ANALYSIS OF BIOSURFACTANT PRODUCTION POTENTIAL BY *Aspergillus niger* MUTANT STRAIN 11T53A14.

Julyane L. Azeredo[1*], Luana F. Santos[1] & Gizele C. F. Sant'Ana[1]

[1]*Universidade do Estado do Rio de Janeiro - UERJ - Instituto de Química – Departamento de Processos Bioquimicos*
** Email: azeredojulyane@gmail.com*

ABSTRACT

The aim of this study was to investigate the potential of biosurfactant production by the filamentous fungus *Aspergillus niger*. The use of biosurfactants is considered advantageous over synthetic surfactants due to their more sustainable characteristics. However, the process remains complex and economically unfeasible. Therefore, this study aimed to explore optimal cultivation conditions to maximize biosurfactant production. A submerged cultivation (CS) method was employed, with nutrient adjustments made to maximize consumption. It was initially observed that increasing the concentration of soybean oil as a carbon source improved outcomes, reducing surface tension and forming an emulsion layer. These findings suggest that the strain possesses significant potential for biosurfactant production and biotechnological applications, highlighting the need for further research to enhance process efficiency.

Keywords: *Aspergillus niger*. Biosurfactant production. Submerged Cultivation.

1. INTRODUCTION

Biosurfactants are essential molecules in the chemical industry and play a crucial role in reducing surface tension and promoting emulsification (MULLIGAN, 2001). This ability is widely utilized in the petroleum sector, where they assist in the remediation of oil spills at sea, as lower surface tension allows the oil slick to be broken down into smaller droplets, which are then more easily consumed by marine microorganisms (MULLIGAN, 2001). Unlike synthetically produced surfactants that accumulate in the environment, biosurfactants are biodegradable, and their synthesis uses cheap and residual substrates, making them an even more sustainable choice (MUKHERJEE, 2006).

The fungus *Aspergillus niger* is known for producing valuable secondary metabolites and has garnered significant applicability in research due to its lower pathogenic tendency (FONTES et al., 2008; AMARAL, 2010). Moreover, the GRAS (Generally Recognized as Safe) status of A. niger ensures the safety of its products for handling and contact with the human body. The production of biosurfactants by microorganisms occurs through secondary metabolism reactions, as these organisms need to metabolize water-insoluble compounds. These secondary reactions perform essential functions, such as cell adhesion and motility, facilitating surface colonization and movement; differentiation, promoting cellular specialization in microbial communities; accessibility to substrates and carbon molecules, emulsifying hydrophobic compounds for use as a source of nutrients; and energy storage, acting as reserves that can be metabolized in conditions of nutrient scarcity (Van Hamme et al., 2016).

Despite presenting several advantages, the described process is still considerably expensive and complex, making it less competitive compared to synthetic surfactants. The costs associate with fermentation, separation and characterization of biossurfactants, in addition to the need to use specific and high quality nutrients to optimize production, and also the high investment in research and development to increase de production scale are significant challenges until finally becoming competitive with synthetic surfactants. Therefore, research is necessary to make its production feasible, making the process more efficient and economically viable using strategies such as optimizing fermentation conditions or alternative substrates to reduce production costs.

2. MATERIAL & METHODS

FUNGAL STRAIN

The mutant fungal strain Aspergillus niger 11T53A14 was provided by Embrapa Agroindústria de Alimentos, Rio de Janeiro, Brazil. The strain was maintained, activated, and propagated (inoculum preparation) according to procedures described by Couri & Farias (1995).

BIOSURFACTANT PRODUTION

The biosurfactant production was carried out in submerged culture. Initially, the culture medium was prepared with 0.05g of soybean oil, 0.3g of yeast extract, 0.1g of KH_2PO_4, 0.49g of $MgSO_4.7H_2O$, 0.39g of $Na_2HPO_4.7H_2O$, and 0.3g of $FeCl_3.6H_2O$

diluted in 100mL of distilled water. The CS was performed in a 250 ml Erlenmeyer flask inoculated with 10^7 conidia/ml, at 32°C and 170 rpm for 168 hours. Samples were taken at time intervals (24h, 48h, 168h) to measure biosurfactant production.

In addition to these components, another experiment was conducted with the aim of maximizing biosurfactant production. For this purpose, the amount of the carbon source was increased, using 2% oil in one assay and a combination of oil and 1% glucose in another.

EMULSIFICATION INDEX AND SUPERFICIAL INDEX

The emulsification index (IE) was conducted according to procedures described by MET Silva, 2019. There was only one change regarding the ratio. For this analysis, 3 mL of kerosene and 2 mL of cell-free culture medium were used.

After 24 hours, the formula below was used to calculate the IE.

$$\mathrm{IE}(\%) = \frac{\text{emulsified layer height(mm)}}{\text{Total height of the liquid column(mm)}} * 100$$

The surface tension was measured using the pendant drop tensiometer OCA (Dataphysics). The needle diameter used was 1,644 mm, and the analysis was conducted at 32°C. Previously, it was necessary to measure the density (densitometer - Kyoto DA-640B) of the samples, as the previous equipment required this parameter.

RESULTS & DISCUSSION

This study aimed to explore the biosurfactant production potential of a mutant strain 11TA53A14, belonging to the genus Aspergillus niger, and to identify optimal conditions for this production. Prior to the experiment, a literature review was conducted. A previous investigation by Kannahi (2012) examining various temperatures and pH levels revealed that biosurfactant production peaked around pH 7, with a decline in production observed at pH 8. Similarly, temperatures above 37°C were found to reduce production. Collectively, these findings suggested that the most favorable conditions fell within a pH range of 7 and temperatures ranging from 28°C to 37°C. Consequently, submerged cultivation was performed under these conditions.

Initially, $FeCl_3$ was included in the culture medium based on insights from a study by Colin, V. L., Baigori, M. D., & Pera, L. M. (2010), which proposed that the interaction between iron and phosphate could enhance emulsifying capacity. The presence of an appropriate concentration of ferric chloride was hypothesized to boost biosurfactant production, thereby improving the efficacy of biotechnological processes employing these compounds.

However, subsequent analyses revealed that the presence or absence of $FeCl_3$ had no significant impact on biosurfactant production. Unexpectedly, the surface tension of the medium increased in the presence of ferric chloride. Furthermore, no formation of an emulsified layer was observed during the initial emulsification assay (IE). Consequently, $FeCl_3$ was excluded from the composition of the culture medium in subsequent experiments.

An effective strategy to optimize biosurfactant production entails evaluating the carbon source. Given its pivotal role in secondary metabolite synthesis, a thorough assessment of the carbon source employed was imperative. Thus, the decision was made to elevate the concentration of soybean oil to 2% while simultaneously incorporating 1% glucose into the culture medium.

Significant reduction in surface tension was observed in the medium containing a higher concentration of soybean oil (2%), decreasing from 53.13 mN/m to 41.16 mN/m across the conducted experiments. Additionally, emulsification index analysis revealed the formation of an emulsion layer after 48 hours (EI = 10 ~ 16%) and 168 hours (EI = 6 ~ 7%). These findings indicate biosurfactant production in the culture medium comprising solely soybean oil (2%), underscoring the biotechnological potential of this strain.

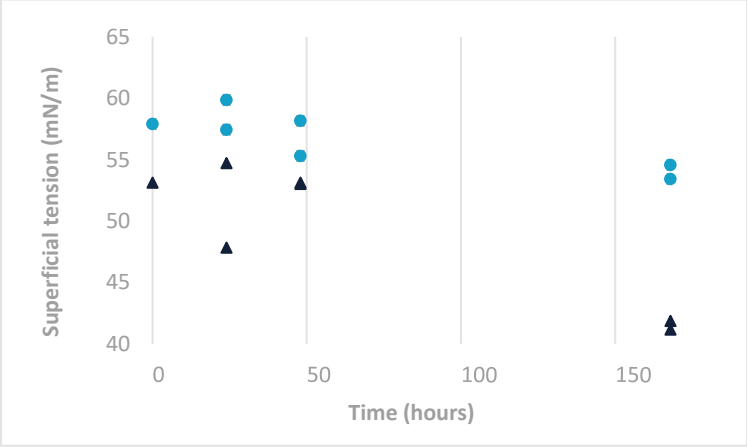

Figure 1 Graphical Analysis of Surface Tension for Evaluating Carbon Sources in Culture Media with Oil(▲) and Combined Oil/Glucose(●)

3. CONCLUSION

While conducting carbon source maintenance, it was observed that the mutant strain Aspergillus niger 11T53A14 produced biosurfactant in a medium containing exclusively soybean oil (2%), indicating its biotechnological potential. However, it is evident that this production has not yet reached its maximum potential. To achieve more significant results, a more comprehensive experimental design is necessary, one that evaluates not only the other components of the medium but also the ideal concentration of the carbon source. This will pave the way for more efficient and sustainable production, highlighting Aspergillus niger as a valuable tool in industrial biotechnology.

REFERENCES

[1] MULLIGAN, C. N.; YONG R. N.; GIBBS, B. F. Surfactant-enhanced remediation of contaminated soil: A review. Engineering Geology, v. 60, 2001.

[2] MUKHERJEE, S.; DAS, P.; SEN, R. Towards commercial production of microbial surfactants. Trends in Biotechnology, v. 24, n.11, 2006.

[3] FONTES, G. C.; AMARAL, P. F. F.; COELHO, M. A. Z. Produção de biossurfactantes por levedura. Química Nova, v. 31, 2008.

[4] SILVA, A. F. C., FERREIRA, B. CASTRO, C. T. 2023. Lat. Am. J. Biochem. Process. 27 (1). 429-440.

[5] GREEN, T. PARKER, R. 1999. Interpreting landscapes. *In:* Evolution of Bioinformatics. DAWSON, L. (ed). 2nd ed. Pearson, Los Angeles. 180-205.

[6] COURI, S.; FARIAS, A. X. Genetic manipulation of Aspergillus niger for increased synthesis of pectinolytic enzymes. Rev. Microbiol, v. 26, n. 4, p. 314-7, 1995.

[7] KANNAHI, M; SHERLEY, M. Biosurfactant production by pseudomonas putida and Aspergillus niger from oil contaminated site. International Journal of Chemical and Pharmaceutical Sciences v.3(4), 2012.

[8] Colin, V. L., Baigori, M. D., & Pera, L. M. (2010). Bioemulsifier production by Aspergillus niger MYA 135: Presumptive role of iron and phosphate on emulsifying ability. *World Journal of Microbiology and Biotechnology, 26*(12), 2291–2295. https://doi.org/10.1007/s11274-010-0409-4

ACKNOWLEDGEMENTS

We would like to express our sincere gratitude to Professor Gizele Cardoso, CETREINA, and all individuals involved in this project. Your support and collaboration were essential for the development of this work.