T.P. n°5 : Study of the effect of temperature on Invertase

Principle :

The initial reaction rate is measured in the presence of enzyme and substrate at constant concentration, in a buffered medium with a pH of 4.7. The temperature of the reaction medium is varied.

Equipment :

- ✓ Boiling water bath (95°C) ;
- ✓ Water bath set to 60°C ;
- ✓ spectrophotometer + cuvettes ;
- ✓ Oven set to 40°C ;
- ✓ Test tubes ;
- ✓ Beakers ;
- ✓ Pipette and micropipette ;
- ✓ Hot plates ;
- ✓ Vortex agitator ;
- ✓ Magnetic agitator and stir bars ;
- ✓ Thermometer.

Reagents :

- ✓ Diluted enzyme extract (1/50) ;
- ✓ Acetate buffer at pH 4.7 ;
- ✓ 0.1 M sucrose solution ;
- ✓ DNA reagent ;
- ✓ Distilled water ;
- ✓ Ice cubes.

Procedure :

Prepare five test tubes for each temperature condition as follows :

Temperature condition	**1**		**2**		**3**		**4**		**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									
Incubation temperature (10 min)	**0° C**		**Température de laboratoire**		**40° C**		**60° C**		**90° C**	
Enzyme extract	0	0,1	0	0,1	0	0,1	0	0,1	0	0,1

Contact time	Shake and incubate for : **1, 3, 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. Leave to cool.
Distilled water (ml)	6

Note : For each temperature 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 room temperature.
- ✓ Read the absorbances (OD) at 540 nm against the blank (tube B).

Work to be done :

- ✓ Using the calibration curve created earlier, calculate the rate values (Vi) for each experiment (in µM/L/min).
- ✓ Plot the curve Vi = f(T°).
- ✓ Interpret the results obtained.