Title: OPTIMIZATION OF CELULASE AND XYLANASE PRODUCTION BY *Trichoderma* sp.

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Abstract:

The high cost of producing lignocellulolytic enzymes such as cellulase and xylanase, is considered one of the main obstacles to their implementation in industrial processes. Research focused on enzyme production by microorganisms have focused their efforts on the use of agro-industrial by-products as a substrate for high-value and low cost products manufacture. Thus, the aim of this study was to optimize the production of cellulase and xylanase by *Trichoderma* sp. and perform partial characterization of these enzymes. Experiments were conducted in duplicate, solid state fermentation with *Trichoderma* sp. at a concentration of 10^6 spores/mL, at 90 grams of wheat bran, 50 % moistened with Mandels and Weber medium. Flasks were incubated at 37 °C for 7 days. Every 24 hours, two grams of crude enzyme were harvested by addition of 10 mL citrate buffer (50 mM, pH 4.8). After one hour incubation at room temperature (25 °C) the material was filtered using Whatman nº 1 filter paper and centrifuged at 10.000 rpm for 5 minutes at 4 °C. The cellulolytic and xylanolytic activity was measured by 3,5-dinitro salicylic acid (DNS) method (GHOSE , 1987). To optimize the production of cellulase and xylanase, we have used the Response Surface Methodology, by analyzing three variables, such as: inoculum concentration (10^5 to 10^7), moisture content (40% to 60 %) and incubation temperature (32ºC to 42ºC). The flasks were removed after 120 h and the crude filtrate extract was analyzed for cellulase and xylanase activity. To analyze the profile of cellulase and xylanase produced by *Trichoderma* sp., electrophoresis was performed in polyacrylamide gel (SDS-PAGE) combined with zymogram assay. The microorganism evaluated in this study exhibited higher production of CMCase, β-glucosidase and xylanase after 120 h (0.91 U/g), 144 h (94.11 U/g) and 168 h (366.64 U/g) of cultivation, respectively. After the optimization analyses of enzyme production by Response Surface Methodology, the highest enzymatic production founded were 14.36 U/g of CMCase, 69.74 U/g of β -glucosidase and 411.03 U/g of xylanases. SDS-PAGE and zymogram demonstrated the production of different proteins and isoforms of cellulase and xylanase, respectively. The response surface methodology, together with the use of lignocellulosic substrate, demonstrated optimal strategies to promote an increased production of these enzymes.