

Purification of Alkaline Xylanase Produced by Thermophilic *Bacillus Pumilus*

Kapilan. R¹ and Arasaratnam. V²

¹Department of Botany, University of Jaffna, Sri Lanka

²Department of Biochemistry, University of Jaffna, Sri Lanka

Xylanases are very active under alkaline and thermostable conditions and are widely used in industries to bleach craft pulp and to increase the brightness in paper industry, to improve the digestibility of animal feed and to clarify the juices in food industry. The objective of the study is to purify the xylanase produced by *Bacillus pumilus* by ammonium sulphate precipitation and ion exchange chromatography using DEAE –Sephacel and to determine the kinetic properties and stability of the purified xylanase. *Bacillus pumilus* which can grow and produce xylanase above 40°C and pH 9.0 was selected for this study. The spent medium contained 27.91U/mL xylanase activity and 1.49mg/mL protein. Highest specific activity (33.65U/mg protein) was precipitated with 50% (NH₄)₂SO₄ saturation. The recovery of xylanase by (NH₄)₂SO₄ precipitation was 94.79% showing specific activity of 33.16U/mg protein. The dialyzed enzyme was purified with DEAE-Sephacel and eluted with 0.8M NaCl. The specific activity of xylanase was increased from 33.16 to 222.6U/mg protein, which was 6.7 fold higher than that of the crude xylanase with 84.16% yield. When the purified xylanase was subjected to polyacrylamide gel electrophoresis, the sample gave a single band. This single band indicated that there were no contaminants in the purified sample and further purification steps were not needed. The molecular weight of the purified xylanase is 55.38KDa.

Key words: Alkaline xylanase, *Bacillus pumilus*, DEAE-Sephacel, Purification.