PRODUCTION OF CERULENIN BY SAROCLADIUM ORYZAE BRM 59907 USING CRUDE GLYCEROL

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ABSTRACT

Thanks to the increasing production of biodiesel in Brazil and worldwide, crude glycerin emerges as a promising renewable resource for obtaining high-value-added bioproducts. However, many bioprocesses cannot directly employ it in microorganism cultivation due to its impurities, which may exert inhibitory effects on cell growth or the production of the target metabolite. This study tested the substitution of glycerol (>99%) with crude glycerin (72% glycerol) in the submerged cultivation of *Sarocladium oryzae* BRM 59907 for cerulenin production. The presence of contaminants in glycerol, such as salts and methanol, did not negatively affect the inoculum propagation stage but did reduce the cerulenin concentration in the production medium. Crude glycerin proved to be an adequate carbon source for this bioprocess, provided that the concentrations of its contaminants can be previously determined.

Keywords: Crude Glycerine. Biodiesel co-product. Cerulenin. Submerged cultivation.

1 INTRODUCTION

Glycerol is a raw material obtained by the transesterification of vegetable oils and animal fats in biodiesel refineries, and it shows great promise as an alternative substrate for microorganism cultivation. Currently, Brazil is the largest producer of this biofuel in South America and the third largest internationally[1], generating glycerol primarily in the form of crude glycerin. Also known as raw glycerin, this material typically contains high levels of impurities such as bases (KOH and NaOH), inorganic salts (NaCl and K_2SO_4), free fatty acids, monoglycerides, diglycerides, triglycerides, methyl esters, methanol and water. Therefore, before being used in various industrial sectors (food, pharmaceutical, hygiene and cosmetics, etc.), crude glycerin must undergo purification processes that increase the final cost of glycerol. In this context, numerous efforts have been made to directly utilize crude glycerin in biotechnological processes, such as the production of high-value-added bioproducts.

Cerulenin is a secondary metabolite produced by *Sarocladium oryzae* and is known as a natural inhibitor of the enzyme fatty acid synthase (FAS). This compound has demonstrated antibacterial and antifungal activity against various pathogenic strains and is typically produced through submerged cultivation in a medium containing high-purity glycerol and glucose as carbon sources. In this study, the potential to substitute glycerol with crude glycerin in the propagation of the *Sarocladium oryzae* BRM 59907 strain and in its cerulenin production medium was investigated.

2 MATERIAL & METHODS

The potential replacement of glycerol with crude glycerin in inoculum propagation culture media of *Sarocladium oryzae* BRM 59907 (30 g L^{-1} glycerol, 10 g L^{-1} glucose, 5 g L^{-1} peptone, 3 g L^{-1} NaCl, 9,00 mg L^{-1} Ca^{2+}, 1,75 mg L^{-1} Mg^{2+} and 1,30 mg L^{-1} K^{+}) was tested by adding contaminants to this medium component according to the average levels reported in literature[2]. The crude glycerin, containing sodium, potassium and phosphorus salts, as well as methanol, was analyzed for its performance in biomass production and carbon sources consumption after cultivation for 72 h at 28 ºC and 150 rpm. Glycerol and glucose concentrations were 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. Cerulenin concentration was estimated through a bioassay with *Saccharomyces cerevisiae*, and cellular biomass was measured using the dry mass method. The experiments were conducted in sextuplicate, and means were compared using two-tailed Student's t-test for independent samples ($p < 0.05$).

Additionally, the use of crude glycerin was evaluated in an optimized medium for cerulenin production (20 g L^{-1} glycerol, 5 g L^{-1} glucose, 2,5 g L^{-1} peptone, 0,025 g L^{-1} NaCl and 0,029 g L^{-1} $FeCl_3$) with culture harvested after 48 h. The raw material from the biodiesel biorefinery, containing approximately 72% w/v glycerol and 20% w/w moisture, was used to compare biomass and cerulenin production with high-purity glycerol. The experiments were performed in quintuplicate, and the difference between means was assessed using two-tailed Student's t-test for independent samples ($p < 0.05$).

3 RESULTS & DISCUSSION

Contaminants in crude glycerin, such as phosphate, methanol, and lipids, did not inhibit the growth of *Sarocladium oryzae* nor alter the carbon source consumption profile. There was no statistically significant difference in the mean biomass produced (fig. 1a) and the final concentrations of glucose and glycerol (fig. 1b) in the inoculum after 72 hours. The production of (73 ± 10) mg/L and (72 ± 7) mg/L of cerulenin was also observed during the inoculum propagation with glycerol and crude glycerin, respectively, indicating that the biosynthesis of this secondary metabolite is already induced during the pre-inoculum cultivation period. Although methanol is known to be toxic to many microorganisms, inhibiting their growth even at low concentrations, it is possible that this alcohol completely evaporated after autoclaving the medium at 121°C for 15 minutes[3,4]. Therefore, its prior presence in crude glycerin likely did not compromise inoculum propagation.

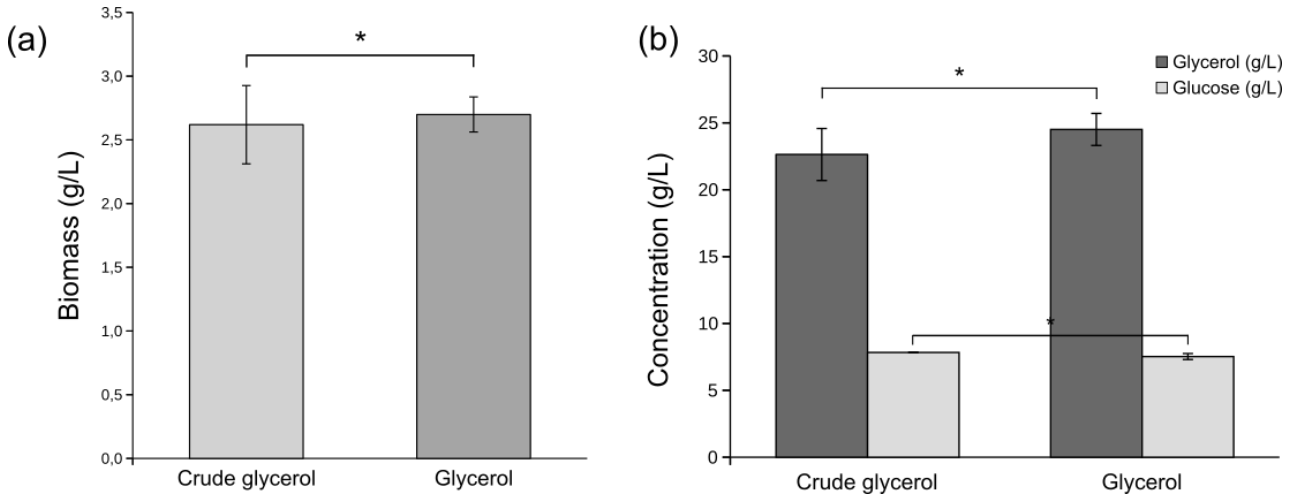

Figure 1 Comparison of (a) biomass production and (b) carbon sources final concentration using crude glycerine and high-purity glycerol. Bars represent the respective replicate standard deviations. *two-sided p value < 0.05.

In the production medium, no statistically significant difference was observed in biomass growth (fig. 2a). The BRM 59907 strain was able to produce cerulenin in the presence of crude glycerin, reaching pH (4.16 ± 0.05) in culture media after 48 h. However, its average concentration in the filtrate ((83 ± 10) mg/L) was approximately 20% lower compared to (116 ± 7) mg/L obtained in the other condition (fig. 2b). The levels of calcium, magnesium, phosphate, and sulfur in crude glycerin may be responsible for this slight reduction. Previous studies with the *S. oryzae* KF-140 strain showed that the use of dibasic potassium phosphate (K_2HPO_4), calcium carbonate ($CaCO_3$), and magnesium phosphate ($Mg_3(PO_4)_2$) during cultivation also inhibited cerulenin production[5,6].

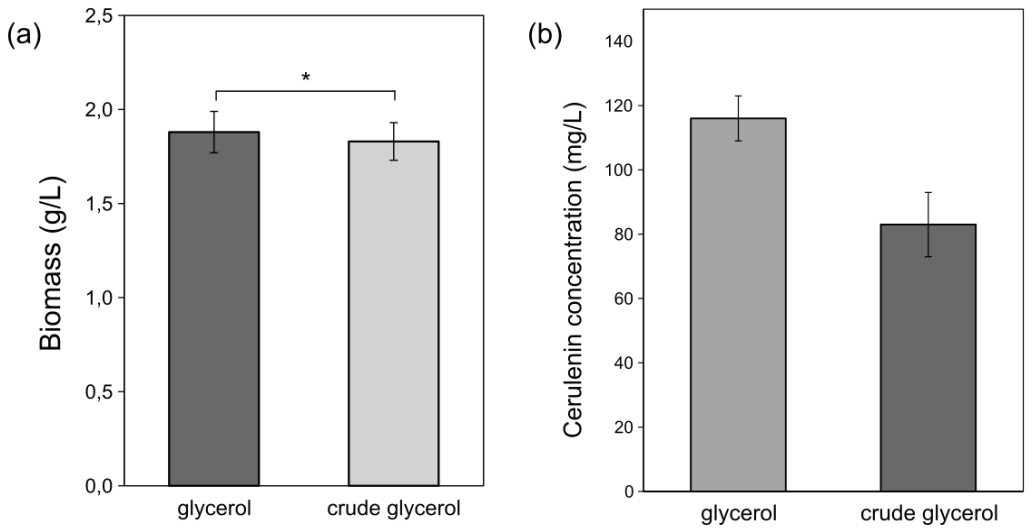

Figure 2 (a) Biomass and (b) cerulenin production comparison using crude glycerol and high-purity glycerol. Bars represent the respective replicate standard deviations. *two-sided p value < 0.05 with the equal variances t-test.

Although this strategy presents an alternative for large-scale cerulenin production, the replacement of glycerol with crude glycerin in the production medium is limited to its intended application. For instance, EU Regulation No. 68/2013, which sets maximum contaminant levels in raw materials for animal feed, stipulates that crude glycerin may contain a maximum of 0.5% methanol and 4% non-glycerol organic matter[7]. Therefore, it may be necessary to analyze these contaminants in crude glycerin beforehand or ensure their removal after the bioprocess.

4. CONCLUSION

Crude glycerin proved to be an adequate substitute for glycerol in the inoculum propagation stage, as it did not hinder cell growth and was relatively well tolerated by *Sarocladium oryzae* BRM 59907. Utilizing this biodiesel co-product did not require additional purification steps, such as the removal of salts and/or methanol, reducing the production cost of cerulenin while making the bioprocess more sustainable. However, the presence of salts in crude glycerin reduced the final concentration of the bioproduct of interest, limiting the use of this alternative carbon source. These results encourage further studies on the efficiency of glycerol utilization for cerulenin production.

5. REFERENCES

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