

TITLE: EFFECT OF ABIOTIC FACTORS IN THE PRODUCTION OF FPASES BY *Bacillus* sp. IN SUBMERSAL CULTURE USING TOBACCO POWDER RESIDUE

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ABSTRACT:

The tobacco powder is a by-product of tobacco processing and symbolizes a concern at the level of final disposal. Their conversion into bioproducts represent an alternative from the economic and environmental points of view. The productive yield of enzymes depends on evaluations of the interrelationship of a variety of abiotic factors that contribute to the optimization of cellulase production. Thus, it is justified to evaluate the best abiotic factors for conduction of the submerged culture using an isolate of the genus *Bacillus* sp. through the Plackett-Burman experimental design (PB), to the enzyme production FPase. The PB design evaluated $N + 1$ experiments, and 3 central points, totaling 15 trials considering variables described as significant. The nutritional medium was obtained by infusing the tobacco powder residue in different concentrations. The inoculum was prepared in sterile BHI broth, maintained at 35°C, overnight under agitation (160 rpm), with initial concentration determined by optical density (0.9 to 1). The planning matrix and the evaluation of the responses were obtained through Chemoface software v1.61. The factors were evaluated in the high (+) and low (-) levels and included: temperature (65 and 45 ° C); agitation (200 and 120 rpm); pH (5 and 9); residue (45 and 15%); inoculum (10 and 2%); NaCl (5 and 0%) and Tween80 (5 and 0%). Results in the production of FPase, in 48 hours of fermentation showed a wide variation of enzyme production, 3.4914 to 1.89 IU ml⁻¹, emphasizing the importance of variation of factors studied. All factors were considered significant ($p \leq 0.05$), and pH and NaCl concentration showed a negative effect on FPase production. Industrially the wide pH range found for production of cellulases may be an important feature due to the stability conferred to the enzyme. The next step of this research is to define the optimum levels for cellulolytic enzymatic production and their interactions through Central Compound (CC) planning using the variables considered significant in the PB planning (Temperature, agitation, residue concentration, inoculum and Tween). The NaCl concentration and pH will be maintained at the low level (-1) due to its negative effect on FPase production.

Keywords: *Bacillus*, Experimental design, FPase.