1.0 Abstract

Considerable savings in the cost of large scale saccharification of dextrinized starch can be achieved by repeated use of immobilized amyloglucosidase. Of the various methods used for immobilizing amyloglucosidase, physical binding on ion exchangers is the simplest and most preferred method for large scale operation. Although this method causes mild inactivation of enzyme, it permits easy reloading of carrier with the enzyme.

The conditions for physical immobilization of amyloglucosidase on DEAE - cellulose and for hydrolysis of starch by the immobilized enzyme were optimized. The iso-electric pH of amyloglucosidase (Aspergillus niger) was between 3.5 and 4.0. The optimum pH for immobilizing amyloglucosidase on DEAE - cellulose was 7.0 and at this pH, 95% of the added amyloglucosidase units (i.e., immobilization yield = 95%) and 78% of the added protein were immobilized to the carrier. When DEAE-cellulose immobilized amyloglucosidase (0.172 mg protein ml^{-1}) was incubated with 2 % (W/V) soluble starch in 0.01M acetate buffer (pH 4.5) at $57^{o}C$, the activity yield was 26.3 %. (activity yield = observed activity / expected activity X 100). When using DEAE-cellulose immobilized amyloglucosidase

for the hydrolysis process, the agitation speed of 120 rpm was employed to reduce the "Nernst film" diffusional effects.

To avoid the desorption of immobilized amyloglucosidase along with buffer or buffer - substrate, DEAE - cellulose immobilized amyloglucosidase was crosslinked with 0.2 % (W/V) glutaraldehyde solution for 2h at pH 7.0 and 30 °C. However, crosslinking with glutaraldehyde has reduced the activity of immobilized amyloglucosidase by 85 %. The time required for the equilibration of amyloglucosidase with DEAE - cellulose in 0.01M phosphate buffer (pH 7.0) was 4h. In batchwise agitation with 4h of equilibration, the amyloglucosidase immobilized per 1 ml (0.25 g dry weight) DEAE - cellulose in 0.01M phosphate buffer (pH 7.0) was 1325 AIG Units (69.3 mg protein). However, during "in - column" loading of amyloglucosidase to DEAE - cellulose bed (6.5 x 1.5 cm) the amyloglucosidase immobilized per 1 ml of DEAE - cellulose was only 17.4 AIG Units (0.9 mg protein).

Minimum effective distribution coefficient (E.C) (0.16) and high immobilization yield (86%) could be obtained when added amyloglucosidase is within the adsorption limit of 351 AIG Units ml^{-1} for the volume ratio of enzyme:carrier as 4:5 (E.C = enzyme units in the mobile phase / enzyme units in the stationary phase). Effec-

tive distribution coefficient (E.C) remained constant when 0 - 62.7 % of the available capacity of DEAE - cellulose was utilized for immobilization. Therefore to minimize the enzyme wastage and to obtain good immobilization yield (86 %), utilizing the binding capacity of the carrier below 62.7 % of the available capacity is more preferable. The activity of immobilized amyloglucosidase (1 ml) was proportional to the amount of added protein (within the range of 0 - 19 mg) or to added amyloglucosidase (within the range of 0 - [illegible] units). In the upper limit, the amount of protein and amyloglucosidase immobilized were 11.3 mg ml^{-1} carrier and 266.8 [illegible] carrier respectively and the activity and specific activity were 45 AMG Units ml^{-1} and 3.6 AMG Units mg^{-1} protein respectively when soluble starch was used as substrate. Best DEAE-cellulose immobilized amyloglucosidase preparation could be obtained by using the enzyme concentration of 249 AMG Units ml^{-1} if the volume ratio of carrier : enzyme ratio is 5:4.

An increase (2.7 fold) in the activity of immobilized enzyme was observed when soluble starch was replaced by dextrinized starch (DE 36). Dextrinized starch (DE 36, 16 % W/V) was hydrolyzed to 98% in 1h (at pH 4.5 and 57 °C) by 1 ml of immobilized amyloglucosidase (containing 11.3 mg protein). The immobilized amyloglucosidase showed highest activity at pH 4.0 in the pH

range of 4.0 - 7.0. However at pH 4.0, only 66 % of the initial activity was observed after two saccharification cycles (1h each). Although the efficiency of hydrolysis was low (< 60 %) the enzyme retained by DEAE - cellulose was high (> 97.5 %) for 3 consecutive reaction cycles (1h each) at pH 6.0. Among the pH values considered, pH 4.5 was found to be suitable for the hydrolysis (> 85 %) and the enzyme retentivity (>95 %). Immobilization of amyloglucosidase on DEAE - cellulose has lowered the temperature optimum from 60 °C to 52 °C. It was observed that immobilized amyloglucosidase can be used for efficient saccharification of dextrinized starch at 52 °C.