

# Creating connections between biotechnology and industrial sustainability

August 25 to 28, 2024 Costão do Santinho Resort, Florianópolis, SC, Brazil

Choose an item

# TECHNOLOGICAL STRATEGY FOR PRODUCING BIOSURFACTANTS IN BRAZIL FOR THE COSMETIC INDUSTRY: SHORT AND MEDIUM STAGES

Paula M. P. Freire<sup>1</sup>, Luciana R. B. Gonçalves<sup>1</sup> & Guilherme A. Lobo<sup>1</sup>

<sup>1</sup> Department of Chemical Engineering/Postgraduate in Chemical Engineering, Federal University of Ceara/ Fortaleza, Brazil.

\* paulamaria@alu.ufc.br, lrg@ufc.br

## **ABSTRACT**

This work aims to explore and identify the production routes of patented biosurfactants or those in the process of being requested in Brazil. Emphasis is placed on biochemical production using heterogeneous biocatalysis (enzymatic routes), and the investigation is expanded to a global level. The Technological Roadmap was used as the methodology for this study conducted in May 2024. The results revealed that in Brazil, there are 3 granted patents and 12 in the application process, with the alternative production route being predominant. This highlights microbial production using agro-industrial wastes. Although the production of biosurfactants by the enzymatic route was not identified in Brazil, a global patent was found. The study indicates the growing development of alternative routes and suggests a future focus on research and patents due to the sustainability and viability of these processes. However, the exploration of integrated bioprocesses remains open, as no patents applying enzyme systems were found in Brazil. This suggests an innovative potential for investment.

Keywords: Biosurfactant 1. Production routes 2. Cosmetic 3.

#### 1 INTRODUCTION

Products and excipients for ecologically sustainable pharmaceutical and cosmetic formulations are continuously being targeted. In this scenario, the production of biosurfactants that constitute many of these products is growing. In 2022, the global biosurfactants market was estimated at US\$1.2 billion, with a forecast growth of 11.2% between 2022 and 2027, reaching US\$1.9 billion by 2027¹. Brazil has the potential to become a significant competitor in the field of research due to its studies involving renewable raw materials, such as industrial and agricultural waste².

Biosurfactants can form micelles at the interface of different solvent polarities and reduce the surface tension between different liquid-liquid and liquid-gas systems. They comprise a long hydrophobic carbon chain linked to a hydrophilic polar chain. The production of biosurfactants can occur via chemical routes or alternative routes, such as the use of microorganisms (bacteria, yeast, fungi), techniques for genetic manipulation of microorganisms, and biochemical pathways through enzymes<sup>3</sup>.

The use of enzymatic catalysis (biocatalysis) in the production of biosurfactants offers several advantages. These include the high selectivity of enzymes, the use of sustainable methods, reduced reliance on fossil-based solvents and chlorinated compounds, the utilization of green and safer materials, the production of natural products, and energy savings<sup>4</sup>. Additionally, biocatalysis can be further improved through enzyme immobilization techniques. These techniques address common limitations of the process such as enzymatic stability, enzyme reuse, and high process costs. They can enhance enzyme selectivity and specificity, control product formation and enzyme recovery, and improve overall production efficiency<sup>5</sup>.

The primary objective of this study is to examine the chemical and alternative pathways that have been patented in Brazil. We aim to determine if there have been any patent requests or grants for the alternative pathway involving the use of enzyme immobilization methodology. Additionally, we seek to ascertain whether global patents have been sought or granted for the production of biosurfactants using this pathway. Our ultimate goal is to create a Technological Roadmap that reflects the current landscape of Brazilian patents in this particular field

#### 2 MATERIAL & METHODS

In the present study, the Technology Roadmap (TRM)<sup>6</sup> methodology to develop a roadmap of patents granted in Brazil and around the world for the production of biosurfactants in the cosmetic industry using the alternative route of the biochemical pathway with immobilized enzymes. Following the determination of the theme, the study was segmented into three stages: pre-prospective, prospective, and post-prospective.

The pre-prospective phase corresponds to the preliminary study to understand the topic. The prospective phase involves guided search, with the selection of keywords and the use of patent inclusion and exclusion criteria, as well as the organization of information and knowledge management. The inclusion criterion for patents was the production of biosurfactants for cosmetic purposes, covering both requested and granted patents. The exclusion criteria were patents that did not produce biosurfactants but only used them as components or in product formulation processes and patents on biosurfactants that were not intended for the cosmetic industry.

The last phase, the post-prospective, was intended to construct the technological roadmap, which was divided into two phases: a short-term one involving patents already submitted and accepted and a medium-term one covering patents submitted and still in the analysis process<sup>6</sup>.

Data was collected in May 2024 using the Google Patents database, and the keyword in Portuguese was "Biosurfactant," in addition to the combination of keywords in English: biosurfactant and enzyme immobilization. The search results were exported to an Excel spreadsheet to identify patents related to the topic. The data generated was also confirmed in Espacenet Patents. The Google Slides program was used to create the script. It is a free program that includes Google Docs Editors.

#### 3 RESULTS & DISCUSSION

A search on Google's patent database revealed 55 patents for biosurfactant production using the Portuguese keyword "Biosurfactant." Still, none of them used the biochemical route by enzymatic immobilization for biosurfactant production in Brazil. These patents have been proven in terms of application and initial issues. After that, only 15 patents remained based on their target destination, the cosmetics industry. They were double-checked in the Espacenet database to verify the data found. After systematizing them in Excel spreadsheets, the script was created (Figure 1), identifying the main biosurfactant production routes in Brazil.

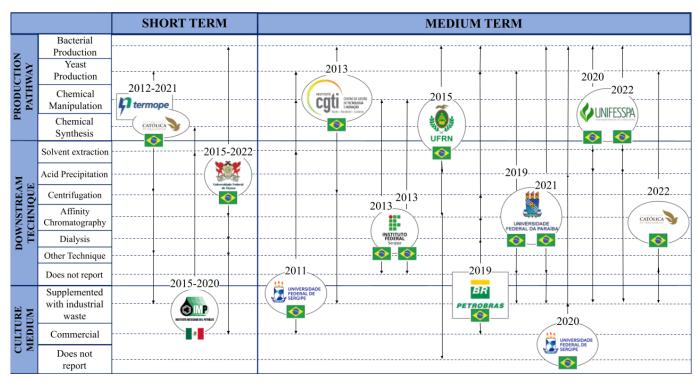


Figure 1 Technological Roadmap of biosurfactant production granted in Brazil for use in cosmetics.

Three patents were already accepted, while the others are still under analysis. Over time, it has been possible to identify the growing advances in this technology, which started more than 10 years ago and has been embraced by many research institutions, especially with the alternate biological route since 2015. Furthermore, the players found using a Portuguese keyword are 84,6% (11) institutions of research and only 15,4% (2) of industries. It is essential to highlight the presence of a partnership between a company and a federal university for an already accepted patent and also the presence of 3 institutions owning the rights to two patents at the same time, even though they are very similar to each other. It was found and a Mexican patent was accepted in Brazil, but no other international player was identified.

The Technological Roadmap also provides the following information: 73,33% (11) of the patents were identified using an alternative biological pathway for biosurfactant production, 20% (3) used the chemical route, and 6,66% (1) used a combination of both. Even though many reports argue that the best form of analytical study is the use of free suspensions of microbial cells as the best alternative<sup>1</sup>, this result represents the success of biological production for this purpose, in particular, the bacterial strains, which meant 58,33% (7) of them, compared to 25% (3) of yeast strains cultivated, and 16% (2) that used both. As for the chemical route, 66,6% (2) included the production by manipulating sodium hydroxide with an already produced biosurfactant to generate a biosurfactant salt, 33,3% (1) synthesized a new biosurfactant through a chemical reaction of reagents.

The downstream methods observed were: acid precipitation, present in 25% (5) of the patents, followed by solvent extraction, present in 20% (4), affinity chromatography and centrifugation with the same frequency of 15% (3), dialysis with 10% (2), and

finally, microfiltration, ammonium sulfate precipitation and precipitation through pH variation, with 5% (1) each. These results show a non-standardized methodology for this purpose, probably due to different biochemical characteristics among the interest molecules or their production course, indicating a possible challenge to obtain this substance empirically through experiments.

The study for the culture medium for cell cultivation revealed that 66,66% (8) of the patents used culture medium supplemented with agro-industrial or industrial waste, 25% (3) commercial medium, and 8,33% (1) did not use them. This is attributable to the presence of a major microbial route for production that demands a culture medium and also a national effort to exercise the United Nations Sustainable Development Goals 9, which stands for Industry, Innovation, and Infrastructure, and also SDG 12 for Responsible Consumption and Production<sup>6.7</sup>.

When expanding the search to a global level and using the keywords "biosurfactant" and "enzyme immobilization," the search returned 14 patents. However, only one patent (7.14%) used an immobilized enzyme to produce biosurfactants. Another 11 patents (18.57%) used enzyme immobilization methods but did not produce biosurfactants or use biosurfactants to compose products. Furthermore, 2 patents (14.28%) produced biosurfactants by bacterial cultivation without using immobilized enzymes.

The patent for the use of a biochemical route with an immobilized enzyme was requested by the Republic of Korea. Its code is KR20010001813A, and the application was filed in 1999, but it was discontinued in 2001. The immobilized enzyme, produced using the sol-gel immobilization process, was used to create a biosurfactant in a tubular reactor (PFR) with a mixed organic/inorganic membrane. This process produced malt-pure oligosaccharide from starch, allowing for the continuous manufacture of biosurfactants, with 96% of the total product being generated due to the production of only one component of the reducing sugar.

This work provides insight into the evolution of publications and research related to the identification and selection of the most effective microorganisms for producing biosurfactants in a cost-effective manner. It also focuses on optimizing processes for bioprocessing, thereby enabling the market acceptance of these biomolecules<sup>1</sup>. Furthermore, the roadmap helps to identify alternative routes for biosurfactant production that still need exploration, encouraging further research and publications in this field. Among these alternative routes, agro-industrial waste, submerged fermentation, solid-state fermentation, genetic engineering, and synthetic biology stand out, and integrated bioprocesses using enzymatic systems worldwide <sup>8,9</sup>.

## 4 CONCLUSION

The increase in patents for biosurfactant production in Brazil over the last five years reflects significant technological innovation. By utilizing agro-industrial waste as substrates for culture media, Brazil has not only reduced production costs but also promoted sustainability. Although the biochemical route through enzymes has not yet evolved enough to generate a national patent, the potential for promoting sustainable production processes makes it an open field for further exploration, research, and patenting.

#### REFERENCES

- <sup>1</sup> KADAM, V., DHANORKAR, M., PATIL, S., SINGH, P. 2024. Advances in the co-production of biosurfactant and other biomolecules: statistical approaches for process optimization. Journal Of Applied Microbiology. 135 (2) 1-13
- <sup>2</sup> SILVA, N. A., LUNA, M., SANTIAGO, A., FRANCO, L., SILVA, G., SOUZĂ, P., OKADA, K., ALBUQUERQUE, C., SILVA, C;. CAMPOS-TAKAKI, G. 2014. Biosurfactant-and-Bioemulsifier Produced by a Promising Cunninghamella echinulata Isolated from Caatinga Soil in the Northeast of Brazil. International Journal Of Molecular Sciences. 15 (9) 15377-15395
- <sup>3</sup> ZAGO, E., JOLY, N., CHAVERIAT, L., LEQUART, V., MARTIN, P. 2021. Enzymatic synthesis of amphiphilic carbohydrate esters: influence of physicochemical and biochemical parameters. Biotechnology Reports, 30 631-642
- <sup>4</sup> ZAGO, E., JOLY, N., CHAVERIAT, L., LEQUART, V., MARTIN, P. Enzymatic synthesis of amphiphilic carbohydrate esters: influence of physicochemical and biochemical parameters. Biotechnology Reports, [S.L.], v. 30, p. 631-642, jun. 2021. Elsevier BV. http://dx.doi.org/10.1016/j.btre.2021.e00631.
- <sup>5</sup> PINHEIRO, B.B., RIÓS, N. S., ZANATTA, G., PESSELA, B. C., GONÇALVES, L. R. B. Aminated laccases can improve and expand the immobilization protocol on agarose-based supports by ion exchange. Process Biochemistry, [S.L.], v. 133, p. 292-302, out. 2023. Elsevier BV. http://dx.doi.org/10.1016/j.procbio.2023.09.008.
- <sup>6</sup> S., DA SILVA, A.L.R. 2016. Technology Roadmap-Planejamento Estratégico Para Alinhar Mercado-Produto-Tecnologia. Interciência. Rio de Janeiro. Brazil.
- PEDERSEN, C. S. 2018. The UN Sustainable Development Goals (SDGs) are a Great Gift to Business! Procedia CIRP, 69 (1) 21-24
   BANAT, I. M., FRANZETTI, A., GANDOLFI, I., BESTETTI, G., MARTINOTTI, M. G., FRACCHIA, L., SMYTH, T. J., MARCHANT, R.
- Microbial biosurfactants production, applications and future potential. Applied Microbiology And Biotechnology. 87 (2) 427-444
- <sup>9</sup> SANTOS, D., RUFINO, R., LUNA, J., SANTOS, V., SARUBBO, L. 2016. Biosurfactants: multifunctional biomolecules of the 21st century. International Journal Of Molecular Sciences. 17 (3) 401-432.

#### **ACKNOWLEDGEMENTS**

This study was funded by the Brazilian agencies Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).