T.P. n° 3 : Determination of the initial rate of an enzymatic reaction

Principle :

To measure the initial speed, the experiment is carried out in the presence of enzyme and substrate at constant concentration, in a buffered medium with a pH of 4.7 and thermostated at 37°C. The contact time between the substrate and enzyme is varied and the concentration of the product formed (inverted sugars) is measured.

Equipment :

- ✓ Test tubes and rack ;
- ✓ Pipettes and micropipettes ;
- ✓ Pipettors ;
- ✓ Beakers ;
- ✓ Balance ;
- ✓ Watch glass and spatula ;
- ✓ Agitator and magnetic stir bar ;
- ✓ Oven set to 37°C ;
- ✓ Boiling water bath ;
- ✓ Vortex agitator ;
- ✓ Spectrophotometer and cuvettes.

Reagents :

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

Procedure :

- ✓ Prepare the tubes according to the table below. :

Tube number	**0**	**1**	**2**	**3**	**4**	**5**
0.1M sucrose solution (ml)	1					
Distilled water (ml)	1					
Acetate buffer pH 4.7 (ml)	1					
Preincubation (min)	5 min at 37 °C					
Diluted enzyme extract (1/50) (ml)	0	0,1				

Contact time (min)	**0**	**1**	**2**	**4**	**6**	**10**
DNS reagent (ml)	2					
Incubation	Homogenize, seal the tubes with aluminum foil, and heat in a boiling water bath for **5 minutes**. Allow to cool, then add :					
Distilled water (ml)	6					

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

Work to be performed :

- ✓ Trace the curve DO = f(t)
- ✓ Trace the curve [invert sugar] = f(t)
- ✓ Calculate the initial reaction speed (Vi) in µmol of hydrolyzed sucrose.L^{-1}.min^{-1} (L = liter of reaction medium)).