1. ABSTRACT

The production of glucose oxidase (GOD) from *A.niger* (CISIR N_4) was undertaken because of its commercial importance in producing gluconic acid, in desugaring of egg products, in determining glucose and in removing oxygen from certain foods and beverages.

When submerged cultivation of *A.niger* was carried out at the initial pH of 5.6, at 30^{o}C and at an aeration rate of 100 bubbles min^{-1}, pellet form of mycelial growth and a GOD activity of 0.51 μmoles min^{-1} in the mycelia of 1 ml culture fluid were observed. When the culture was agitated at 160 rpm, the GOD activity in the mycelial extract increased to 2.3 μmoles min^{-1} and the activity reached a maximum in 48h rather than in 72h with aeration. Further the presence of GOD activity in the mycelial extract and not in the culture fluid indicates that GOD is an intracellular enzyme. Hand grinding with sand or Triton X - 100 (0.1% V/V) extracted the intracellular GOD completely from the mycelia. As pH influences the GOD production, 0.02M, 0.1M and 0.5M phosphate buffers with and without $CaCO_3$ (5.67 gl^{-1}) were tried to maintain the pH of the medium. Phosphate buffer (0.5M) alone or phosphate buffer (0.02M) with $CaCO_3$ was able to maintain the pH at about 5.6. However the maximum GOD activity of 4.51 μmoles min^{-1} in the mycelia of 1 ml culture fluid was obtained in 0.1M phosphate buffer containing $CaCO_3$. When fermentation studies were carried out with different nitrogen sources such as KNO_3 (2.5 gl^{-1}), $(NH_4)_2HPO_4$ (1.65 gl^{-1}) and peptone (2.4 gl^{-1}) containing 0.35 gl^{-1} elemental nitrogen in 0.1M phosphate buffer (pH 5.6) and $CaCO_3$ (5.67 gl^{-1}), maximum GOD activity of 9.91 μmoles min^{-1} in the mycelia of 1 ml culture fluid and 1339 μmoles min^{-1} g^{-1} mycelia were attained with peptone. A comparison of the carbon sources at 30 gl^{-1} showed that the GOD activity at 48h in the mycelia of 1 ml culture fluid was decreased from 10 to 6.3 μmoles min^{-1} and the activity per gram mycelia decreased from 1198 to 680.8 μmoles min^{-1} when glucose was substituted with sucrose. When the effect of glucose concentration (15, 30 and 50 gl^{-1}) was studied, the maximum activity of 11.2 μmoles min^{-1}

in the mycelia of 1 ml culture fluid and 5600 μmoles min^{-1} g^{-1} mycelia were observed at 24h with 15 gl^{-1} glucose. Finally when glucose (15 gl^{-1}) was supplemented with dextrinized starch (5 gl^{-1}; DE 15) GOD activity in the mycelia of 1 ml culture fluid and per gram mycelia were increased to 16.8 and 5814 μmoles min^{-1} respectively at 24h. The growth at 24h was also increased from 2.0 to 2.9 gl^{-1}. In the optimized medium the GOD activity observed in the mycelia of 1 ml culture fluid at 24h and 40h were 16.9 and 23.7 μmoles min^{-1}. The harvesting time was selected as 40h as there was a 17% increase in GOD activity in the mycelia of 1 ml culture fluid. By improving the culture conditions, the GOD activity in the mycelia of 1 ml culture fluid was increased from 0.51 to 23.7 μmoles min^{-1}.

GOD was purified 6.7 fold and specific activity increased from 195 to 1306 μmoles min^{-1} mg $protein^{-1}$ when the purification was carried out on DEAE - cellulose by ion exchange chromatography. The recovery of GOD activity was 56.7%. GOD showed zero order kinetics for 20 min at 37^{o}C and a linear increase in initial reaction rate was observed in the range of 0 - 4 μg protein. Broad temperature optimum between 35^{o} - 45^{o}C and pH optimum of 5.0 were observed when the GOD activity was measured in the coupled reaction with POD. The effect of temperature on enzyme stability showed that the enzyme lost 0.0%, 0.0% and 10% of the initial activity at 30^{o}, 37^{o}and 45^{o}C respectively when incubated at pH 5.6 for 6h while the enzyme lost 3%, 9% and 12% of the activity after 3 days. The effect of pH on enzyme stability showed that the loss of activity after 6h at pH 5.6, 5.0 and 3.5 were 0.0%, 5% and 10% of the initial activity when incubated at 30^{o}C while the loss of activity after 3 days were 5%, 7% and 86% respectively. These results show that GOD was more stable at 30^{o}C than at 37^{o} and 45^{o}C and at pH 5.6 than at pH 5.0 and 3.5.