TITLE: OPTIMIZATION OF THE PRODUCTION OF CHITINASE BY THE FUNGUS *ASPERGILLUS NIVEUS* IN SUBMERGED FERMENTATION USING RESIDUES FROM FISHING INDUSTRY

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ABSTRACT:
Chitinases are enzymes capable of cleaving β-1.4 links between N-acetylglucosamine units from chitin. They have biotechnological application in the control of phytopathogens and herbivorous pests, and as antifungal enzyme. Chitin can be found in the exoskeletons of arthropods and in cell walls of fungi. Fishing industry is responsible by the production of rich chitin waste, polluting the environment. The eco-friendly destination of this waste is a challenge. According to this, the aim was to optimize the production on chitinases by *Aspergillus niveus* using chitin waste under submerged fermentation (FSbm). Initially, the FSbm was performed using Minimal medium (MM), initial pH 6.0, supplemented with 0.5-2% (m/v) shrimp shells (particle size >1 mm) for 96 h, at 30ºC, and 100 rpm. The influences of agitation (AGI), time of cultivation (TCU), concentration of shrimp shells (CSU), amount of spores (ASP), temperature (TEM) and pH (IPH) of cultivation were analyzed using the Fractional Factorial Design (FFD). The influences of the independent variables TEM (20-40°C), AGI (20-180 rpm), TCU (24-120 h) and ASP (10^4 - 10^8 spores/mL of medium) were significant (R^2=0.93), and they were selected to be analyzed using a Central Composite Rotatable Design (CCRD) to determine the best values of each variable for chitinase production. After the vacuum filtration, the cell-free filtrate was used as the source of extracellular enzyme. Chitinase activity was determined using 1 mmol L^{-1} of 4-nitrophenyl-β-D-N,N',N"-triacetylchitotriose in 100 mmol L^{-1} sodium acetate buffer, pH 5.0. The concentration of 0.5% (m/v) of shrimp shells in MM was the best for chitinase production (0.14 U ml^{-1}). The variables TCU, TEM, AGI and ASP in the linear and quadratic model, as well as the interaction between TEM and AGI influenced the chitinase production (R^2=0.85). The critical points of the variables were 35.5°C, 180 rpm, 109 h and pH 6.5. The predicted and experimental values of enzyme activity were 0.84 U mL^{-1} and 1.4 U mL^{-1}, 10-fold higher than the values obtained initially. Thus, it was possible to increase chitinase production using statistical tools enabling the use of this enzyme in future biotechnological applications and to demonstrate that *A. niveus* is a interesting chitinase producer using chitin waste, a low-cost substrate, minimizing its environmental impact.

KEYWORDS: chitinase, enzyme, shrimp shell, filamentous fungi, chitin waste

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