T.P. n°1 : Extraction of invertase and realization of a calibration curve for the DNS method

Introduction :

Invertase, also known as saccharase or β-fructofuranosidase (EC 3.2.1.23), is an enzyme capable of hydrolyzing the β-fructofuranosidic bond of sucrose, releasing its constituents : glucose and fructose.

Yeasts (Saccharomyces cerevisiae, Saccharomyces carlbergensis) possess a saccharase that allows them to use sucrose as a nutrient substrate in the same way as oses. This enzyme is intracellular. Its extraction requires the cell membrane to be broken. It is found in the soluble fraction of yeast.

DNS Reagent :

In an alkaline and warm environment, yellow 3,5-dinitrosalicylic acid (3,5-DNS) is reduced by reducing sugars to orange-red 3-amino 5-nitrosalicylic acid. It should be noted that the calibration curves do not always pass through the origin.

Thus, the enzymatic activity of invertase is studied by measuring the reducing sugars released after hydrolysis using the DNS method.

Equipment :

- ✓ Test tubes ;
- ✓ Pipettes and micropipettes ;
- ✓ Beakers ;
- ✓ Centrifuge tubes ;
- ✓ Freezer bottles ;
- ✓ Balance ;
- ✓ Oven (heater) ;
- ✓ Centrifuge ;
- ✓ Water bath ;
- ✓ Vortex mixer ;
- ✓ agitator and magnetic stir bars
- ✓ Spectrophotometers and cuvettes.

Reagents:

- ✓ Baker's yeast (Saccharomyces cerevisiae) ;
- ✓ 0,1M sodium bicarbonate solution ;
- ✓ Sodium acetate buffer pH 4.7 ;
- ✓ DNS reagent ;
- ✓ 0,1 M sucrose solution (table sugar) ;
- ✓ 0.005 M glucose and fructose solution ;

Preparations :

- ✓ 0,1 mol/l sucrose : 34.2 g of table sugar in one liter of distilled water.
- ✓ 0.005 M glucose and fructose solution : 1 g glucose + 1 g fructose in one liter of distilled water.
- ✓ Sodium bicarbonate at 0.1 M : 8.4 g of sodium bicarbonate in 1 liter of distilled water.
- ✓ Sodium acetate buffer at 0.05 M pH 4.7 : 4.1 g of sodium acetate in 1 liter of distilled water + diluted acetic acid (5%).

Procedure :

- ✓ Suspend 10g of baker's yeast in 40ml of 0.1M sodium bicarbonate ;
- ✓ Incubate at 35-40°C for 24 hours ;
- ✓ Centrifuge at 5000 rpm for 5 minutes ;
- ✓ Collect the supernatant (containing soluble molecules, including invertase) ;
- ✓ Throw away the pellet formed from cellular debris ;
- ✓ Note the total volume of supernatant (test tube) ;
- ✓ Store the supernatant in a cool place (for further handling).

Preparation of the calibration range :

Perform a calibration range according to the table below :

Tube number	**0**	**1**	**2**	**3**	**4**	**5**
0.005M glucose + fructose solution (ml)	0	0,2	0,4	0,6	0,8	1
Distilled water (ml)	1	0,8	0,6	0,4	0,2	0
Acetate buffer pH 4.7 (ml)	1					
0.1 M sucrose solution (ml)	1					
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 room temperature.
- ✓ Read the absorbances (OD) at 540 nm against the blank (tube 0).

Work to perform :

Trace the calibration curve : OD = f([inverted sugar] in µM).