Investigation of dehydrogenase activity in yeast

Specification reference:

A level Component: 1.1

Importance of ATP

Introduction

Yeast performs aerobic respiration when there is access to oxygen, towards the top of a suspension in a test tube, and anaerobic respiration lower down. Dehydrogenase activity removes hydrogen atoms from intermediates in both types of respiration and transfers them to hydrogen acceptors. If an artificial hydrogen acceptor is added to the suspension, it will accept the hydrogen atoms and undergo a colour change on being reduced. The time taken for the indicator to change colour can be used as a measure of the rate of dehydrogenase activity.

Use the method below to carry out an investigation into dehydrogenase activity in yeast

Apparatus

Redox indicator: methylene blue (0.05 g/100cm^3)
Yeast suspension (100 g/dm^{-3})
30 ^{o}C Water bath
Test tube
Cork for test tube
10 cm^3 syringe
1 cm^3 syringe
Stop clock

Method

1. Place 10 cm^3 of the yeast suspension into a test tube.
2. Place test tube in water bath for 5 minutes, to equilibrate to 30 ^{o}C.
3. Add 1 cm^3 indicator.
4. Invert the test tube once, to mix.
5. Replace the test-tube in the water bath.
6. Time how long the indicator takes to change colour.

Risk assessment

Hazard	Risk	Control measure
Redox indicator is harmful	Skin irritation and staining	Avoid skin contact
	Eye irritation if transferred to eye	Use eye protection
Electric shock from waterbath	Electric shock if unplugged with wet hands	Use dry hands to unplug after use

Teacher/ Technician notes

The concentration of yeast stated is only a guideline, as the activity of yeast can vary greatly. The dried yeast should be mixed to a thin paste with distilled water and then made up to the desired volume. This should be done an hour before the practical starts to allow fermentation to start and then it should be kept in a water bath or incubator at 35°C.

The students should be told to keep the first tube as a reference colour for the end point of the reaction.

This could be used as an alternative to the investigation into factors affecting the rate of respiration in yeast. There is no need for both practicals to be completed as full investigations.

Other indicators can be used as shown below:

Indicator	**Concentration / g 100 cm^{-3}**	**Colour when**	
		oxidised	**reduced**
methylene blue	0.1	dark blue	colourless
DCPIP (dichlorophenol indophenol)	0.1	dark blue	colourless
TTC (triphenyl tetrazolium chloride)	0.5	colourless	red

Sample results

Temperature of water bath (°C)	**Time taken for yeast to turn methylene blue colourless (s)**			
	Trial 1	**Trial 2**	**Trial 3**	**Mean**
30	641	651	633	642
40	443	441	412	432
50	291	233	306	277
60	136	119	139	131
70	198	208	187	198

Further work

- This could be used as an alternative to the investigation into factors affecting the rate of respiration in yeast. There is no need for both practicals to be completed as full investigations.

Practical techniques

- use appropriate apparatus to record a range of quantitative measurements (to include mass, time, volume, temperature, length and pH)

- use laboratory glassware apparatus for a variety of experimental techniques to include serial dilutions