TITLE: Production and optimization of pectinolytic enzymes of *Paecilomyces formosus* when grown in coffee processing residues

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ABSTRACT:

Coffee is the second largest traded commodity in the world and it is one the largest plantation crops of Brazil. A total of the 51, 37 millions of bags were produced and benefited throughout Brazilian territory. Coffee husks and coffee pulp are agro-industrial wastes produced after the processing of coffee by dry and wet methods, respectively. These residues contain great amounts of nutrients which makes them an ideal substrate for the biotechnological processes, including the production of lignocellulose-degrading enzymes. This study aimed to produce pectinolytic enzymes from the filamentous fungus *Paecilomyces formosus* when grown in liqueurs obtained by *Liquid Hot Water* pretreatment of coffee husks. For these experiments, factorial design and surface response were performed. The parameters for enzyme production and optimization its culture conditions involved physical, chemical and biochemical factors such as biomass concentration, time and temperature of substrate pretreatment, incubation temperature, addition of nitrogen, pH of the liquor and shaking incubation. The experimental matrix consisted of 29 different experiments, varying the level of selected parameters. The central composite had neutral conditions for pretreatment (14 minutes, 3% biomass concentration and 185°C) and incubation (28°C, 120 rpm, 0.5 g/L of nitrogen, pH 7) for 10 days. The enzyme activities were analyzed using the 3, 5-dinitrosalicylic acid (DNS) method, by measuring the amount of total reducing sugars liberated. The results demonstrated that higher pectinase activity (0.65 ± 0.04 IU/ml) was reached after 4 days of fungus cultivation, while the highest xilanase activity (0.80 ± 0.04 IU/ml) remained stable after 8 days of fungus cultivation. Mannanase and carboxymethyl cellulase activities were 0.16 ± 0.02 IU/ml and 0.10 ± 0.02 IU/ml after 8 and 10 days of fungus cultivation, respectively. The results above suggest that the pretreatment was responsible for a better enzyme access to substrate structure, decreasing the steric hindrance.

Keywords: Coffe husks, pectinases, xilanases, *Liquid Hot Water*, *Paecilomyces formosus.*

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