Purification Of Xylanase From *Bacillus Pumilus* And It's Characterization

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ABSTRACT

This study is aimed at purification and characterization of xylanase produced by thermophilic and alkalophilic *Bacillus pumilus* isolated from corncob decaying soil. . Xylanase with thermostable and alkalo tolerant properties are needed for the application in paper pulp industry as the bio-bleaching process. Culture supernatant (120.6Umg^{1}) of *B. pumilus* the xylanase was purified by ammonium sulphate precipitation and Sephadex G 75 gel filtration. With different concentrations of $(NH_4)_2SO_4$, maximum amount of xylanase was precipitated at 50% of $(NH_4)_2SO_4$ saturation. This $(NH_4)_2SO_4$ precipitated sample was dialysed against distilled water for 24h and the sample (824.72 Umg^{-1}) was loaded to Sephadex G 75 column and eluted with 0.5M Tris buffer at the flow rate of 0.5mL/min.

Eluted fractions which showed highest xylanase activity were pooled together (2250.13 Umg^{-1}), separated by Sodium Dodecyl Sulphate polyacrylamide (SDS) gel electrophoresis. Purified xylanase showed 2250.13 Umg^{-1} specific activity and the purification fold was18.6. The specific activity of the initial crude xylanase was 120.62 Umg^{-1} with a recovery yield of 34 %. The enzyme appeared as a single band on SDS-PAGE gel with the molecular mass of approximately 25kDa. Accurate molecular mass was determined as 25.42kDa by electrospray mass spectrometry (ES-MS). Purified xylanase showed zero order kinetics for 4 min and gave highest xylanase activity [193.7 (±0.26) UmL^{-1}] at 60^{o}C and pH 8.4. Purified enzyme showed high specific activity against xylan and showed no activity with carboxymethyl cellulose, starch and Avicel. Two step purification method was able to provide this purified xylanase with no amylase and cellulase activities. Due to the purity and activity at alkaline pH and at 60^{o}C this enzyme can be used for biobleaching of paper pulp.

Key words: Gel filtration, precipitation, purification, xylanase and xylan