TITLE: Use of the coffee pulp for the production of β-glucosidase by *Bacillus subtilis* in submerged fermentation

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ABSTRACT

Coffee is among the most preferred non-alcoholic drinks, and its consumption is distributed globally. During the coffee fruiting process, however, a large amount of waste is generated in the form of pulp, mucilage, husks, and water waste. Countries whose economy is based on agricultural activities generate a great deal of liquid and solid waste. Thus, it is important to develop new alternatives for using this waste rather than disposing it in the environment. The waste could be fermented to obtain organic acids, microbial biomass, and enzymes such as cellulases. The pulp and mucilage have the chemical composition to support the growth of microorganisms and the production of value-added product. Thus, this work focused on optimizing β-glucosidase production with *Bacillus subtilis* CCMA 0087 using pulp as the carbon source and enzyme inductor. The response surface methodology (RSM) based on a central composite rotatable design (CCRD) was employed for this optimization. The methodology used in the optimization process was validated by testing the best conditions obtained and comparing them with the values predicted by the model. Incubation temperature, coffee pulp concentration and pH were the three independent variables evaluated in relation to b-glucosidase production. The results demonstrated that there were significant relationships between pH and temperature and coffee pulp concentration for bglucosidase production. The obtained results enabled us to determine regression coefficients for the b-glucosidase response. Although fermentation occurred over 5 days of culture, b-glucosidase production was only significant experimentally in the first 24 h. The model parameters were significant ($P \leq 0\ 05$) at a 95% signifi- cance level, with the exception of the linear pH. The highest β-glucosidase production (22.59 UI ml^{-1}) was reached in 24 h of culturing at coffee pulp concentration of 36.8 g l^{-1}, temperature of 36.6ºC, and pH of 3.64.

Keywords: Cellulase; Enzyme; Sub-product coffee processing; *Bacillus*; Fermentation biotechnology.

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