

## T.P. n°2 : reactor with invertase immobilized in alginate balls

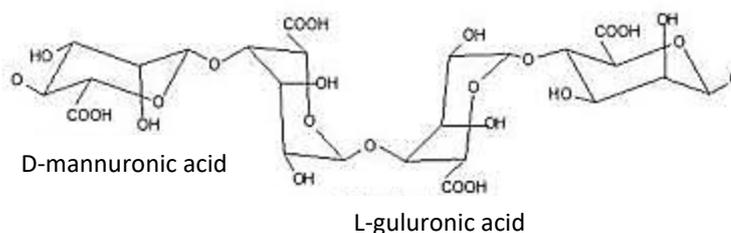
### Principle :

The raw extract obtained from baker's yeast cells (*Saccharomyces cerevisiae*), containing invertase, is immobilized by physical retention : it is encapsulated in alginate balls.

### Sodium alginate :

Sodium alginate is a compound extracted from certain brown algae, such as *Macrocystis pyrifera* and other Laminaria species.

Alginic acid is a linear polymer, consisting entirely of  $\alpha$  D-mannuronic acid and  $\beta$  L-guluronic acid in varying proportions. The bond is formed via  $\beta$ -1-4.



### Equipment :

- ✓ Erlenmeyer flasks + beakers ;
- ✓ Test tubes ;
- ✓ Pipettes and micropipettes ;
- ✓ Test tube ;
- ✓ 5 ml syringe ;
- ✓ Support ;
- ✓ Precision balance, spatula and watch glass ;
- ✓ Agitator and magnetic stir bar ;
- ✓ Heating plate ;
- ✓ Oven set to 40°C ;
- ✓ Boiling water bath (95°C) ;
- ✓ Vortex agitator ;
- ✓ Spectrophotometer and cuvettes.

### Reagents :

- ✓ Baker's yeast extract (*Saccharomyces cerevisiae*) diluted to (1/50) ;
- ✓ Sodium acetate buffer pH 4.7;
- ✓ Sodium alginate ;
- ✓ DNS reagent ;
- ✓ 0,1 M sucrose (table sugar) ;
- ✓ 0.15 M calcium chloride salt.

### Procedure :

#### 1. Preparation of a 0.1 M sucrose solution :

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

**2. Preparation of a 0.15 M calcium chloride solution :**

Dissolve 16.65 g of CaCl<sub>2</sub> in 100 mL of distilled water.

**3. Preparation of a 2% alginate gel :**

- ✓ Dissolve 1 g of sodium alginate powder in 50 ml of distilled water.
- ✓ Heat the mixture to 45°C.

**4. Immobilization of yeast extract in alginate gel :**

- ✓ Combine 3 ml of the alginate solution and 0.5 ml of the yeast extract at a temperature of 45°C in a warmed syringe ;
- ✓ Drop this mixture into 100 ml of the CaCl<sub>2</sub> solution, gently stirred, 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 balls containing the yeast extract ;
- ✓ Leave the balls to rest for 30 minutes in the CaCl<sub>2</sub> solution ;
- ✓ Filter the balls, rinse them with distilled water, and drain them.

**5. Study of the enzymatic activity of invertase immobilized in a bioreactor :**

- ✓ Prepare a mixture of : 50 mL sucrose + 150 mL acetate buffer ;
- ✓ Pre-incubate the mixture at 40°C for 5 minutes.

**Setup with a beaker :**

- ✓ Place 100 mL of the previously prepared mixture in a beaker ;
- ✓ Place the beaker on a magnetic agitator ;
- ✓ Add the alginate balls to the beaker while agitating moderately ;
- ✓ Take 2 mL samples of the mixture after 2, 4, 6, 8, and 10 minutes ;
- ✓ Add 1 mL of the DNS reagent ;
- ✓ Stir well and heat in a boiling water bath (95°C) 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 :**

Calculate the initial reaction rate (Vi) in mmol of hydrolyzed sucrose.L<sup>-1</sup>min<sup>-1</sup> (L = liter of reaction medium).