WJEC Wales Biology A Level

SP 3.1: Investigation of dehydrogenase activity in yeast using artificial hydrogen acceptors

Practical notes

Introduction

In the presence of oxygen, yeast respires **aerobically**. **Dehydrogenase** enzymes remove **hydrogen atoms** from certain molecules and transfer them to **hydrogen acceptors** such as NAD.

Artificial hydrogen acceptors such as **methylene blue** accept hydrogen atoms. They become reduced and undergo a visible colour change (e.g. methylene changes from **blue** to **colourless**). This allows the rate of dehydrogenase activity in organisms such as yeast to be investigated.

Equipment

- Methylene blue
- Yeast suspension
- 10 cm^3 syringe
- 1 cm^3 syringe
- 35°C Water bath
- Boiling tube
- Bung
- Stopwatch

Risk assessment

Hazard	Risk	Precaution	Emergency
Broken glass	Cuts	Keep glassware away from the edge of the desk	Dispose of broken glassware carefully; elevate cuts and apply pressure; do not remove glass from cuts; seek medical assistance
Methylene blue	Irritation to eyes	Wear safety goggles	Flood eye(s) with tap water; seek medical assistance
Yeast suspension	Irritation to eyes	Wear safety goggles	Flood eye(s) with tap water; seek medical assistance

Method

1. Using the **10 cm^3 syringe**, add **10 cm^3** yeast suspension to the boiling tube.

2. Place the boiling tube in the **35°C** water bath for 10 minutes to allow it to **equilibrate**. *35°C is the optimum temperature for respiratory enzymes in yeast cells.*

3. Using the **1 cm^3 syringe**, add **1 cm^3** methylene blue indicator to the boiling tube. Insert a bung and invert to mix.

4. Place the boiling tube in the 35°C water bath and start the stopwatch. Time how long it takes for the solution to turn from **blue** to **colourless**.

Results

The rate of dehydrogenase activity can be **estimated** using **rate = 1/t**.

The effect of different factors (e.g. temperature) on the rate of dehydrogenase activity can be determined by changing **one variable only**, and observing its effect on the rate.