

ABSTRACT

The present study is concerned with the production of alkaline proteases by *Bacillus subtilis* PTCC-19 provided by (PTCC) FBRC laboratory of the PCSIR Lahore by submerged fermentation technique. Shake flask cultivation conditions were optimized for alkaline protease production by *Bacillus subtilis* PTCC-19. A fermentation media (Poultry feed waste 4%, Peptone 0.8%, K₂HPO₄ 0.2%, NaCl 0.02%, CaCl₂ 0.02%, and MgSO₄ 0.02%) that supported enzyme generation was used. It was observed that the temperature had the great effect on alkaline protease production, at 37°C and decreased significantly when temperature was above 37°C. The influence of inoculum size and age was so remarkable that at optimum, a crude filtrate with an enzyme activity (234.85±2.86) was yielded in the medium pH (10) inoculated with 10% (V/V) inoculum of culture. The effect of different level of salts (NaCl, CaCl₂, MgSO₄) on production and activity of alkaline protease was studied in growth medium. When the enzyme was incubated with different buffers for 24h, the protease was found to be stable over a broad pH range from 6 to 10, which retained more than 90% of its activity even after 24h, at pH 10 however more than 80% activity was lost below pH 8 or above 12. By analyzing the thermal stability, the protease was found to be stable up to 55°C for 1h incubation, but lost approximately 45% of its activity at 60°C even after 30 min incubation. Effect of metal ion on enzyme activity was also determined. The metal ions, Ca²⁺, Mg²⁺, Fe²⁺, activated the protease however, Cu²⁺ had a slight inhibitory effect on enzyme activity.