ABSTRACT

The parent and mutant strains of *A. niger* were grown under varied conditions for maximum production of the α-amylases and effect of pH, temperature, carbon sources and inoculum sizes was determined. The fungi gave best results with 4% (w/v) maize starch when grown at 30 °C, pH 5. The μ of parent and mutant strains was 0.165 h^{-1} and 0.139 h^{-1}, while their t_d values were 4.2 h and 4.98 h, respectively. The Q_p of mutant strain on 4% maize starch concentration was maximum (3868 IU L^{-1} h^{-1}) as compared to the other concentrations. Whereas, parent strain gave maximum Q_p on 3% maize starch (1023 IU L^{-1} h^{-1}). The Q_{ep} value of parent strain was maximum on 1% maize starch *i.e.* 4.74 mg L^{-1} h^{-1}, whereas mutant strain gave maximum value at 5% maize starch concentration *i.e.* 16.1 mg $L^{-1}h^{-1}$. On the other hand, Q_x value of parent strain was maximum on 5% maize starch (400 mg L^{-1} h^{-1}), while mutant showed highest rate of biomass formation on 4% maize starch concentration(Q_x = 1332 mg L^{-1} h^{-1}).

Specific growth rate (μ) and doubling time (t_d) for biomass production were calculated for the parent and mutant strains grown at 30 °C, pH 5.0 on different inoculum's sizes *i.e.* 0.92, 1.15, 1.28, 1.47, 1.66, 1.84 mg cells ml^{-1}. The μ of parent strain was maximum at 1.47 mg cells ml^{-1}, which was 0.247 h^{-1}, while mutant's μ was maximum at inoculum level 1.84 mg cells ml^{-1} and the value obtained was 0.04 h^{-1}. The t_d values of parent and mutant strains were 2.8 h and 17.3 h, respectively. The yield of the enzyme for both strains of *A. niger* was maximum on 1.84 mg cells ml^{-1} and the values for parent and mutant strain obtained were 15.48 IU mg^{-1} cells and 11.57 IU mg^{-1}cells, respectively. The trend of q_p for parent and mutant strains on different inoculum sizes indicated that the maximum specific product formation was on 1.84 mg cells ml^{-1} inoculum size and values of q_p for parent and mutant strains were 0.23 IU mg^{-1} cells h^{-1} and 0.46 IU mg^{-1} cells h^{-1}, respectively.

The fungal strains were grown at 30 °C on different pH *i.e.* 3, 4, 5, 6, 7 and 8. The μ of parent was maximum at pH 8 and the value obtained was 0.67 h^{-1}. The μ of mutant strains was maximum at pH 3 and the value obtained was 0.541 h^{-1}. The t_d values were 1.03 h and 1.28 h, respectively. The maximum yield of enzyme shown by mutant strain was also at pH 5, and the value calculated was 4.29 IU mg^{-1}cells. The trend of q_p for parent and mutant strain on different pH indicated that the maximum specific product

formation by mutant strain was at pH 5 with a value of 0.1716IU mg^{-1}cells h^{-1}, while the parent strain showed maximum value of yield of product formation at pH 8 *i.e.* 0.20IU mg^{-1} cells h^{-1}.

The μ for parent strain was maximum at 40 °C (0.1016 h^{-1}), while mutant gave maximum μ at 30 °C (0.04 h^{-1}). The t_d *i.e.* biomass doubling time values of parent and mutant strains were 6.82 h and 17.32 h, respectively. The yield of the enzyme by both parent and mutant strains of *A. niger* was maximum on 30 °C and the values calculated were 5.8 IU mg^{-1}cells and 4.29 IUmg^{-1}cells. The trend of q_p for parent and mutant strain on different temperatures indicated that the maximum specific product formation by mutant strain was at 30 °C with a value of 0.172 IU mg^{-1}cells h^{-1}, while the parent strain showed maximum value of yield of product formation at 27 °C *i.e.* 0.052 IU mg^{-1} cells h^{-1}.

Crude α-amylase was purified by subjecting it to ammonium sulfate precipitation, Hiload anion exchange and hydrophobic interaction chromatography on Pharmacia Fast Protein Liquid Chromatography (FPLC) unit. The purity of α-amylase was at homogeneity level and was 11.9 fold purified. Three isoforms of α-amylases were separated on HIC. The isoform-I was eluted at 1.87M $(NH_4)_2SO_4$, while isoform-II was eluted at 0.78M $(NH_4)_2SO_4$. Interestingly, isoform-III showed its onset of elution when the ammonium sulfate gradient was finished completely. The adsorption of isoform-III was so strong that the hydrophilic phosphate buffer had to apply for prolonged period. The form-I was present as traces i.e. in a minute quantity. Isoform-II was in moderate amount, while majority of α-amylases belong to isoform–III. Therefore, form-II and form–III were characterized in detail for effect of temp, pH, substrate and thermo-stability.

Temperature optimum of isoform-II was 45°C while that of isoform-III was 70°C. Isoforms-II and III showed *E*a of 32.49 kJ mol^{-1} and 5.55 kJ mol^{-1}, respectively. The isoform-II showed almost same pH optimum range (7.5-8.0) at 35°C to 40°C, while at 45°C it showed a shift in pH optimum towards acidic side (6.5-7.0). The pH optimum of isoform-III at 35°C, 37°C, 40°C and 50°C was almost same (7.4-8.3), whereas at 45°C it also showed shifting of optimum pH towards acid side like isoform-II and gave

maximum activity at pH 6.8 to 7.7. The pK_a values of active site residues controlling maximum velocity (V_{max}) were determined using Dixon plot. With increase in temperature, the pK_a values showed an increasing and decreasing trend, but cumulatively there was an increase in pK_a values.

The values of V_{max} indicated that isoform-III was 32 folds more active as compared to isoform-II. The K_m for isoform-II was smaller than that of isoform-III. It indicated that the affinity of soluble starch binding with isoform-II was higher than isoform-III. Specificity constant (V_{max}/K_m) for isoform-II and –III was 1786 and 40241, respectively.

The half lives of both isoforms showed an increasing trend from 45°C to 53°C, but above 53°C the enzymes showed a decrease in t½ values. The α-amylase isoform-III was comparatively more stable than form-II and gave half life of 156 min at 53°C as compared to 115 min of form-II. The energy of activation for irreversible thermostability "$Ea_{(d)}$" of form-II and –III was 32.88 and 22.48 KJ mol^{-1}, respectively. The ΔH*, ΔG* and ΔS* of form-II at 53°C was 30.17 kJ mol^{-1}, 105.03 kJ mol^{-1} and -229.64 J $mol^{-1}K^{-1}$, respectively. Similarly, ΔH*, ΔG* and ΔS* values for isoform-III at 53°C were 19.77 kJ mol^{-1}, 105.85 kJ mol^{-1} and -264.08 J $mol^{-1}K^{-1}$, respectively. Broadly speaking, on the basis of half life and Gibbs free energy (ΔG*), it was concluded that isoform-III was more stable as compared to the form-II.