

T.P. n°4 : Study of pH effect on invertase

Principle :

The initial reaction rate is measured in the presence of enzyme and substrate at constant concentration, in a thermostat-controlled environment at 37°C. The pH of the reaction medium is varied.

Equipment :

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

Reagents :

- ✓ Diluted enzyme extract (1/50) ;
- ✓ Acetate buffer at different pH levels ;
- ✓ 0.1 M sucrose solution ;
- ✓ DNS reagent ;
- ✓ Distilled water.

Procedure :

In fact, this practical requires the preparation of buffer solutions with pH values ranging from 2.5 to 7.5. The procedure consists of preparing five (5) tubes for each pH value : one blank tube (B) and four test tube (E), as shown in the table below :

buffer pH	2,5		4		4,7		6		7,5	
	B	E	B	E	B	E	B	E	B	E
Distilled water (ml)	1	0,9	1	0,9	1	0,9	1	0,9	1	0,9
Sucrose 0.1 M (ml)	1									
Buffer (ml)	1									
Preincubation	Preincubate the tubes for 5 minutes at 37°C.									

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

N. B. : For each pH value, prepare a series of 5 tubes (one blank tube B and 4 tubes E 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 B).

Work to do :

- ✓ Using the calibration curve performed previously, calculate the rate values (V_i) for each experiment (in $\mu\text{M/L/min}$).
- ✓ Trace the curve $V_i = f(\text{pH})$.
- ✓ Interpret the result obtained.