and yeast extract (X^6), according to the concentrations established by the factorial planning fraction $2^{(6-2)}$ (Table 1).

Table 1. Concentration of independent variables in different trials

Trials	Independent Variables					
	X_1	X_2	X_3	X_4	X_5	X_6
1	0,2	2,0	2,0	1,0	0,1	0,2
2	2,0	2,0	2,0	1,0	1,0	0,2
3	0,2	20,0	2,0	1,0	1,0	2,0
4	2,0	20,0	2,0	1,0	0,1	2,0

5	0,2	2,0	20,0	1,0	1,0	2,0
6	2,0	2,0	20,0	1,0	0,1	2,0
7	0,2	20,0	20,0	1,0	0,1	0,2
8	2,0	20,0	20,0	1,0	1,0	0,2
9	0,2	2,0	2,0	10,0	0,1	2,0
10	2,0	2,0	2,0	10,0	1,0	2,0
11	0,2	20,0	2,0	10,0	1,0	0,2
12	2,0	20,0	2,0	10,0	0,1	0,2
13	0,2	2,0	20,0	10,0	1,0	0,2
14	2,0	2,0	20,0	10,0	0,1	2,0
15	0,2	20,0	20,0	10,0	0,1	2,0
16	2,0	20,0	20,0	10,0	1,0	2,0

After this procedure the erlenmeyers were incubated in a shaker at 200 RPM and with temperature control at 28°C for 144 hours following the methodology of Rufino et al. (2007).

2. 3 EMULSIFYING INDEX DETERMINATION

The emulsifying index (E_{24}) was determined following the methodology of Cooper and Goldenberg (1987). Emulsifier activity was measured by adding 2 mL of hexane to 2 mL of centrifuged fermented medium and vortexing at high speed for 2 min. Measurements were made 24 h later. The emulsion index (E_{24}) is the height of the emulsion layer, divided by the total height, multiplied by 100.

3 RESULTS AND DISCUSSION

Biosurfactant production was evidenced by the results obtained by emulsifying index in the fermented medium by *K. marxianus* (Table 2). The condition with the lowest emulsifying index was found in trial 2 (3.33%), consisting of 2% soybean oil; 2% corn oil; 2% glycerol; 1% glucose; 1% ammonium sulfate, and 0.2% yeast extract. On the other hand, the condition with the highest emulsifying index was found in trial 12, with emulsifying index of 60%, consisting of 2% soybean oil; 20% corn oil; 2% glycerol; 10% glucose; 0.1% ammonium sulfate, and 0.2% yeast extract.

Table 2. Percentage of the emulsifying index of *K. marxianus*

Independent Variables						Dependent Variable	
Trials	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	Emulsifying Index (E ₂₄)

1	0,2	2,0	2,0	1,0	0,1	0,2	8,33
2	2,0	2,0	2,0	1,0	1,0	0,2	3,33
3	0,2	20,0	2,0	1,0	1,0	2,0	33,33
4	2,0	20,0	2,0	1,0	0,1	2,0	56,66
5	0,2	2,0	20,0	1,0	1,0	2,0	16,66
6	2,0	2,0	20,0	1,0	0,1	2,0	40,0
7	0,2	20,0	20,0	1,0	0,1	0,2	10,0
8	2,0	20,0	20,0	1,0	1,0	0,2	10,0
9	0,2	2,0	2,0	10,0	0,1	2,0	33,33
10	2,0	2,0	2,0	10,0	1,0	2,0	13,05
11	0,2	20,0	2,0	10,0	1,0	0,2	40,0
12	2,0	20,0	2,0	10,0	0,1	0,2	60,0
13	0,2	2,0	20,0	10,0	1,0	0,2	4,00
14	2,0	2,0	20,0	10,0	0,1	2,0	50,0
15	0,2	20,0	20,0	10,0	0,1	2,0	50,0
16	2,0	20,0	20,0	10,0	1,0	2,0	8,33

According to the statistical analysis at 95% confidence level ($p \geq 0.05$), the independent variables that produced a negative effect were glycerol (X_3) and ammonium sulfate (X_5), the latter statistically significant at the level of 95% confidence ($p \geq 0.05$) (Table 3).

Table 3. Estimates of the effects of each variable and significance with $p \geq 0.05$ for the emulsifying index

Trials	Effects	p
Soybean oil	10,5487	0,142718
Corn oil	17,2863	0,027274*
Glycerol	-1,7963	0,790653
Glucose	15,6338	0,041208*
Ammonium sulfate	-16,8688	0,030271*
Yeast extract	13,0462	0,078257

* is significant at 5% ($p \geq 0.05$).

On the other hand, the variables corn oil (X_2) and glucose (X_4) showed a significant positive effect ($p \geq 0.05$). Thus, as the concentrations of corn oil (X_2) and glucose (X_4) increase, the emulsifying index also increases. The variables, soybean oil (X_1) and yeast extract (X_5) had a positive effect, however, were not statistically significant ($p \leq 0.05$). However, if the concentrations of ammonium sulphate increase, a decrease in the emulsifying index occurs, thus due to its negative and significative effect ($p \geq 0.05$).

According to Gudiña et al. (2017), several microorganisms produce biosurfactants. The production efficiency is determined by the microorganism genetics, as well as the type of substrate, available nutrients, carbon source, environmental conditions, and other factors.

Casas and Ochoa (1999) evaluated the production of biosurfactants from different species of yeasts and concluded that the glucose concentration produced a positive effect on the biosurfactant production and promoted an increase in the emulsification index. Accorsini et al. (2012) studied the biosurfactant production by a consortium of yeasts, and at the end of the trials found that soybean oil and glucose are excellent sources of carbon for biosurfactant production.

The results found by Dikit et al. (2010) and Abreu et al. (2012) corroborate with the results found in this work. These authors obtained emulsifying indices ranging from 49 to 64.3% and 62.5% ($\pm 0.88\%$), respectively, in extract containing mannoprotein. The ability of the yeast to form emulsions is related to mannoprotein, i.e. the hydrophilic part of mannose, which binds with protein part, is hydrophobic, and forms an amphipathic structure, responsible for the emulsion (LUKONDEH et al., 2003). And it is due to these hydrophilic and hydrophobic portions of mannoproteins that they are able to reduce the surface and interfacial tension of immiscible compounds (BERTON et al., 2011), which may help in the processes of fixing oily residues by the active microorganisms present in the environment.

Because they are produced by different microbial species and under different culture conditions, biosurfactants have molecules with different chemical structures and specific properties, which should be considered at the time of their application. Therefrom, biosurfactants can be used in the oil industry for bioremediation of contaminated soils, cleaning of oil reservoirs and improved oil recovery. They are also applied in therapeutic treatments; hygiene products; formulation of cosmetics; in agriculture, in herbicide and pesticide formulation; in mining, in the ore flotation separation; in the food, paper, textile and ceramic industry (NITSCHKE and PASTORE, 2002).

Research activities and scientific publications on the subject are always increasing, focusing on understanding and optimizing the process of biosurfactant production by different microbial species in addition to the use of several low-cost

renewable resources and the discovery of new producing strains (CLAUS and VAN BOGAERT, 2017).

4 CONCLUSION

According to the experimental conditions and based on the results, the strain *Kluyveromyces marxianus* (CCT - 3172) demonstrated potential for biosurfactant production. The results serve as a basis for the fermentation process optimization by using surface response methodology (SRM), excluding ammonium sulfate to eliminate its negative and significant effect ($p \geq 0.05$) in the process.

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REFERENCES

- ABREU, R. et al. Obtenção e caracterização de manoproteínas da parede celular de leveduras de descarte em cervejaria. **Acta Scientiarum Biological Sciences**, 34 (1):77-84, 2012.
- ABDEL-MAWGOUD, A. M.; ABOULWAFI, M. M.; HASSOUNA, N. A. H. Optimization of surfactin production by *Bacillus subtilis* isolate BS5. **Applied Biochemistry and Biotechnology**, 3 (150): 305-325, 2008.
- ACCORSINI, F. R. et al. Biosurfactants production by yeasts using soybean oil and glycerol as low cost substrate. **Brazilian Journal of Microbiology**, 43: 116-125, 2012.
- AGUIAR, G. P. S. et al. Influência do Meio Mineral na Produção de Biossurfactantes. *Revista de Engenharia e Tecnologia*, 7 (1): 115-119, 2015.
- ALMEIDA, D. G. de et al. Utilização de planejamento experimental na produção de biossurfactante pela levedura *Candida tropicalis* a partir de resíduos industriais. p. 2686-2693. In: Anais do XX Congresso Brasileiro de Engenharia Química - COBEQ 2014 [Blucher Chemical Engineering Proceedings, v.1, n.2]. São Paulo. 2014.

ALMEIDA, D.de; BASTOS, C.G.; AMARAL, M.A.; MAGALHÃES, J.T. de; PARREIRA, A.G.; GONÇALVES, D.B.; CARVALHO, F.S.; SILVA, J.A. da and GRANJEIRO, P.A.; Métodos de Extração e Atividade Antimicrobiana do Biossurfactante Produzido por *Bacillus subtilis*- atcc19659, p. 33-34. In: Anais da V Jornada Acadêmica Internacional de Bioquímica [Blucher Biochemistry Proceedings, v.1, n.1]. São Paulo: Blucher, 2014.

AL-WAHAIBI, Y. et al. Biosurfactant production by *Bacillus subtilis* B30 and its application in enhancing oil recovery. **Colloids and Surfaces B: Biointerfaces**, 114: 324-333, 2014.

AMARAL, P. F. F. et al. Production and characterization of a bioemulsifier from *Yarrowia lipolytica*. **Process Biochemistry**, 41: 1894-1898, 2006.

AMÉZCUA-VEGA, C. et al. Effect of culture condition on fatty acids composition of a biosurfactant produced by *Candida ingens* and changes of surface tension of culture media. **Bioresource Technology**, 98: 237-240, 2007.

BARROS, F. F. C.; QUADROS, C. P. de; PASTORE, G. M. Propriedades emulsificantes e estabilidade do biossurfactante produzido por *Bacillus subtilis* em manipueira. **Ciência e Tecnologia de Alimentos**, 4 (28): 979-985, 2008.

BARTH, G.; GAILLARDIN, C. Physiology and genetics of the dimorphic fungus *Yarrowia lipolytica*. **FEMS Microbiology Reviews**, 19: 219-237, 1997.

BERTON, C.; GENOT, C.; ROPERS, M. H. Quantification of unadsorbed protein and surfactant emulsifiers in oil-in-water emulsions. **Journal of Colloid and Interface Science**, 354 (2): 739-748, 2011.

BICCA, F. C.; FLECK, L. C.; AYUB, M. A. Z. Production of biosurfactant by hydrocarbon degrading *Rhodococcus ruber* and *Rhodococcus erythropolis*. **Revista de Microbiologia**, 30: 231-236, 1999.

BUENO, G. F. et al. Evaluation of the pre-inoculum utilization in biosurfactant production by solid state fermentation. **HOLOS Environment**, 11: 88-88, 2011.

CASAS, J. A.; GARCÍA-OCHOA, F. G. Sophorolipid production by *Candida bombicola*: Medium composition and culture methods. **Journal of Bioscience and Bioengineering**, 88 (5): 488-494, 1999.

- CAVALERO, D. A.; COOPER, D. G. The effect of medium composition on the structure and physical state of sophorolipids produced by *Candida bombicola* ATCC 22214. **Journal of Biotechnology**, 103: 31-41, 2003.
- CLAUS, S.; VAN BOGAERT, I. N. A. Sophorolipid production by yeasts: a critical review of the literature and suggestions for future research. **Applied Microbiology Biotechnology**, 101: 7811–7821, 2017.
- COLLA, L. M.; COSTA, J. A.V. Obtenção e aplicação de biossurfactantes. **Vetor**, 13: 85-103, 2003.
- COOPER, D.G.; GOLDENBERG, B.G. Surface-Active agents from two *Bacillus* species. **Applied and environmental microbiology**, 53 (2): 224-229, 1987.
- DAVEREY, A.; PAKSHIRAJAN, K. Sophorolipids from *Candida bombicola* using mixed hydrophilic substrates: Production, purification and characterization. **Colloids and Surfaces B: Biointerfaces**, 79: 246–253, 2010.
- DECESARO, A. et al. Produção de biossurfactantes por microrganismos isolados de solo contaminado com óleo diesel. **Química Nova**, 36 (7): 947-954, 2013.
- DE FRANÇA, I. W. L. de et al. Avaliação de substratos de baixo custo na produção de biossurfactantes do tipo lipopeptídeos. p. 2288-2295. In: Anais do XX Congresso Brasileiro de Engenharia Química - COBEQ 2014 [Blucher Chemical Engineering Proceedings, v.1, n.2]. São Paulo, SP. 2014.
- DIKIT, P. et al. Emulsifier properties of the mannoprotein extract from yeast isolated from sugar palm wine. **Science Asia**, 36: 312-318, 2010.
- DOS SANTOS, R.H.Z. and JÚNIOR, A.E. Co-produção de biomassa e biossurfactantes de *Bacillus subtilis* reciclando alimentos, resíduos de biomassa e da síntese de biodiesel. In: XVI Encontro de Iniciação Científica e I Encontro de Iniciação em Desenvolvimento Tecnológico e Inovação. PUC-Campinas, SP. 2011.
- FONTES, G. C.; AMARAL, P. F. F.; COELHO, M. A. Z. Produção de biossurfactante por levedura. **Química Nova**, 31 (8): 2091-2099, 2008.
- FONTES, G. C. et al. Renewable resources for biosurfactant production by *Yarrowia lipolytica*. **Brazilian Journal of Chemical Engineering**, 29 (3): 483-493, 2012.
- FREITAS, B. G. et al. Estudo das propriedades do biossurfactante formulado produzido por *Candida bombicola* utilizando resíduos de baixo custo. **Blucher Chemical Engineering Proceedings**, 1 (2): 2701-2708, 2015.

GASPARIN, F. G. M. et al. Produção de lipase e biossurfactante por isolado de efluente de laticínio. **BBR-Biochemistry and Biotechnology Reports**, 1 (1): 28, 2012.

GOUVEIA, E. R. et al. Bactérias Produtoras de Biossurfactantes. **Revista Biotecnologia Ciência e Desenvolvimento**, (30): 39-45, 2003.

GUDIÑA, E. J. et al. Potential therapeutic applications of biosurfactants. **Trends in Pharmacological Sciences**, 34 (12): 667-675, 2013.

JIMÉNEZ-PEÑALVER, P. et al. Production of sophorolipids from winterization oil cake by solid-state fermentation: optimization, monitoring and effect of mixing. **Biochemical Engineering Journal**, 115: 93-100, 2016.

JIMÉNEZ-PEÑALVER, P. et al. Production and characterization of sophorolipids from stearic acid by solid-state fermentation, a cleaner alternative to chemical surfactants. **Journal of Cleaner Production**, 172: 2735-2747, 2018.

JOSHI, S.; YADAV, S.; DESAI, A. J. Application of response-surface methodology to evaluate the optimum medium components for the enhanced production of lichenysin by *Bacillus licheniformis* R2. **Biochemical Engineering Journal**, 41: 122–127, 2008.

KONISHI, M.; YOSHIDA, Y.; HORIUCHI, J. Efficient production of sophorolipids by *Starmerella bombicola* using a corncob hydrolysate medium. **Journal of Bioscience and Bioengineering**, 119 (3): 317-322, 2015.

KURTZMAN, C.P. et al. Production of sophorolipid biosurfactants by multiple species of the *Starmerella (Candida) bombicola* yeast clade. **FEMS Microbiology Letters**, 311: 140-146, 2010.

LIU, Z. et al. Efficient sophorolipids production via a novel *in situ* separation technology by *Starmerella bombicola*, **Process Biochemistry**, 81: 1-10, 2019.

LUKONDEH, T., ASHBOLT, N. J.; ROGERS, P. L. Evaluation of *Kluyveromyces marxianus* FII510700 grown on a lactose-based medium as a source of a natural bioemulsifier. **Journal of Industrial Microbiology and biotechnology**, 30 (12): 715-720, 2003.

MEDEIROS, A. B. P. et al. Aroma compounds produced by *Kluyveromyces marxianus* in solid-state fermentation on a packed bed column bioreactor. **World Journal Microbiology & Biotechnology**, 17: 767-771, 2001.

- MORAES, K. A. D. et al. Produção de biossurfactante por *Bacillus* sp. em meio mínimo contendo glucose. **Enciclopédia Biosfera**, 11 (22): 3084-3094, 2015.
- NITSCHKE, M.; PASTORE, G. M. Biossurfactantes: propriedades e aplicações. **Química Nova**, 25 (5): 772-776, 2002.
- NITSCHKE, M.; PASTORE, G. M. Avaliação de resíduos agroindustriais como substratos para a produção de biosurfatantes por *Bacillus*. **Boletim da Sociedade Brasileira de Ciência e Tecnologia de Alimentos**. 6 (31): 63-67, 2003.
- PEREIRA-FILHO, E. R.; POPPI, R. J.; ARRUDA, M. A. Z. Emprego de planejamento fatorial para a otimização das temperaturas de pirólise e atomização de Al, Cd, Mo e Pb por ETAAS. **Química Nova**, 25 (2): 246-253, 2002.
- PERALTA-ZAMORA, P., MORAES, J. L. de; NAGATA, N. Por que a atimização variada? **Engenharia Sanitária e Ambiental**, 10 (2): 106-110, 2005.
- RUFINO, R. D. et al. Characterization and properties of the biosurfactant produced by *Candida lipolytica* UCP 0988. **Electronic Journal of Biotechnology**, 17: 34-38, 2014.
- RUFINO, R. D.; SARUBBO, L. A.; CAMPOS-TAKAKI, G. M. Enhancement of stability of biosurfactant produced by *Candida lipolytica* using industrial residue as substrate. **World Journal of Microbiology and Biotechnology**, 23: 729-734, 2007.
- SARUBBO, L. A. et al. Bioemulsifier production in batch culture using glucose as carbon source by *Candida lipolytica*. **Applied Biochemistry Biotechnology**, 95: 59-67, 2001.
- SOUZA, K. S. T. et al. New glycolipid biosurfactants produced by the yeast strain *Wickerhamomyces anomalus* CCMA 0358. **Colloids and Surfaces B: Biointerfaces**, 154: 373-382, 2017.
- SHAH, M. U. H. et al. Production of sophorolipids by *Starmerella bombicola* yeast using new hydrophobic substrates. **Biochemical Engineering Journal**, 127: 60-67, 2017.
- VAN BOGAERT, I. N. A. et al. Microbial production and application of sophorolipids. **Applied Microbiology and Biotechnology**, 76: 23-34, 2007.
- ZHANG, J. et al. Production of lipopeptide biosurfactants by *Bacillus atrophaeus* 5-2a and their potential use in microbial enhanced oil recovery. **Microbial Cell Factories**, 15 (1): 168-179, 2016.