CERULENIN PRODUCTION SCALING BY SUBMERGED CULTIVATION OF SAROCLADIUM ORYZAE BRM 59907

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ABSTRACT

The present study aims to obtain, on a bioreactor scale, the secondary metabolite cerulenin produced by strain *Sarocladium oryzae* BRM 59907. The production results demonstrated an average cerulenin concentration of 66 mg/L after 48 h and 103 mg/L in 72 h. Within 24 hours, no significant amounts of cerulenin were found. Therefore, scaling is a viable possibility and, through future experimental adjustments, it may be possible to optimize the methodology used to increase the concentration produced and reduce the time in which the plateau of this concentration is reached, which contributes to the economicity of the process and encompasses the circular economy since glycerol, coming from the biodiesel production chain, is used as the carbon source.

Keywords: Cerulenin. Bioprocess optimization. Glycerol.

1 INTRODUCTION

The Doughnut Economy model, proposed by British economist Kate Raworth, points to a safe and fair space for humanity. Within the socio-environmental limits established in the Doughnut's visual structure, the social foundation is reached and the ecological ceiling is not exceeded[1]. The social aspects present in the model are directly related to the Sustainable Development Goals (SDGs) that make up the United Nations agenda. Among these aspects, there is responsible consumption and production, themes that encompass sustainable agriculture.

In this context, the secondary metabolite cerulenin from *Sarocladium oryzae*[3] is very attractive as a biobased antimicrobian agent. The production of this substance in a bioreactor using glycerol as a carbon source makes the production system cyclical while incorporating a product from the biodiesel production chain, and increases the added value of glycerol from the perspective of obtaining a new biotechnological product of interest. This production is based on the cultivation of *S. oryzae* and is carried out for 72 hours in an 8L tank with a total volume of 4L of medium. The metabolite of interest is secreted into the extracellular environment by the fungus, which facilitates the obtaining and dosing of the product of interest.

2 MATERIAL & METHODS

First of all, a pre-inoculum (30 g L^{-1} glycerol, 10 g L^{-1} glucose, 5 g L^{-1} peptone, 3 g L^{-1} NaCl and 1,75 mg L^{-1} Mg^{2+}) was prepared by cultivation at 28°C and 150 rpm during 72h. In the next step, a culture medium to produce cerulenin was prepared and added to the bioreactor vessel to be autoclaved. Finally, one percent of the pre-inoculum volume was added in the bioreactor vessel along with the cerulenin production medium (20 g L^{-1} glycerol, 2,5 g L^{-1} peptone and 0,025 g L^{-1} NaCl) , totalizing 4L (Figure 1b). The culture medium was aerated with 1 vvm of air flow at 28 ºC and 150 rpm. Throughout the cerulenin production, aliquots were taken to measure the biomass accumulation by dry weight method and to determine carbon source consumption and the pH of the medium.

At the end of the production, the mycelium was vacuum filtered (Whatman n. 54 quantitative filter and 0.22 µm membrane) and the filtrate was stored at 4 ºC for posterior analysis. Glycerol consumption was determined by High-Performance Liquid Chromatography using an Agilent Infinity 1260 HPLC system (Agilent Corp., USA) equipped with an Aminex HPX-87H column (BioRad – 300 mm x 7.8 mm) and a refractive index detector (RID) at 45 ºC, with mobile phase 5 mmol/L H_2SO_4 at a flow rate of 0.6 mL/min. (Figure 1a). The concentration of cerulenin was determined by a bioassay with *Saccharomyces cerevisiae* Fleishmann strain using Omura method with modifications[3]. The software WebPlotDigitizer[4] was used to determine the area of the inhibition halos. Cerulenin was detected in culture media through Thin Layer Chromatography (TLC) on silica gel G60 plates developed with solvent system of ethyl acetate-acetic acid (100:0.1), and the spots were revealed by iodine vapor.

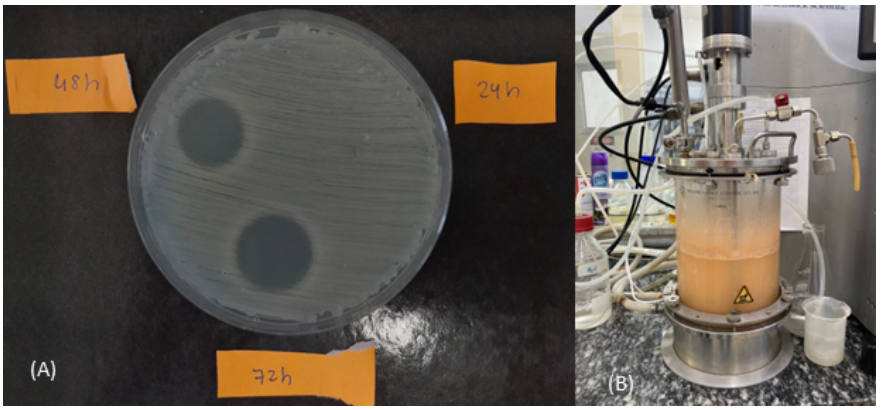

Figure 1 (A) *Saccharomyces cerevisiae* Fleischmann® plate with cerulenin inhibition halos; (B) Bioreactor with culture medium in 72h of process.

3 RESULTS & DISCUSSION

Below are the results of the analyzed parameters (Table 1). Firstly, It is possible to observe the pH and glycerol concentration in the culture medium decrease with the production of cerulenin. The glycerol is consumed while the concentration of biomass increases. The pH probably decreases because of the organic acids produced by the microorganism metabolism. It is known that the optimal growth conditions of *S. oryzae* occur at a lower pH (about pH 4). Consequently, the acidification of the culture medium by the metabolism of the microorganism itself contributes to its growth.

The cerulenin concentration had its biggest value in 72h hours, according to previous experiments conducted in Erlenmeyer flasks. Therefore, it was found that expanding the scale of production is a viable proposal that can undergo experimental adjustments to be optimized. Finally, the TLC using a cerulenin standard for comparative purposes confirmed the presence of cerulenin in the samples of the bioreactor production (Figure 2).

Table 1 . Tests results and experimental parameters control

Time of cultive (h)	Glycerol (g/L)	pH	Biomass (g/L)	Conidia concentration/(mL)	Cerulenin (mg/mL)
0	20,07	6	0	~10^5	0
24	18,7	4,8	1,2	1,4 x 10^7	0
48	16,17	4,2	1,8	2,8 x 10^7	65,49
72	12,89	4	2	5,2 X 10 ^7	102,39

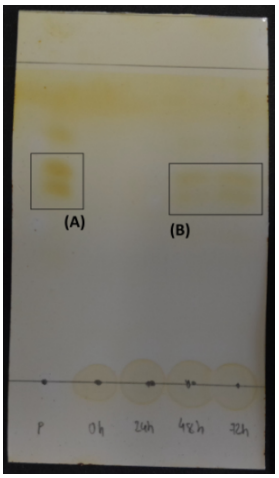

Figure 2 (A) Cerulenin standard solution bands after mobile phase elution; (B) Cerulenin bands from the 48h and 72h samples, from left to right

4 CONCLUSION

After analyzing the results, it is concluded that it will be necessary, in the future, to repeat the production in bioreactor to ensure the reproducibility of the results. In addition, adjustments will be made in the experimental conditions in order to optimize production, aiming to reduce the time to reach the cerulenin concentration plateau.

However, the possibility of scaling the process, usually carried out in Erlenmeyer flasks, is already a great advance to achieve the main objective of promoting a gradual increase in the economicity of the cerulenin production. There may be a great industrial interest in this optimized production, since the biotechnological application of cerulenin, from the perspective of a sustainable agriculture, encompasses not only economic issues, but also socio-environmental factors, such as the preservation of nature and the well-being of humanity.

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ACKNOWLEDGEMENTS

Special acknowledgements to UFRJ, to the supervisors João Rogério Borges de Amorim Rodrigues, Maria Fernanda dos Santos Mota, Ana Cristina Pinheiro de Lima, Marcio Vinicius de Carvalho Barros Cortes, Elisa d'Avila Costa Cavalcanti, Denise Freire and to the entire workplace of the Laboratório de Biotecnologia Microbiana (LaBiM).