

## Practical work 2: Testing of the invertase activity by the Fehling reagent

### Introduction (must be written in the report)

Invertase activity can be tested using Fehling's reagent by measuring the amount of reducing sugars (glucose and fructose) produced from sucrose hydrolysis. It is a qualitative test

Fehling's reagent is made by mixing two separate solutions :

- **Fehling's A:** A deep blue aqueous solution of *copper(II) sulfate*.
- **Fehling's B:** A colorless solution of *potassium sodium tartrate* (Rochelle salt) in *sodium hydroxide*, making it strongly alkaline.

These two components are stable separately but must be mixed just before use to form the active reagent.

Fehling's reagent is primarily used in:

- **Fehling's test:** A qualitative test to detect *reducing sugars* (like glucose and fructose) and *aldehydes*. It produces a brick-red precipitate when a positive result occurs.

### Principle of the Test

Invertase catalyzes the hydrolysis of sucrose into glucose and fructose, both of which are reducing sugars. **Fehling's reagent reacts with these reducing sugars**, forming a brick-red precipitate of copper(I) oxide ( $\text{Cu}_2\text{O}$ ), which indicates enzyme activity.

### Materials Required

- **Invertase enzyme solution (Extracted during practical work 1)**
- **Fresh yeast solution (1g/20ml)**
- **Sucrose solution 1g for 100 ml distilled water (typically 5% in 80 mM acetate buffer, pH 4.5)**
- **Fehling's A and B solutions :**
  - *Fehling A:* Copper (II) sulfate solution
  - *Fehling B:* Alkaline tartrate solution (Rochelle salt + NaOH)
- **Water bath (60–70°C)**
- **Test tubes and pipettes**

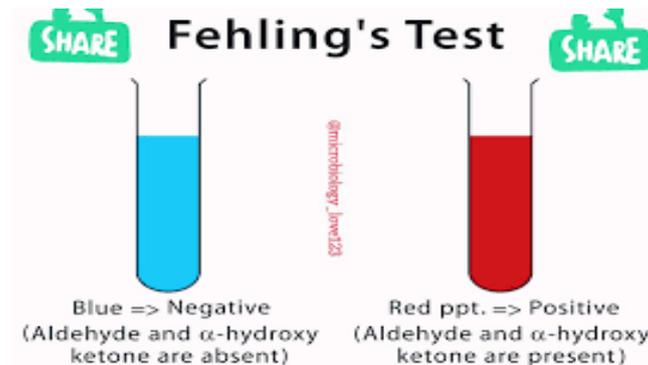
### Procedure

1. **Incubation :**
  - Mix invertase (Yeast solution) with sucrose solution.
  - Incubate at optimal temperature (**usually 60°C**) for a set time (e.g., **10–30** minutes).
2. **Reaction with Fehling's reagent:**
  - Add equal volumes of Fehling A and B to the reaction mixture.
  - Heat the mixture in a boiling water bath for 2–5 minutes.
3. **Observation :**

- A **brick-red precipitate** indicates the presence of reducing sugars, confirming invertase activity.

**The result of Fehling's test, which is commonly used to detect invertase activity through the presence of reducing sugars.**

When invertase breaks down sucrose into glucose and fructose, Fehling's reagent reacts with these reducing sugars to form a brick-red precipitate of copper(I) oxide, as seen below:



### Notes :

- The **brick-red precipitate** confirms the presence of reducing sugars (glucose + fructose).
- The **intensity of color** or **amount of precipitate** correlates with enzyme activity.