

PRODUCTION, PURIFICATION AND CHARACTERIZATION OF PROTEASE ENZYME FROM *BACILLUS SUBTILIS*

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ABSTRACT

Production and partial purification of protease enzyme by *Bacillus subtilis* was the aim of this study. *Bacillus subtilis* was allowed to grow in broth culture for purpose of inducing protease enzyme. Optimal conditions for protease production by *Bacillus subtilis* were; an optimum substrate concentrations 0.5 %; optimum incubation period, 30 h.; optimum incubation temperature was 40 °C; the optimum pH was 7.0; the best buffer for production of protease enzyme was phosphate buffer. An optimum inoculum size was 1 ml⁻¹ from stock suspension of *Bacillus subtilis* (7×10^3 / ml⁻¹); an optimum inoculum age 24 h. 250 ml⁻¹ was the optimum fermentor (flask) capacity (aeration); the best-extracted volume 150 ml⁻¹. The best broth ingredient was beef extract and NaCl; An optimum carbon sources was lactose; an optimum nitrogen source for protease production was (NH₄)₂ SO₄; Valine was the best amino acids to production of protease enzyme; the utilized organic acids, acetic, citric, lactic acid decreased protease production at different concentrations. The protease enzyme was purified by ammonium sulfate precipitation and sephadex G 200 filtration. A trial for the purification of protease resulted in an enzyme with specific activity of 6381.75 (units/mg prot/ml⁻¹) with purification folds 7.87 times. The protease activity increased as the increase in enzyme concentration; optimum substrate concentration (gelatin) was 0.5% (w/v); an optimum incubation temperature was 35 °C. Purified protease enzyme had a maximum activity at pH 7.0 of phosphate buffer, and the optimum incubation time was 24 h. Data emphasized the possibility of the production and purification microbial protease enzyme for application under industrial scale.