KINETIC CARACTERIZATION OF THE ALMOND β-GLUCOSIDASE

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Introduction

β-glucosidase (EC 3.2.1.21) is a large group of enzymes within glycosidases. This enzyme catalyses the hydrolysis of the O-β-glycosidic bonds of non-reducing terminal of oligosaccharides and of aril y alquil- β-D glycosides, releasing β-D glucose. β-glucosidases are present widely distributed in nature, that's why they have different biological functions depending of the organism of origin **[1]**. The classification is based on the folding process and sequence, and there are mostly present in the families: 1 (plant and mammal), 3 (bacteria) and 30 **[2].**

Materials and Methods

A commercial solution of sweet almond β-glucosidases from *Prunus dulcis,* p-nitrophenol, p-nitrophenol-β-glycoside, glucose and δ-gluconolactone were used from Fulka. Moreover, NaOH, HCl and phosphate salts were supplied by Panreac. The same schematic diagram (Fig. 1) was used to carry out the experiments.

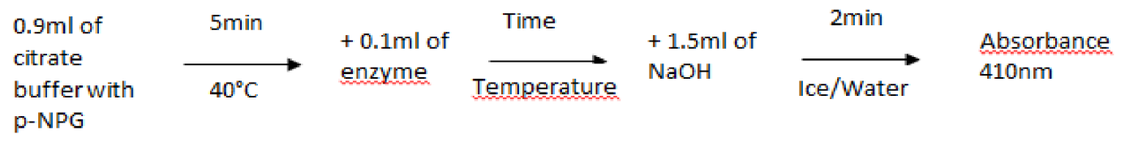


Fig. 1: Schematic diagram

Results

1. Standardization of assays conditions

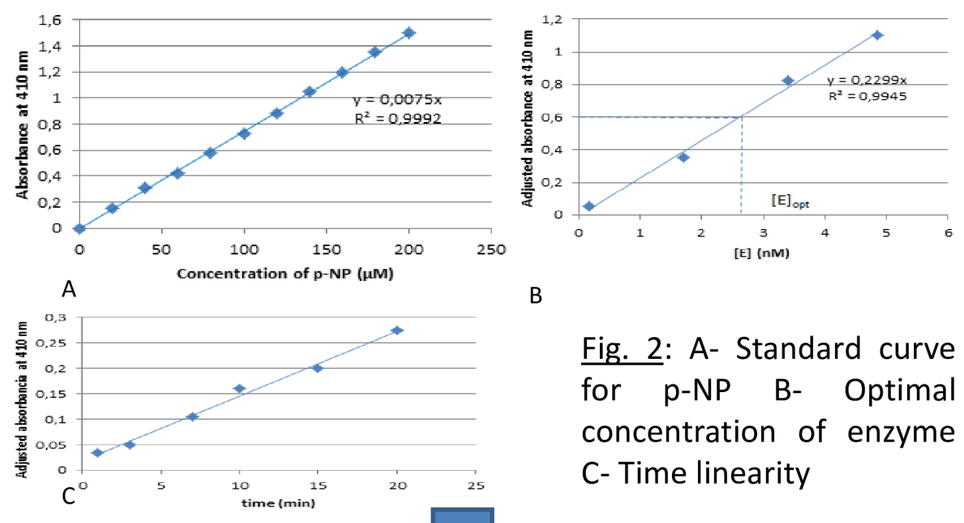
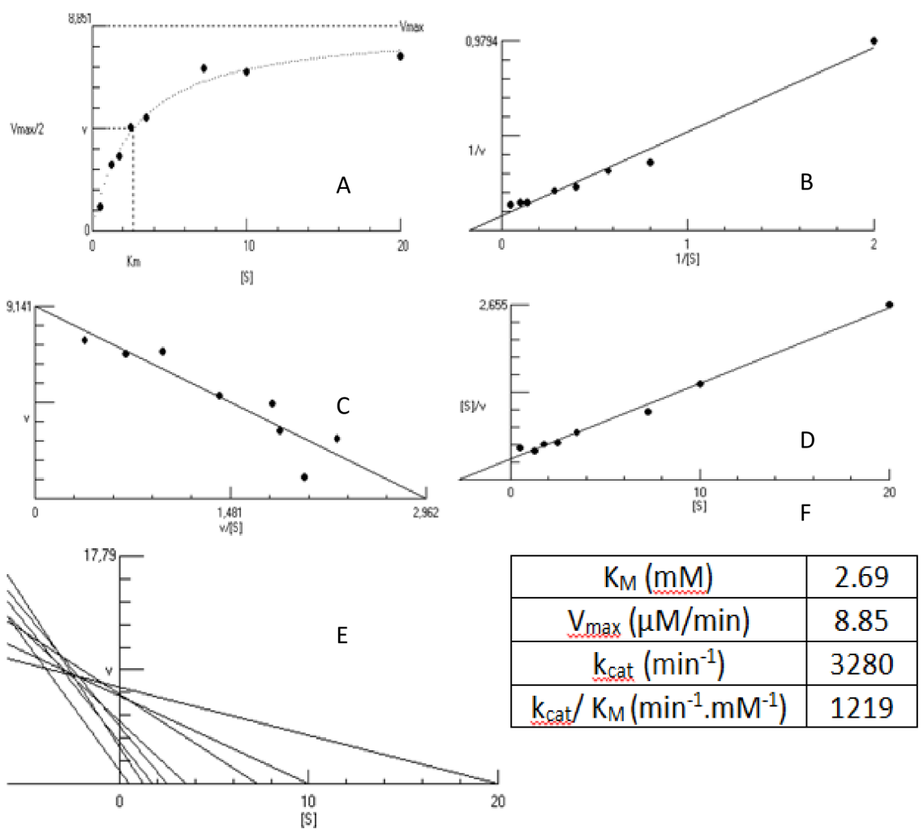


Fig. 2: A- Standard curve for p-NP B- Optimal concentration of enzyme C- Time linearity

2. Determination of kinetic parameters



K_M (mM)	2.69
V_{max} (µM/min)	8.85
k_{cat} (min^{-1})	3280
k_{cat}/K_M ($min^{-1}.mM^{-1}$)	1219

Fig. 3: A- Michaëlis-Menten plot B- Lineweaver-Burk plot C- Eadie-Hofstee plot D-Hanes-Woolf plot E-Eisenthal y Cornish-Bowden plot F- kinetics parameters

3. Temperature effect

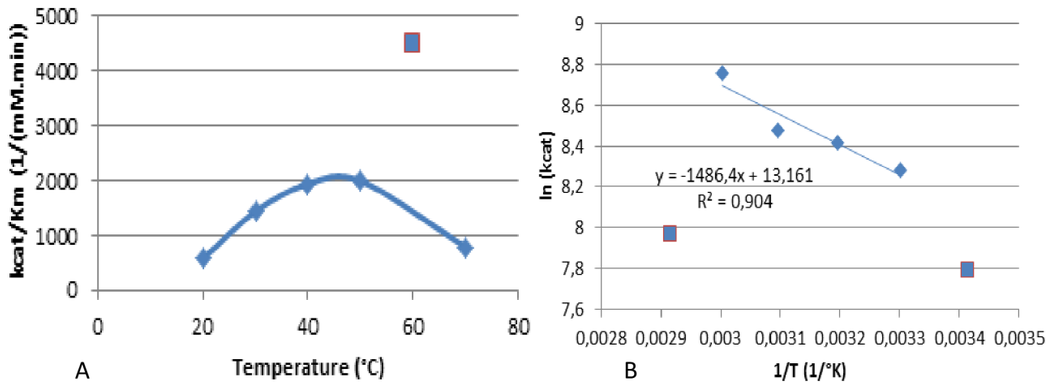


Fig. 4: A- Variation of Kcat/Km with temperature B- Linearization with Arrhenius model

4. Inhibition studies

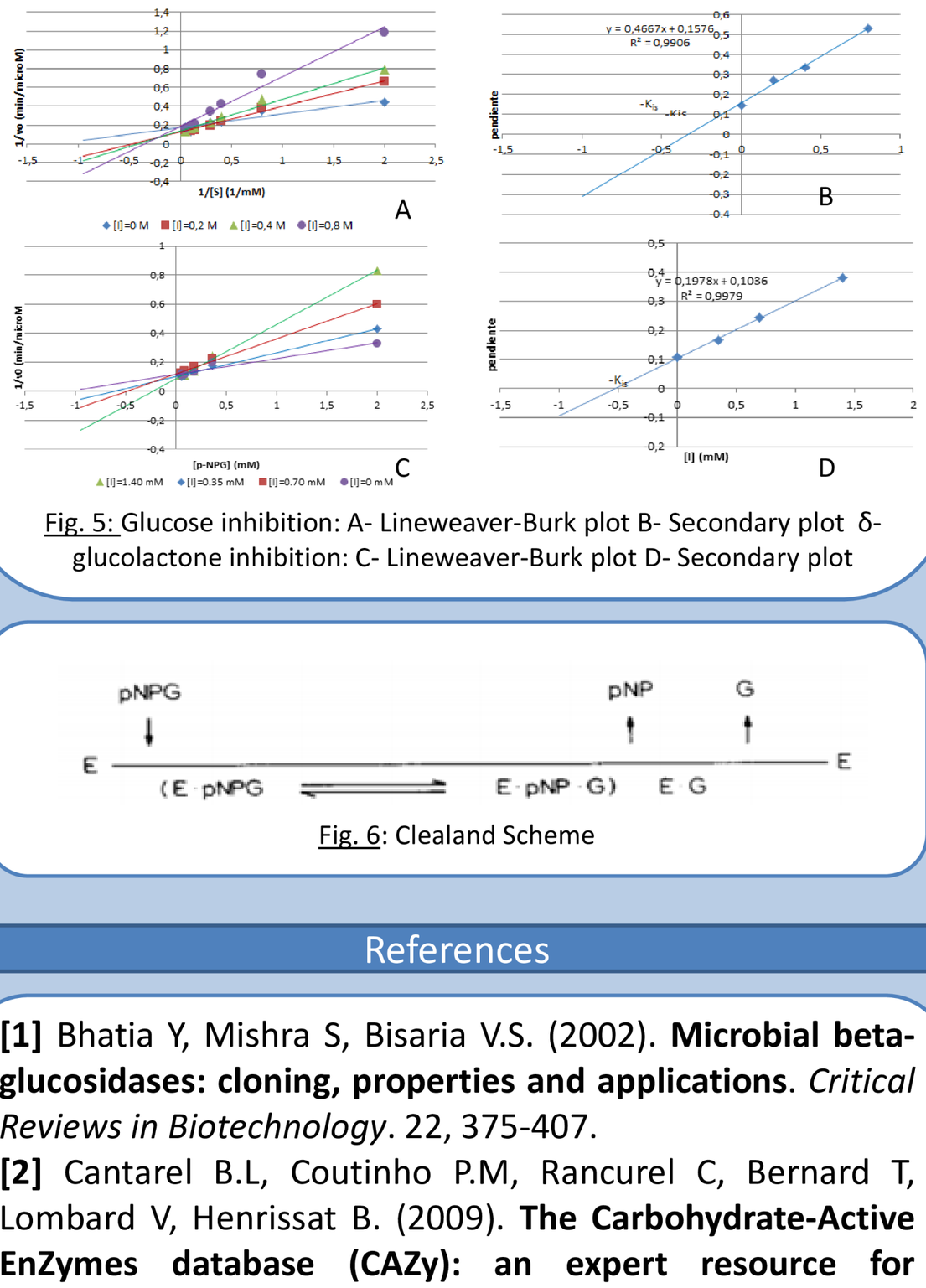


Fig. 5: Glucose inhibition: A- Lineweaver-Burk plot B- Secondary plot δ-glucolactone inhibition: C- Lineweaver-Burk plot D- Secondary plot

Fig. 6: Clealand Scheme

Conclusion

The almond β-glucosidase presents the following kinetic parameters for the transformation of p-NPG on p-NP and glucose: $K_M = 2.69\ mM$, $v_{max} = 8.85\ \mu M.min^{-1}$ and $k_{cat} = 3280\ min^{-1}$. Furthermore, the temperature range for an optimal activity it is besides 40 and 50 °C. Finally, the studies of inhibition show that the δ-gluconolactone is an inibitor more efficient than the glucose. It also allowed to determine the mechanism kinetics Uni Bi for the enzyme.

References

[1] Bhatia Y, Mishra S, Bisaria V.S. (2002). **Microbial beta-glucosidases: cloning, properties and applications**. *Critical Reviews in Biotechnology*. 22, 375-407.

[2] Cantarel B.L, Coutinho P.M, Rancurel C, Bernard T, Lombard V, Henrissat B. (2009). **The Carbohydrate-Active EnZymes database (CAZy): an expert resource for Glycogenomics**. *Nucleic Acids Research*. 37, 233-238.