BIOSURFACTANT PRODUCTION BY *Yarrowia lipolytica* AT DIFFERENT CONCENTRATIONS OF CORN STEEP LIQUOR

Thuane S. Sodré[1], Jully L. Fraga[1] & Priscilla F. F. Amaral[1*]

[1] *By&Bio – By-products to Bioproducts lab., Dep. de Eng. Bioquímica. Universidade Federal do Rio de Janeiro/Escola de Química (UFRJ/EQ), Rio de Janeiro, Brasil.*
** pamaral@eq.ufrj.br*

ABSTRACT

Biosurfactants are molecules with significant interfacial activity properties produced by microorganisms. Aiming to reduce production costs, this research investigated the synthesis of a bioemulsifier by *Yarrowia lipolytica* using only corn steep liquor as a culture medium. The concentration of 20 g/L of corn steep liquor without the addition of ammonium sulfate proved to be most effective, achieving an emulsification index of 73% in 48 hours. However, with the increase in the concentration of corn steep liquor, the formation of an emulsion was observed at the begining, before inoculation. These results show that it is possible to obtain high emulsifying activity with *Y. lipolytica* in a more economical way using only this agro-industrial residue.

Keywords: Corn Steep Liquor. Bioemulsifier. *Yarrowia lipolytica*. Emulsification Index.

1 INTRODUCTION

Biological surfactants are amphiphilic compounds that encompass a wide array of surface-active molecules synthesized by a variety of microorganisms[1]. For instance, they are found in the food industry, agriculture, detergent and cosmetics formulation, as well as tertiary oil recovery. Similar to chemical surfactants, biosurfactants reduce the interfacial and surface tension of immiscible solutions. However, they offer several advantages, including low toxicity, high biodegradability, effectiveness under extreme conditions, and the ability to be regenerated and reused.[1,2]

The raw material used for bioconversion represents 50% of the final product cost. Thus, for biological surfactants to become economically more attractive than synthetic ones, alternative substrates have been investigated, especially agro-industrial residues[3]. Among these, corn steep liquor (CSL) demonstrated excellent performance in the production of bioemulsifier by *Yarrowia lipolytica*[4]. This by-product, obtained during corn starch processing, is used in rural feed and as a nutrient source in industrial fermentation processes due to its rich composition of carbohydrates, proteins and lipids[5].

In the last years, *Y. lipolytica* has been widely utilized in various studies, not only due to its simple cultivation method and ease of genetic manipulation, but also for its ability to consume and ferment different carbon sources to synthesize industrially important metabolites. This strictly aerobic yeast is considered non-pathogenic and classified as biologically safe (GRAS – Generally Recognized as Safe) by the United States Food and Drug Administration (FDA)[6,7].

In this context, the present study aimed to analyze the production of bioemulsifier by the yeast *Yarrowia lipolytica* IMUFRJ 50682 with an increase in the concentration of CSL in the culture medium.

2 MATERIAL & METHODS

Materials and microorganism: A wild type strain of *Yarrowia lipolytica* (IMUFRJ 50682) was employed and kept at 4ºC on YPD-agar medium (w/p: yeast extract, 1.0%; peptone, 2.0%%; glucose, 2.0%; agar, 4.0%). For the biosurfactant production test, media containing Corn Steep Liquor (CSL) at different concentrations were used. CSL, a by-product of the corn wet-milling process, is rich in organic acids, amino acids, vitamins, and sugars, making it a valuable and cost-effective nutrient source for fermentation and biosurfactant production[5].

Biosurfactant production: Yarrowia lipolytica cells (IMUFRJ 50682) were transferred to 500 mL shake flasks containing 200 mL of YPD medium (w/p: yeast extract, 1.0%; peptone, 2.0%%; glucose) and cultivated at 28 ºC in a rotary shaker at 160 rpm. After 72 h of cultivation, these cells were centrifuged (2,000 *g*) in a sufficient amount to inoculate 1 mg of the dry weight of cells per mL of biosurfactant production medium. Biosurfactant production carried out in 1000 mL Erlenmeyer flasks containing 500 mL of

production medium with CSL at different concentrations: 5.0 g/L, 10 g/L, 20 g/L, and 30 g/L, with and without ammonium sulfate (10 g/L), in duplicate. The flasks were incubated for 72 h at 28 °C and 250 rpm. Samples were collected at 24 h intervals, centrifuged (2,000 *g*) and stored at -20°C for the analytical procedures.

Analytical methods: Cell concentration was followed by optical density (OD) measurements at 570 nm and the values were converted to g dry weight (d.w.) cells/L using the equation OD*dilution*0,4945 to monitor cell growth. The pH of the cell-free samples was determined by a pH meter (DIGIMED DM-22), calibrated with standard buffer solutions (pH 4.1 and 6.8). The Emulsification Index (EI) of cell-free samples was determined by adding 1 mL of hexadecane with an equivalent volume of the sample, followed by vortex-mixing this mixture for 2 min and leaving it to stand for 24 hours. The EI is given as the percentage of height of the emulsified layer (cm) divided by the total height of the liquid column (cm)[8].

3 RESULTS & DISCUSSION

Cell growth, pH profile and Emulsification index during *Y. lipolytica* cultivation with CSL in the presence and absence of amonium sulfate is shown in Figures 1, 2 and 3, respectively. Results showed that CSL did not promote significant growth for *Y. lipolytica* cells, with only one condition showing a double of its initial concentration (Figure 1d). The presence of amonium sulfate, which was tested to increase the nitrogen source concentration, was also indeficient for cell growth, except for the medium with 10 g/L CSL (Figure 1b). However, pH values exhibited a consistent change across all samples over time, ranging from ±4 to ±7 within the first 24 hours, as depicted in Figure 2. This observation suggests dynamic metabolic activity within the culture medium, which may have implications for bioprocess optimization.

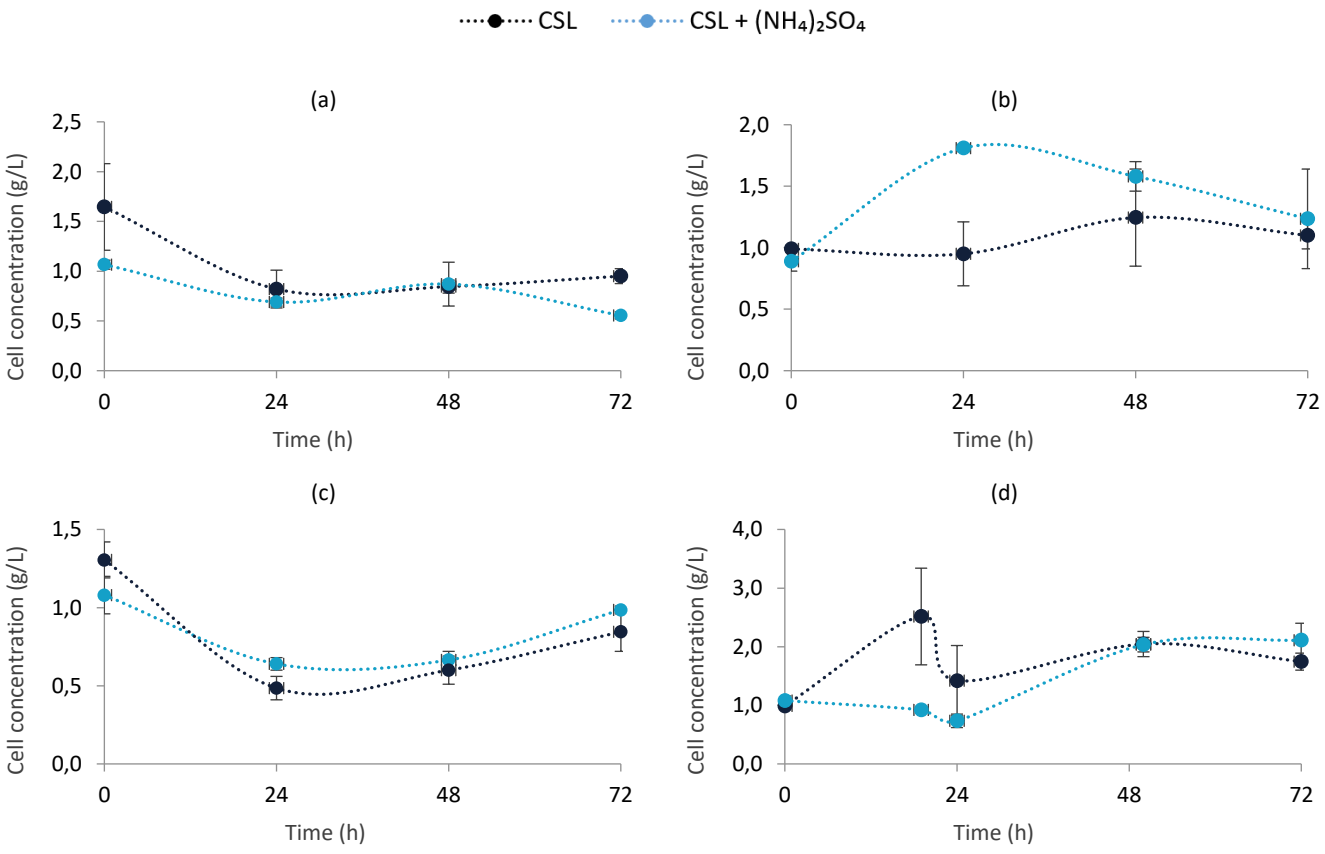

Figure 1 Cell growth during *Y. lipolytica* cultivation at 28°C and 250 rpm in 1L-Erlenmeyer flasks with 200 mL of medium containing (a) 5 g/L corn steep liquor (CSL), (b) 10 g/L CSL, (c) 20 g/L CSL and (d) 30 g/L CSL.

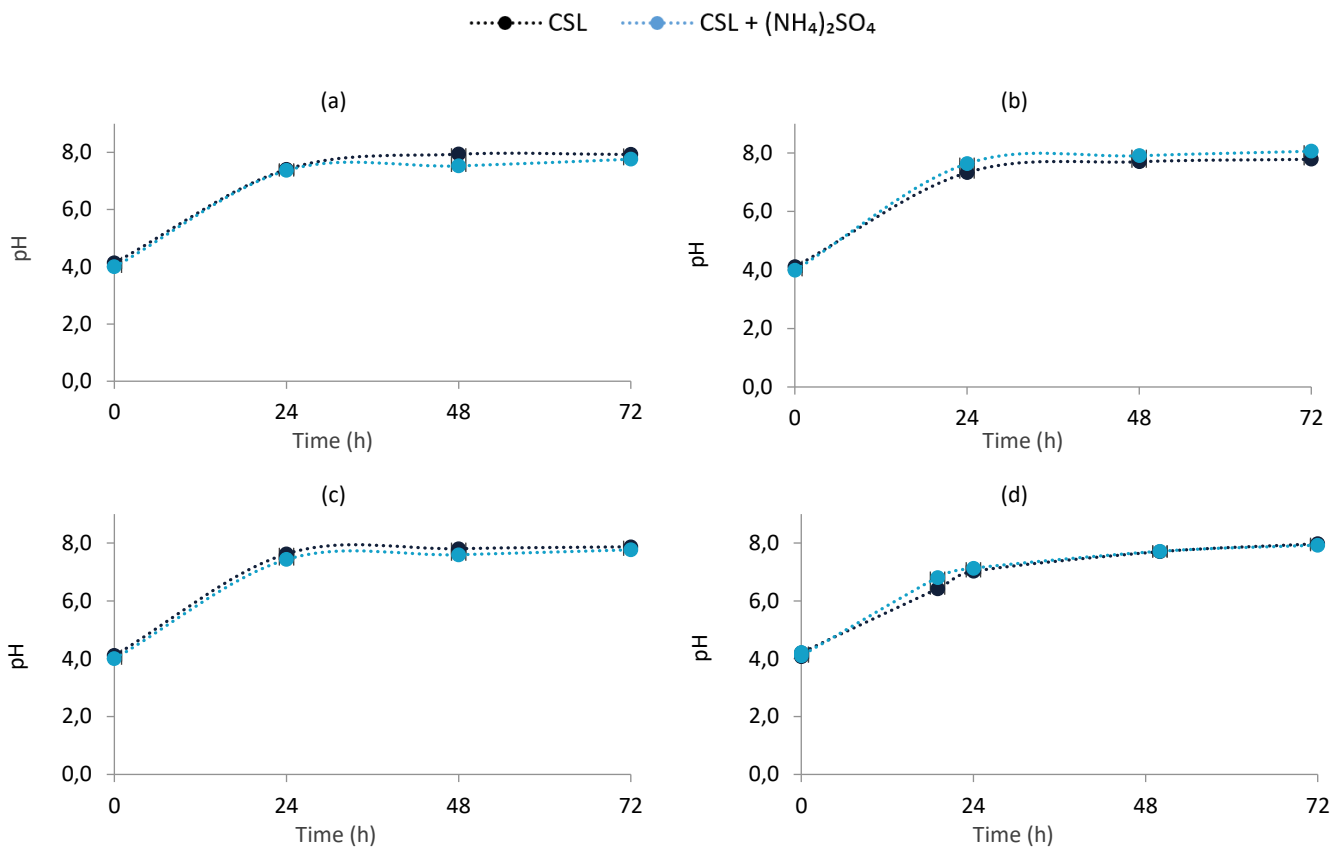

Figure 2 pH during *Y. lipolytica* cultivation at 28°C and 250 rpm in 1L-Erlenmeyer flasks with 200 mL of medium containing (a) 5 g/L corn steep liquor (CSL), (b) 10 g/L CSL, (c) 20 g/L CSL and (d) 30 g/L CSL.

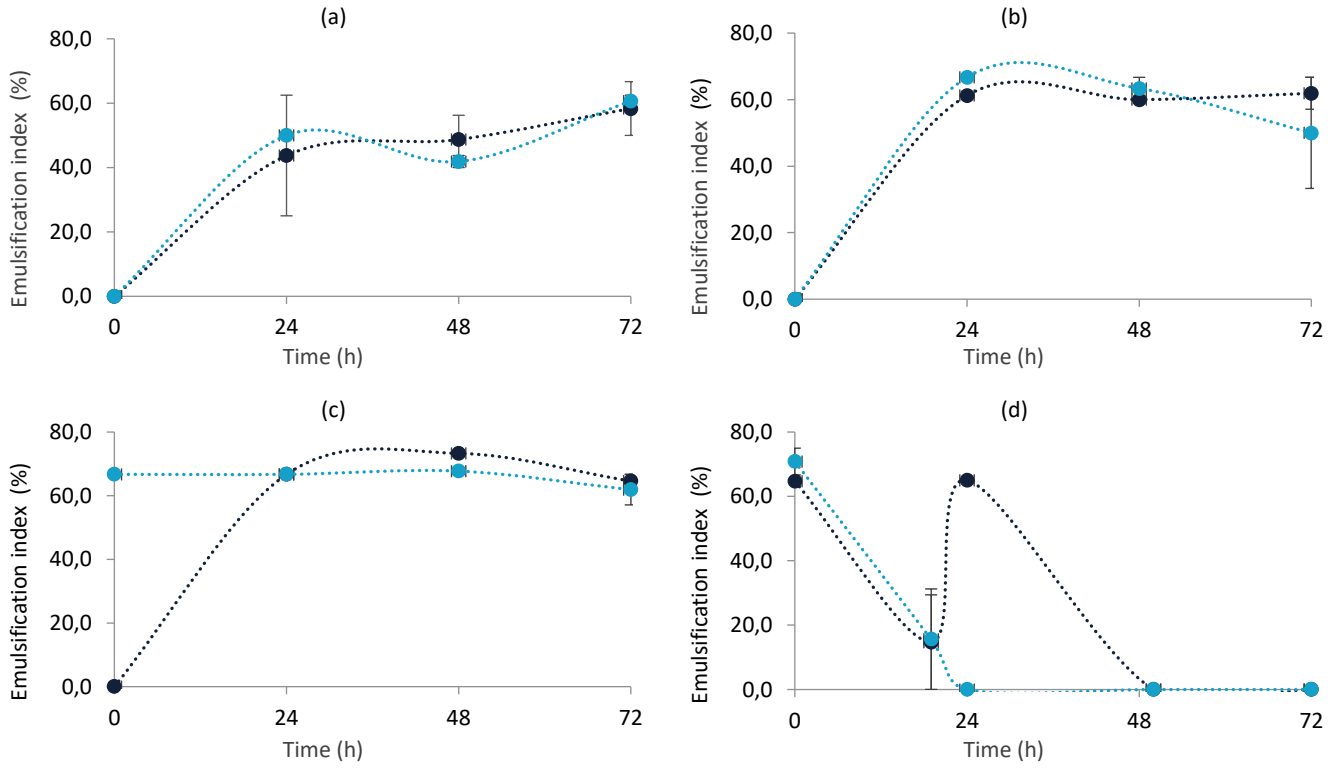

Figure 3 Emusulfication index during *Y. lipolytica* cultivation at 28°C and 250 rpm in 1L-Erlenmeyer flasks with 200 mL of medium containing (a) 5 g/L corn steep liquor (CSL), (b) 10 g/L CSL, (c) 20 g/L CSL and (d) 30 g/L CSL.

Regarding the emulsification index (EI), Figure 3c demonstrates that the medium containing 20 g/L of corn steep liquor without ammonium sulfate recorded the highest EI (73.30%) in 48 hours. However, some samples exhibited high EI before cell addition to the medium, especially those containing ammonium sulfate. This suggests that the residue may contain some unidentified emulsifying molecule.

With the increase in concentration to 30 g/L, inconsistent and incompatible values were observed compared to the other samples. This may have been due to greater precipitation of CSL in the medium, resulting from the elevated concentration. Additionally, the EI also shows a high EI before inoculation. Therefore, the best condition for biosurfactant production would be the medium with 20 g/L of CSL without ammonium sulfate.

4 CONCLUSION

The results obtained in this study indicate that, despite the limited cell growth observed in all tested media, bioemulsifier production was viable, with the medium containing 20 g/L of corn steep liquor without the addition of ammonium sulfate, registering the highest emulsification index (EI) within 48 hours. However, it is crucial to further investigate the emulsification mechanism observed before cell addition to the medium, especially in samples containing ammonium sulfate. This observation suggests the presence of unidentified emulsifying molecules in the residue itself, which may have important implications for understanding the interaction between *Y. lipolytica* and this by-product. In summary, this study highlights the importance of using agro-industrial residues as a nutrient source for bioemulsifier production, demonstrating not only the economic viability of this approach but also its potential to promote environmental sustainability in the biotechnological industry.

REFERENCES

[1] GUDIÑA, E. J et al. Valorization of agro-industrial wastes towards the production of rhamnolipids. Bioresource Technology, 212, 144-150, 2016.

[2] MUTHUSAMY, K., GOPALAKRISHNAN, S., RAVI, T. K., SIVACHIDAMBARAM, P., Biosurfactants: Properties, commercial production and application. Current Science, 94, 736-747, 2008.

[3] Fontes, G. C.; Ramos, N. M.; Amaral, P. F. F.; Nele, M.; Coelho, M. A. Z. Renewable resources for biosurfactant production by Yarrowia lipolytica. Brazilian Journal of Chemical Engineering, 29, (3), 483-493, 2012. Available from: https://doi.org/10.1590/S0104-66322012000300005. Accessed: Mar. 20, 2024.

[4] SANTOS, F. F. et al. Butter whey and corn steep liquor as sole raw materials to obtain a bioemulsifier from Yarrowia lipolytica for food oil-in-water emulsions. Food Technology. Ciência Rural, 51, (4), 2021. Available from: https://doi.org/10.1590/0103-8478cr20200323. Accessed: Mar. 23, 2024

[5] Zhou, K., Yu, J., Ma, Y. et al. Corn Steep Liquor: Green Biological Resources for Bioindustry. Applied Biochemistry and Biotechnology, 194, 3280–3295, 2022. Available from: https://doi.org/10.1007/s12010-022-03904-w. Accessed: Mar. 21, 2024.

[6] Cavallo, E., Nobile, M., Cerrutti, P., Foresti, M.L. Exploring the production of citric acid with Yarrowia lipolytica using corn wet milling products as alternative low-cost fermentation media. Biochem. Eng. J. 155, 107463, 2020. Available from: https://doi.org/10.1016/j. bej.2019.107463. Accessed: Mar. 23, 2024.

[7] P. F. F. Amaral et al. Production and characterization of a bioemulsifier from *Yarrowia lipolytica*. Process Biochemistry, 41, 8, 1894–1898, 2006. Available from: https://doi.org/10.1016/j.procbio.2006.03.029. Accessed: Mar. 23, 2024.

[8] FONTES, G. C. et al. Factorial design to Optimize Biosurfactant Production by Yarrowia lipolytica. Journal of Biomedicine and Biotechnology. 2010. Available from: https://doi.org/10.1155/2010/821306. Accessed: Mar. 20, 2024.

ACKNOWLEDGEMENTS

The authors kindly acknowledge the financial aid and research scholarships given by Conselho Nacional de desenvolvimento Científico e Tecnológico (CNPq).