Recovery of cyclodextrin glucanotransferase (CGTase) using immobilized metal chelating affinity chromatography

Abstract

Immobilized metal affinity chromatography (IMAC) was chosen as a method of purification for the recovery of CGTase from E. coli homogenate. E. coli harbouring the Bacillus sp. G1 gene expressed extracellularly secreted CGTase into ampicillin supplied LB broth. Culture was pre-purified using SnakeSkin dialysis tubing (3.5 MWCO) with an enzyme activity of 147.80 U/mL. Three strategies (A, B and C) were employed for the purification of CGTase using column adsorption chromatography with Ni2+-Sepharose resin. Strategy A employed an elution buffer of 50 mM EDTA, pH 7, Strategy B used 0.1 M imidazole, pH 7 and Strategy C employed 45 mM imidazole pH 7 as the elution buffer. Strategy C was found to be most suitable yielding a total CGTase recovery of 87.04% from an initial activity of 147.80 U/mL.

Keyword: Affinity chromatography; Binding capacity; CGTase; New chromatographic adsorbent; Nickel-Sepharose chelating