

T.P. n°3 : Study of the enzymatic kinetics of invertase (Km and Vmax)**Principle :**

The initial rate of the reaction catalyzed by invertase is determined in the presence of enzyme and substrate at constant concentrations, at pH 4.7 and at a temperature of 37-40°C. The contact time between the enzyme and the substrate is variable.

The kinetic parameters of invertase (Km and Vmax) are measured by varying the substrate concentration.

Equipment :

- ✓ Test tubes ;
- ✓ Racks ;
- ✓ Pipettes and micropipettes ;
- ✓ Beakers ;
- ✓ Precision balance ;
- ✓ Watch glass and spatula ;
- ✓ Oven set to 37°C ;
- ✓ Boiling water bath ;
- ✓ Vortex agitator ;
- ✓ Magnetic agitator and stir bar ;
- ✓ Spectrophotometer and cuvettes.

Reagents :

- ✓ Diluted enzyme extract (1/50) ;
- ✓ 0.05M acetate buffer at pH 4.7 ;
- ✓ 0.1 M sucrose solution ;
- ✓ DNS reagent ;
- ✓ Distilled water.

Procedure :

- ✓ Prepare the tubes according to the table below :

Tube number	0	1	2	3	4
0.1M sucrose solution (ml)	0	0,1	0,2	0,5	1
Distilled water (ml)	2	1,8	1,7	1,4	0,9
Acetate buffer pH 4.7 (ml)	1				

Preincubation	10 min at 37°C	
Diluted enzyme extract (1/50) (ml)	0	0,1
Contact time	Agitate and incubate at 37°C for : 1, 2, 4 et 7 min	
DNS reagent (ml)	2	
Incubation	✓ Homogenize, seal the tubes with aluminum foil, and place in a boiling water bath for 5 minutes. ✓ Allow to cool, then add :	
Distilled water (ml)	6	

N.B. : For each volume of sucrose, prepare 5 tubes (one blank tube and 4 tubes in which the reaction is stopped after 1, 2, 4, and 7 minutes, respectively).

- ✓ Homogenize and let stand for 10 min at ambient temperature.
- ✓ Read the absorbances (OD) at 540 nm against the blank (tube 0).

Work to be performed :

- ✓ For each sucrose concentration, calculate the initial rate (V_i) from the curve [inverted sugar] = $f(t)$ and using the calibration curve (from the previous practical). The unit of V_i is $\mu\text{mol/L/min}$.
- ✓ Calculate the $[S]_0$ values for each experiment.
- ✓ Trace the curves $V_i = f([S]_0)$ and $1/V_i = f(1/[S]_0)$.
- ✓ Determine K_m and V_{max} .