Abstract

β-xylanase (Tnap_0700) gene was cloned from a hyperthermophilic *Thermotoga naphthophila* and expressed in *Escherichia coli*BL21 (DE3) using pET-21a (+) as an expression vector. Different parameters were optimized for maximum β-xylanase expression for example time of induction, IPTG concentration, temperature and pH. The pH and temperature optima for the maximum β-xylanase expression were 7.0 and 37°C, respectively. Recombinant enzyme was purified by immobilized metal ion affinity chromatography. Molecular weight of the purified β-xylanase was 38 kDa calculated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The enzyme was stable at room temperature for 30 days. It was stable at a different range of pH 4.0–9.0 and temperature 50–90°C and after the addition of 1 mM Ca^{+2} and decrease in the presence of EDTA and Cu^{+2}. Organic solvents have no remarkable effect on enzyme after addition of 10-30 %. However, urea and SDS acting as an inhibitor, decreasing the enzyme activity. The β-xylanase enzyme was active to hydrolyze xylan from beechwood forming xylose. Thermostable β-xylanase hydrolyzed complex carbohydrates into monosaccharide components. Thermostable β-xylanase presented considerable properties, which make it an inspiring candidate for different industrial applications specially in the conversion of alternative renewable biomasses into biofuels and ethanol production from lignocelluloses. β-xylanase immobilization on magnetic nanoparticles of iron oxide(Fe_2O_3) decrease the cost for enzyme production. The reusability of immobilized magnetic nanoparticles was observed, and it was used for up to six times in the saccharification process. The binding of β-xylanase on magnetic nanoparticles was performed by using EDC (1-(3-dimethylaminopropyl)-3ethylcarbodiimide hydrochloride) and evaluated by Fourier transform infrared spectroscopy and transmission electron microscopy analysis.