6 Practical 1

Investigating the effect of temperature on dehydrogenase activity in yeast

Safety
The normal safety precautions associated with the use of chemicals and heating apparatus apply.

Apparatus and materials

- boiling tubes
- 10 cm³ syringe
- large beaker (water bath)
- tripod
- Bunsen burner
- test-tube holder
- stirring rod
- 100 cm³ of a 10% suspension of actively respiring yeast
- test-tube rack
- 1 cm³ syringe
- thermometer
- gauze
- heat-resistant mat
- stopwatch
- distilled water
- 10 cm³ of 0.5% triphenyltetrazolium chloride solution

Introduction
Actively respiring yeast contains dehydrogenase enzymes. Normally when the yeast respires, hydrogens are removed from the respiratory substrates and passed to hydrogen acceptors such as NAD. It is possible to use an artificial hydrogen acceptor called triphenyltetrazolium chloride (TTC) to show the activity of these enzymes. TTC is a redox indicator. It is colourless when oxidised and pink when reduced. If TTC is mixed with yeast cells in suspension, some hydrogens will be passed to the TTC, causing it to be reduced and change from colourless to pink.

In this practical, you will:
- carry out the procedure at different temperatures between room temperature and 70 °C, to find out the effect of temperature on the activity of dehydrogenases in yeast.

Procedure

1 Use syringes to place 10.0 cm³ of the yeast suspension into a boiling tube and add 1.0 cm³ of distilled water. This tube will act as a starting-point colour standard for the reaction, so that you can see when a colour change has taken place in the experimental tubes.

2 Prepare a water bath at room temperature. Measure the temperature of the water in the bath.

3 Place 10.0 cm³ of the yeast suspension into a clean boiling tube. Place 1.0 cm³ of triphenyltetrazolium chloride solution (TTC) into a second clean tube.

4 Place the two tubes in the water bath for several minutes to allow them to equilibrate to the temperature of the water. How can you check they have equilibrated?

5 Mix the contents of the tubes by adding the yeast to the TTC. Shake the tube and return it to the water bath. Start the stopwatch.
6 Note the time taken for a definite pink colour to develop, by comparison with the starting-point colour standard. Shake the tube gently at intervals to prevent the yeast settling to the bottom of the tube.

7 Repeat steps 1–6 at five more temperatures between room temperature and 70 °C. At each temperature, be careful to maintain the temperature of the water bath as constant as possible.

8 Present your results as a table. An arbitrary measure of the rate of reaction can be found by calculating the reciprocal of the time taken for the colour to develop (rate = \( \frac{1}{\text{time}} \)).

If you calculate the values of \( \frac{1000}{\text{time (in seconds)}} \), this will give more manageable numbers.

Add these values to the table.

9 Plot a graph of the rate of reaction (arbitrary units) against temperature.

10 a What are the roles of dehydrogenase enzymes in respiration?
   b Describe what the graph tells you about the activity of dehydrogenases in yeast between room temperature and 70 °C.
   c Explain why the activity is affected in this way.
   d What are the main sources of error and limitations of this experiment?
   e How could you improve the design of the experiment?