

TITLE: PRODUCTION AND EFFECT OF pH ON RESIDUAL ACTIVITY OF FPAse FROM *Penicillium simplicissimum*.

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Cellulolytic enzymes are capable of catalyzing the degradation of cellulosic materials to glucose. The cellulolytic complex is composed of several enzymes and the activities can be quantified by different substrates as filter paper being denominated as FPAse (Filter Paper Activity). The cellulolytic complex is produced by various microorganisms, including filamentous fungi, such as *Trichoderma*, *Aspergillus* and *Penicillium*. The aim of this study was to determine the period of better FPAse production by *Penicillium simplicissimum* and the stability of this enzyme against pH. *P. simplicissimum* was cultivated in medium containing cellulose (7.5g/L); yeast extract (0.3 g/L); (NH₄)₂SO₄ (1.4g/L); KH₂PO₄ (2.0g/L); CaCl₂.2H₂O (0.3g/L); MgSO₄.7H₂O (0.3g/L); FeSO₄.7H₂O (5.0mg/L); CoCl₂.6H₂O (20mg/L); MnSO₄.H₂O (1.6mg/L); ZnSO₄.7H₂O (1.4mg/L); Peptone (0.8g/L); Urea (3.0g/L) for a period of 15 days, at 30 °C and 150 rpm, and the fermented filtered medium was analyzed for FPAse production. The influence of pH (3.0 to 8.0) was evaluated in the culture filtrate performed on the day of higher enzyme production, incubating during the period of 0 to 5 hours. After incubation with different pH, the filtrate was again incubated under standard assay conditions for the evaluation of residual activity. The reaction was performed by adding 1 mL of sodium citrate buffer (pH 5, 50 mmol/L) and using as substrate a strip of Whatman # 1 filter paper (1cm x 6 cm), for 60 minutes. The glucose released by the enzyme action was quantified by dinitrosalicylic acid assay. The highest yield of FPAse was obtained on the fourth day of culture, (69.0 µmol/min/mg protein). The pH stability evaluation showed the highest residual activity at pH 5.0 after 1 hour of incubation (73.3%) and the lowest activity was at pH 8 (22.3%). In pH 3, after 2 hours of incubation, the lowest values of residual activity were achieved, (3.9%) and 0.0% on next hours. The highest FPAse residual activity after 5 hours of incubation was at pH 6 (18.8%), followed by pHs 7 and 8 (13.7% and 14.4%, respectively), which presented a stability profile similar. We can conclude that pH 3 negatively influenced the enzymatic stability after 2 hours of incubation, with a drop of 85.7% and no activity after 4 hours of incubation. The pH 5 showed the highest stability after one hour of incubation, maintaining 73% of residual activity and after 5 hours of incubation, the highest residual activity was observed for pH 6 (18.8%).

Keywords: *Penicillium simplicissimum*, cellulolytic enzyme, FPAse, pH stability

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