PRODUCTION OF THE BIOSURFACTANT OF *STARMERELLA BOMBICOLA* AND ITS INCORPORATION INTO THE DEVELOPMENT OF AN ECO-FRIENDLY DETERGENT

Fabíola C. G. de Almeida[1*], Ivison A. da Silva[1], Káren G. O. Bezerra[1], Maria da Glória C. da Silva[1] & Leonie A. Sarubbo[1,2]

[1] *Advanced Institute of Technology and Innovation - IATI, Rua Potira, n. 31, Prado, 50751310, Recife, Pernambuco, Brazil.*
[2] *Catholic University of Pernambuco, Rua do Príncipe, n. 526, Boa Vista, CEP: 50050-900, Recife, Pernambuco, Brazil.*
** Corresponding author's email address: fabiola.almeida@iati.org.br*

ABSTRACT

Biosurfactants are compounds widely studied worldwide due to their biodegradability and environmental compatibility, unlike petrochemical (synthetic) counterparts. This study investigated the biotechnological potential of the yeast *Starmerella bombicola* ATCC22214, experiments were carried out using 1 L Schott flasks containing 300 mL of solid medium supplemented with 3% sweet potato and 5% cotton oil, inoculated with the yeast. The flasks were incubated at 30°C for 8 days under static conditions. Surface tension, yield, emulsification index, stability test, and toxicity were analyzed. The produced biosurfactant was used in a natural detergent formulation for surface cleaning. The biosurfactant production resulted in a reduction of the surface tension from 62.40 ± 0.26 mN/m to 33.40 ± 0.09 mN/m, a yield of 8.5 gL-1, emulsification activity ranging from 30% to 90%, and molecular stability under adverse conditions such as temperature, pH, and salinity. The produced biosurfactant showed no toxicity. The results of the formulated natural detergent were promising, with a 95% dirt removal efficiency.

Keywords: Yeast. Green surfactant. Natural detergent.

1 INTRODUCTION

The search for natural surfactants to replace synthetic surfactants derived from petroleum has sparked significant interest in biotechnology due to the need for environmental preservation. In this scenario, the metabolites produced by microorganisms such as filamentous fungi, yeasts, and bacteria, as well as algae and plants, known as biosurfactants, stand out. Biosurfactants are sets of chemical compounds generated by different microorganisms, with various industrial applications. They are used especially as wetting agents and surfactants in cosmetology, therapeutic preparations, environmental pollution control, and reducing surface tension, among other purposes. They have special advantages over chemical surfactants such as low toxicity, biodegradability, production from renewable substrates, ability to modify structure through genetic engineering or microbiological methods, and stability at extreme pH and temperature values.[1;2]. The biosurfactants market in 2027 is projected to reach $6.5 billion, with applications including their use as cleaning agents, in bioremediation, and in oil recovery, which require less purity and lower quality in terms of substrate origin.[2;3]. In this study, the goal was to investigate the production of biosurfactant by the yeast *Starmerella bombicola* ATCC 22214 cultivated in a low-cost medium for potential application in the cleaning and hygiene sector, incorporating the biomolecule in the formulation of a natural detergent.

2 MATERIAL & METHODS

Microorganism: The yeast *Starmerella bombicola* ATCC 22214 was tested as a producer of the biosurfactant.

Preparation of the inoculum and the biosurfactant production medium: The inoculum was standardized by transferring a culture of young yeast to flasks containing 250 mL of YMB medium and incubated at 30°C for 48 hours with agitation at 200 rpm. After this period, dilutions were performed until reaching a final cell concentration of 10% (v/v). The biosurfactant production was carried out by the yeast using a medium described by Konishi [4]., modified by replacing glucose and yeast extract with organic substrates (3%), such as sweet potato and papaya peel (3%) end Switch from olive oil to cottonseed oil. Biosurfactant production was conducted in 1 L Schott flasks under static conditions at 30°C for 8 days.

Post-production analysis of the biodetergent: After the production period, the cell-free fermentative broth was analyzed for surface tension using a KSV Sigma 700 tensiometer (Finland) with the NUOY ring. Subsequently, the emulsification activity was determined following the method outlined by Cooper and Goldenberg [5]., and toxicity tests with Artemia (surfactant concentrations of 0.3 g/L, 0.6 g/L, and 1.2 g/L) were conducted by Silva [6]., and stability was determined by emulsification activity following the method described by Cooper and Goldenberg [5]. Furthermore, the biodetergent was isolated using ethyl acetate and isopropanol (8:2, v/v), following the procedure described by Daverey & Pakshirajan [7].

Preparation and Use of Natural Detergent in the Commercial Sector: The formulation procedure was carried out as follows: the biosurfactant was added to 10 ml of distilled water for dissolution. Subsequently, the fatty acid diethanolamide was added while stirring, followed by the addition of sodium chloride and potassium sorbate, and the volume was adjusted to 50 mL. The samples were stored in previously sterilized and tightly closed containers. For application, samples of porous stone were uniformly contaminated with 100 µL of oil, then cleaned with the formulated detergent (diluted 1:1 and undiluted). The oil removal was calculated using the formula: Oil removed (%) = (Mc - Mw) / (Mc - Mi), where Mc is the mass of the contaminated stone, Mw is the mass of the stone after cleaning, and Mi is the initial mass of the stone.

RESULTS & DISCUSSION

According to the studies conducted, the medium supplemented with cottonseed oil and organic substrates (sweet potato and papaya peel) provided the production of surfactants capable of reducing the surface tension to 33.40 ± 0.09 mN/m, signifying a 56.8% decrease compared to distilled water tension (72 mN/m), with a yield of 10.5 g/L. The decrease in surface tension and high emulsification percentages indicate that *Starmerella bombicola* ATCC 22214 can degrade the substrate present in the medium, ensuring its growth and biosurfactant production. The data on surface tension and emulsification activity regarding the produced biosurfactant are presented in Table 1. Recent studies evaluated the production of biosurfactant by strains of *Starmerella bombicola* using alternative substrates (sugarcane molasses, used frying oil, corn maceration liquor, and beet molasses), achieving results of reduced surface tension between 30.60 mN/m and 33.00 mN/m.[7;8]. Thus, *Starmerella bombicola* ATCC 22214 can be considered an excellent biosurfactant producer.

Table 1 Surface tension of the biosurfactant produced in different alternative media and their respective emulsification activity.

Surface tension (mN/m)	Emulsification Activity E_{24} (%)		
	n-Hexadecane	Frying oil	Used motor oil
33,40 ± 0,09	45,5 ± 0.1	94,80 ± 0.3	80,00 ± 0.3

Many biosurfactants can be used under extreme conditions, including high temperatures, pH values, and salinity levels.[9;10]. The biosurfactant stability test conducted at different pH values, temperatures, and NaCl concentrations based on the emulsification index demonstrated stability under all test conditions established (Figure 1).

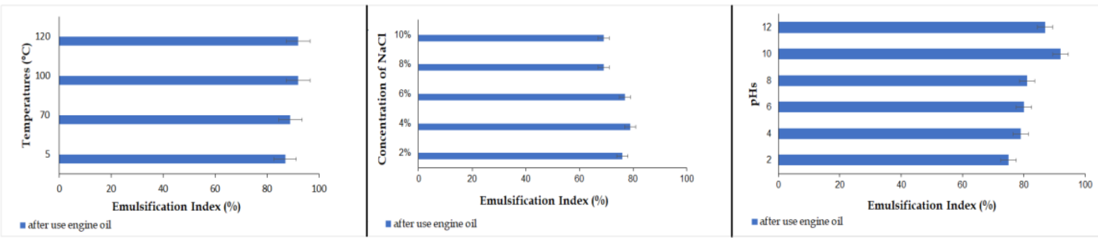

Figure 1 Stability of the biosurfactant from *Starmerella bombicola* ATCC 22214, evaluated under various pH and temperature conditions, and with the addition of NaCl, using the emulsification index.

The toxicity test evaluations of the biosurfactant using microcrustacean larvae (*Artemia salina*) showed satisfactory results. The larvae subjected to concentrations of 0.3 g/L, 0.6 g/L, and 1.2 g/L did not exhibit mortality exceeding 10% during the test (Figure 2).

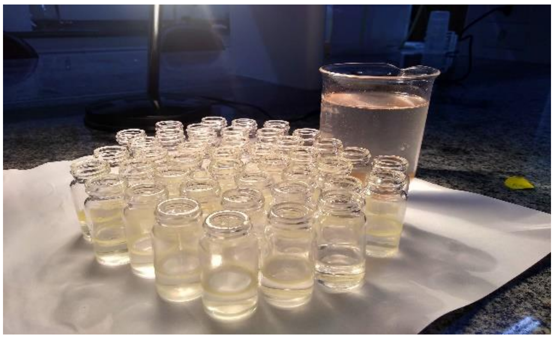

Figure 2 llustration of biosurfactants toxicity testing (0.3 g/L, 0.6 g/L, and 1.2 g/L) using microcrustacean larvae after a 24-hour incubation period.

Biosurfactants have applications in various industrial processes due to their diverse structures and properties. The solubilization capacity relies on the surfactant's ability to enhance the solubility of hydrophobic constituents in the aqueous phase. A natural detergent formulated with biosurfactant produced by *Starmerella bombicola* ATCC 22214 showed stable behavior with organoleptic characteristics of pearly white color, pleasant odor, fluid, and homogeneous consistency. In the test for removing oil compounds adsorbed on solid surfaces (stone), the formulation demonstrated satisfactory results by solubilizing motor oil, achieving a removal rate of 98.42% on all evaluated surfaces (Table 3).

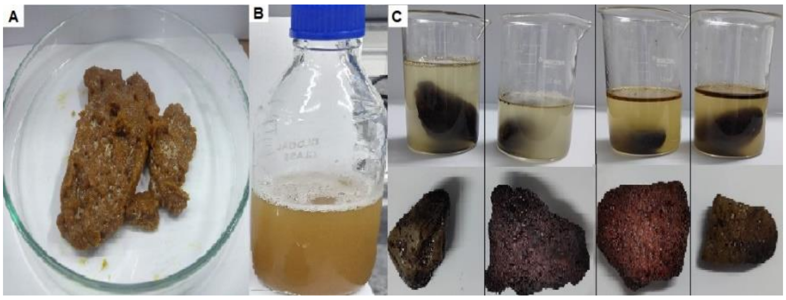

Figure 3 llustration of removing oil from porous surface (C) using a formulated natural detergent (B) with the isolated biosurfactant (A).

3 CONCLUSION

Starmerella bombicola ATCC 22214 shows significant biotechnological potential in biosurfactant production. Furthermore, the results of emulsification, toxicity, and formulation experiments clearly demonstrate the feasibility of applying the biosurfactant as a biotechnological additive in the formulation processes of cleaning and hygiene products, where reducing environmental impacts is a key aspect for maintaining quality of life and social well-being.

REFERENCES

[1] PARTHIPAN P., CHENG L., RAJASEKAR A., & ANGAIAH S., 2021. Microbial Surfactants are Next-Generation Biomolecules for Sustainable Remediation of Polyaromatic Hydrocarbons. Biosurfactants for a Sustainable Future: Production and Applications in the Environment and Biomedicine, p. 139-158.
[2] NITSCHKE M., MARANGON C. A., 2022. Microbial surfactants in nanotechnology: recent trends and applications. Crit Rev in Biotechn, v. 42, n. 2, p. 294-310.
[3] QAZI M. A., WANG Q., DAI Z., 2022. Sophorolipids bioproduction in the yeast *Starmerella bombicola*: current trends and perspectives. Biores Technol, v. 346, p. 126593.
[4] KONISHI M., YOSHIDA Y., HORIUCHI J-I., 2015. Efficient production of sophorolipids by *Starmerella bombicola* using a corncob hydrolysate medium. Journal of Bioscience and Bioengineering, v. 119, n. 3, p. 317-322.
[5] COOPER DG, GOLDENBERG BG, 1987. Surface active agents from two Bacillus species. Applied Enviromental Microbiology, v.53, p. 224-229.
[6] SILVA, N. M. P. R., ALMEIDA, F. C. G., SILVA, F. M. P. R., LUNA, J. M., SARUBBO, L. A, 2020. Formulation of a biodegradable detergent for cleaning oily residues generated during industrial processes. Journal of Surfactants and Detergents. v. 23, p. 1111-1123.
[7] SOUZA, C. V. M., JARA, A. M. A. T., BEZERRA, K. G. O., SILVA, I. A., COSTA, A.F. S., ALMEIDA, F. G. C., SARUBBO, L. A, 2023. Production of the biosurfactent Candida guilliermondii and application in the formulation of a natural detergent. Chemical Engineering Transactions, v. 100, p. 85-90.
[8] PINTO, M.I.S., RIBEIRO, B.G., GUERRA, J.M.C., RUFINO, R.D., SARUBBO, L.A., SANTOS, V.A., LUNA, J.M., 2018. Production in bioreactor, toxicity and stability of a low-cost biosurfactant. Chem. Eng. Trans. 64, 595-600.
[9] BJERK TR, SEVERINO P, JAIN S, MARQUES C, SILVA AM, PASHIROVA T, & SOUTO EB, 2021. Biosurfactants: properties and applications in drug delivery, biotechnology and ecotoxicology. Bioengineering, v. 8, n. 8, p. 115.
[10] KONISHI M., MORITA T., FUKUOKA T., IMURA T., UEMURA S., IWABUCHI H, & KITAMOTO D., 2018. Efficient production of acid-form sophorolipids from waste glycerol and fatty acid methyl esters by *Candida floricola*. Journal Oleo Sciences, n. 67, v. 4, p. 489-496.

ACKNOWLEDGEMENTS

This work was supported by the National Council for Scientific and Technological Development (CNPq), the Foundation for the Support of Science and Technology of the State of Pernambuco (FACEPE) and the Coordination for the Improvement of Higher-Level Education Personnel (CAPES). The authors are grateful to the Centre of Sciences and Technology of the Catholic University of Pernambuco (UNICAP) to the Advanced Institute of Technology and Innovation (IATI) and to the Biosurfatech, Brazil.