613/E2

Optimization of enzyme production and immobilization of a thermo-stable alpha amylase from *Caldimonas manganoxidans NMS 1* isolated from a hot water spring in Sri Lanka

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Thermostable alpha amylase is used in the production of glucose syrup, desizing fabric in textile industry, fermentation industries, paper sizing, detergent and brewing industries. An □-amylase from *Caldimonas manganoxidans NMS 1,* isolated from Nelum-wewa hot water springs, situated in Sewanapitiya in the Polonnaruwa District of Sri Lanka, has been purified to homogeneity as determined by polyacrylamide gel electrophoresis. Kinetic studies of this enzyme have been reported previously. In this study we have optimized the □-amylase production from *Caldimonas manganoxidans NMS 1* and the effect of inhibitors on enzyme activity were studied along with the effect of immobilization on the enzyme. The optimum extracellular α-amylase activity, 56 U/ml at 50 ˚C over a 20 hour incubation period, was observed in a media comprising of 10 % soya powder in a basal media containing 5 g/l NaCl, 0.5 g/l KCl, 0.5 g/l $MgSO_4.7H_2O$, 0.04 g/l $MnSO_4$, 0.3 g/l $FeSO_4$, 0.87 g/l K_2HPO_4, 0.022 g/l $CaCl_2$ and 1 % soluble starch solution while ammonium sulphate (10 % and 20 %), soya powder (20 %) and urea (10 % and 20 %) showed enzyme activities of 1 U/ml, 15 U/ml, 37 U/ml, 1 U/ml and 1 U/ml respectively. Salts of calcium ions (1 mM), manganese ions (1 mM), copper ions (1 mM), and ferrous ions (1 mM) showed 31 %, 31 %, 18 % and 10 % enhancement of α-amylase activity respectively. Sodium ions (1 mM), magnesium ions (1 mM), and zinc ions (1 mM) showed a 33 %, 37 % and 37 % inhibition of α-amylase activity respectively. The surfactant sodium dodecyl sulphate (1 mM) and urea (1 mM) inhibited enzyme activity by 35 % and 10 % respectively. The heavy metal mercuric ions (1 mM) and the chelating agent ethylene diamine tetraacetic acid (1 mM) strongly inhibited α-amylase activity by 49 % and 57 % respectively. Immobilization of enzyme using low melting agarose and agarose showed a retained activity of 43 % and 48 % respectively with respect to purified, un-immobilized α-amylase at 50 ˚C. Immobilization using K-Carrageenan at 37 ˚C showed 48 % retained activity. The α-amylase isolated from *Caldimonas manganoxidans NMS 1* thus has potential applications in industry.

Keywords: *Caldimonas manganoxidans NMS 1,* extracellular α-amylase, enhancement, inhibition, immobilization

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