I. ABSTRACT

The amyloglucosidase enzyme is used for the production of glucose and high fructose syrups from starch in conjunction with alpha amylase and glucoseisomerase. Hence studies were carried out to produce amyloglucosidase by *Aspergillus niger* from locally available raw materials.

Aspergillus niger grown in submerged culture in an orbital shaker is more effective in enzyme production (7 μmoles min^{-1} ml^{-1}) than under aeration (0.18 μmoles min^{-1} ml^{-1}). The optimized medium for maximum amylogluco-sidase production (52 μmoles min^{-1} ml^{-1}) at 30° C and at pH 6.5 contained 2% (W/V) soluble manioc starch and 0.5% (W/V) ammonium sulphate, 6.25% (W/V) peptone and 2% (W/V) soya bean flour as nitrogen sources. Tween 80 0.01% (V/V) and 2% (W/V) dextran had no effect on amyloglucosidase production or release by *Aspergillus niger*.

The growth of the mycelium(monitored by NADPH and dry weight of the mycelium) and amyloglucosidase production reached a maximum at 112 h after inoculation with spores. Thereafter the cellmass and the NADPH level started to decline. The amyloglucosidase production was high at pH 6.5 while the growth of the mycelium was better at pH 4.0 than at pH 6.5.

The production of amyloglucosidase had decreased with fermentation time along with the depletion of reducing sugar level in the medium.

However neither the replacement of the spent medium with a fresh medium nor the addition of soluble starch (3.5% W/V) to the spent medium had shown any effect on amyloglucosidase production. The continuous production of amyloglucosidase depends upon the viability of the mycelium.

Hence spores were added on the fourth and eighthdays of cultivation period at pH 4.0 and 6.5 to replace the old mycelium. In both cases, addition of spores did not help to produce AMG continuously. Furthermore addition of spores (on the fourth and eighth days) and the replacement of the spent medium with the fresh medium (on the fifth day) had no improvement on the continuous AMG production.

In addition to amyloglucosidase the culture supernatant contained alpha amylase (determined by Phadebas amylase test method Pharmacia, Sweden). The rate of glucose production by the crude enzyme was linear upto 40 min at 60°C when incubated with 1% (W/V) starch in 0.02M acetate buffer pH 4.0. The optimum pH and temperature of this crude enzyme were 4.0 and 60°C respectively. Although the temperature optimum was 60°C, the enzyme lost 20% of the activity in 2h and 67% of the activity in 24h at 60°C.

The enzyme lost 20% and 15% of the activity in 24h at 50°C and 45°C respectively. At 37°C it retained 100% activity. The crude amyloglucosidase was stable for 48h in the pH range of 3.0 - 6.0 at 37°C.

The crude enzyme was purified using DEAE- cellulose and the recovery of amyloglucosidase was 58% while the specific activity increased by three fold.