

P021 Activation and thermo-stabilization of *Humicola* sp glucoamylase by manganese ions
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Thermo-stable enzymes can withstand extreme environmental conditions and can resist against detergents, organic solvents, temperature & pH. The operational stability of enzymes is of paramount importance for any bioprocess, which can be improved through various protein engineering techniques. Glucoamylase of *Humicola* sp was novel in a sense that it did not show any inhibition up to 50 mM $MnCl_2$ and presented activation trend. Kinetic constants for soluble starch hydrolysis were determined at various $MnCl_2$ concentrations (1-15 mM): [Control: ($V_{max} = 47.7$ U mg^{-1} Protein min^{-1} , $K_m = 0.26$ mg ml^{-1} & $V_{max}/K_m = 183$) and in the presence of 2.0 mM $MnCl_2$ ($V_{max}=137$ U mg^{-1} Protein min^{-1} , $K_m = 1.0$ mg ml^{-1} & $V_{max}/K_m = 137$)]. The kinetic mechanism for activation of glucoamylase by Mn^{2+} was evaluated as described by Dixon and Webb. The effect of various metals (Ca^{2+} , Mn^{2+} and Zn^{2+}) on irreversible thermostability of *Humicola* sp glucoamylase was also determined. All tested metal ions contributed towards stabilization of the enzyme and Mn^{2+} showed highest half life at 60°C as compared to other metals. Effect of metal ions on kinetic and thermodynamic (ΔH^* , ΔG^* , ΔS^*) parameters of thermo-stability and catalysis were also determined.