

T.P. n°1 : Extraction of invertase from *Saccharomyces cerevisiae*

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.

Centrifugation principle :

Centrifugation is a technique used for the separation of particles from a solution according to their size, density, viscosity of the medium and rotor speed. The particles are suspended in a liquid medium and placed in a centrifuge tube. The tube is then placed in a rotor and spun at a define speed.

Separation through sedimentation could be done naturally with the earth gravity, nevertheless, it would take more time. Centrifugation is making that natural process much faster.

Rotation of the rotor about a central axis generates a centrifugal force upon the particles in the suspension.

A **centrifuge** is a device that separates particles from a solution through use of a rotor. In biology, the particles are usually cells, subcellular organelles, or large molecules.

There are two types of centrifuge procedures ; one is **preparative**, the purpose of which is to isolate specific particles, and the other is **analytical**, which involves measuring physical properties of the sedimenting particles.

Equipment :

- ✓ Erlenmeyer flask ;
- ✓ Centrifuge tubes ;
- ✓ Freezer bottles ;
- ✓ Balance ;
- ✓ Spatula ;
- ✓ Watch glass ;
- ✓ Oven (heater) ;
- ✓ Centrifuge.

Reagents :

- ✓ Baker's yeast (*Saccharomyces cerevisiae*) ;
- ✓ 0.1M sodium bicarbonate solution ;

Preparations :

- ✓ Sodium bicarbonate at 0.1 M : 8.4 g of sodium bicarbonate in 1 liter of distilled water.

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).