

## PREPARATION AND CHARACTERIZATION OF DEAE-CELLULOSE IMMOBILIZED AMYLOGUCOSIDASE

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In the industrial production of dextrose syrups, immobilized amyloglucosidase offers an advantage over the soluble enzyme as immobilized enzyme can be recovered for reuse. Since physical immobilization is the simplest and most preferred method, amyloglucosidase was physically immobilized on DEAE-cellulose.

In a comparative study of equal amounts (0.172 mg protein) of soluble amyloglucosidase and DEAE-cellulose immobilized amyloglucosidase, activities of soluble and immobilized enzyme on the hydrolysis of soluble starch at pH 4.5 and at 57°C were 1.76 and 1 units (1 unit produces 1 mg glucose min<sup>-1</sup> respectively under zero order kinetics. DEAE-cellulose immobilized amyloglucosidase showed optimal activity when agitated at 120 rpm.

Conditions for physical immobilization of amyloglucosidase on DEAE-cellulose and hydrolytic actions of the immobilized enzymes were optimized. Optimum pH value for immobilization of amyloglucosidase was pH 7.0 at which 86% of added enzyme protein was immobilized. Despite the fact that ion-exchange process is instantaneous, time is required to reach an equilibrium when using a porous carrier due to diffusional problems. Thus, the equilibrium time required for physical immobilization of amyloglucosidase was 4 h. With increasing concentrations of soluble amyloglucosidase in the reaction mixture for immobilization, activity of DEAE-cellulose immobilized amyloglucosidase increased whereas its specific activity decreased. When the soluble amyloglucosidase in the immobilization medium was 62.2 U ml<sup>-1</sup> per ml carrier, the optimum activity of DEAE-cellulose immobilized amyloglucosidase was 45 U ml<sup>-1</sup> and its specific activity was 3.6 U mg<sup>-1</sup> protein. As starch (7% W/V) at 95°C is not readily soluble a dextrinized starch (20% W/V) of DE 36 was prepared by incubating starch 20% (W/V) with heat stable amylase at 95°C for 1 h. Activity of DEAE-cellulose immobilized amyloglucosidase was enhanced by 2.7 fold when dextrinized starch was used as substrate instead of normal starch. Immobilized enzyme preparation (11.3 mg protein, 1 ml) hydrolyzed dextrinized starch DE 36 (16% W/V) to 98% in 90 min which was comparable to the activity of soluble enzyme at pH 4.5 and at 57°C. DEAE-cellulose immobilized amyloglucosidase showed optimal activity at 52°C.