

T.P. n°1 : immobilization of invertase in agar fibers

Principle :

The raw extract obtained from baker's yeast cells (*Saccharomyces cerevisiae*), containing invertase, is immobilized by physical retention: it is an inclusion in agar gel fibers.

Equipment :

- ✓ Erlenmeyer flask + beaker ;
- ✓ Test tubes ;
- ✓ Pipettes and micropipettes ;
- ✓ Graduated tube ;
- ✓ 5 ml syringe ;
- ✓ Support ;
- ✓ Hot plate ;
- ✓ Precision balance, spatula, and watch glass ;
- ✓ Agitator ;
- ✓ Oven set to 40°C ;
- ✓ Boiling water bath (95°C) ;
- ✓ Vortex agitator ;
- ✓ Filter or sieve ;
- ✓ Spectrophotometer and cuvettes.

Reagents :

- ✓ Baker's yeast extract (*Saccharomyces cerevisiae*) diluted to (1/50) ;
- ✓ Sodium acetate buffer pH 4.7 ;
- ✓ Agar ;
- ✓ DNS reagent ;
- ✓ 0.1 M sucrose ;
- ✓ 5 M sodium chloride salt.

Procedure :

1. Preparation of a 0.1M sucrose solution :

Dissolve 3.42 g of sucrose in 100 ml of distilled water.

2. Preparation of a 5M sodium chloride solution :

Dissolve 29.22 g of NaCl in 100 ml of distilled water.

3. Preparation of a 2% agar gel :

- ✓ Dissolve 2 g of agar powder in 100 ml of distilled water.
- ✓ Bring to a boil for 2 to 3 minutes.
- ✓ Allow the solution to cool to 60°C.

4. Immobilization of yeast extract in agar gel :

- ✓ In a warmed syringe, introduce 3 ml of the agar solution and 0.5 ml of the yeast extract at a temperature of 60°C ;
- ✓ Drop this mixture into 100 ml of NaCl solution at a temperature of 0°C to 20°C (to do this, place the beaker containing the salt solution in ice). This results in the formation of fibers containing the yeast extract ;
- ✓ Leave the fibers to rest for 30 minutes in the NaCl solution ;
- ✓ Filter the fibers, rinse them with distilled water, and drain them.

5. Demonstration of the enzymatic activity of immobilized invertase :

Prepare the following tubes :

	Tube 0	Tube 1	Tube 2	Tube 3	Tube 4
Acetate buffer pH 4.7	3 ml	3 ml	3 ml	3 ml	3 ml
0.1 M sucrose solution	1 ml	1 ml	1 ml	1 ml	1 ml
Pre-incubation	Place the tubes in the oven for 5 minutes at 40°C				
Enzyme	0	Put the agar fibers into the tubes (equal amounts)			
Reaction time	0	2 min	4 min	7 min	10 min

- ✓ Return the tubes to the oven at 40°C for 2, 4, 7, and 10 minutes.
- ✓ Stop the reaction by filtering the contents of the tubes into other tubes.
- ✓ Add 2 mL of DNS reagent.
- ✓ Shake well and heat in a boiling water bath for 5 minutes.
- ✓ Allow to cool, then add 6 ml of distilled water.
- ✓ Homogenize and allow to stand for 10 minutes at room temperature.
- ✓ Read the absorbance (DO) at 540 nm against the blank (tube 0).

Work to do :

- ✓ Trace the curve $DO = f(t)$.
- ✓ Trace the curve $[\text{invert sugar}] = f(t)$ using the calibration curve.
- ✓ Calculate the initial reaction rate (V_i) in $\text{mmol of hydrolyzed sucrose} \cdot \text{L}^{-1} \cdot \text{min}^{-1}$ (L = liter of reaction medium)
- ✓ Interpret the results obtained.