INVESTIGATING DEHYDROGENASE ACTIVITY

This activity supports the delivery of:

- Higher Human Biology, Unit 1, KA7
- Higher Biology, Unit 2, KA2
- Advanced Higher Biology, Unit 1, KA1

Higher Biology | Higher Human Biology | Advanced Higher Biology

Teacher Guide

This activity supports the use of specified apparatus and techniques including:

- beaker
- test tube
- stopwatch
- water bath
- colorimeter

Technique: Using substrate concentration or inhibitor concentration to alter reaction rates.

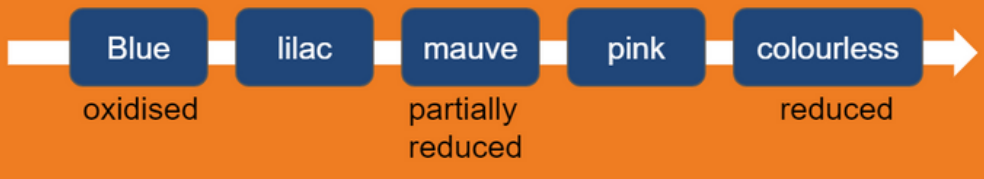
Experiment Introduction

In this experiment, we monitor the action of dehydrogenase enzymes as a measure of respiration rate. Our model organism is yeast, which will be immobilised for this colorimetric assay.

During glycolysis, glucose is broken down to pyruvate in the cytoplasm. Dehydrogenase enzymes remove the hydrogen ions, in a process called oxidation, and transfer the hydrogen ions to a coenzyme.

In this experiment, we use resazurin dye to pick up this hydrogen. Resazurin changes colour (indicated below) during its reduction. It can therefore be used as an indicator of respiration: the time taken for resazurin to change colour will indicate the rate of respiration.

Possible independent variables include respiratory substrate, substrate concentration (which will be outlined clearly in this guide), temperature of incubation and type of yeast.

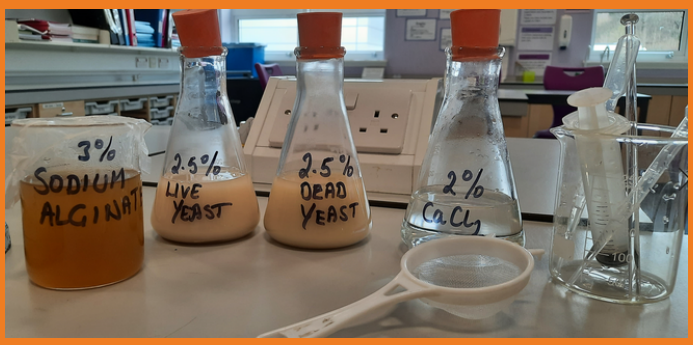


Stage 1: Making Immobilised Yeast

To enable colorimetric measurements, yeast must be immobilised using sodium alginate.

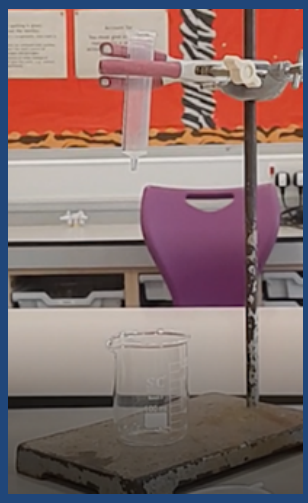
Materials

- 3% sodium alginate solution
- 2% calcium chloride solution
- 2.5% yeast solution
- 10ml syringe barrel
- Bijou bottles (to store beads)
- Deionised water
- 3ml pipette
- Syringe
- Small beaker
- Tea strainer
- Stirring rod
- Clamp stand



click for the video tutorial

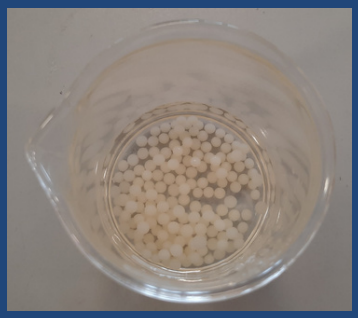
1. Clamp the barrel of the 10ml syringe so that it is about 20cm above the top of the plastic cup. Add 30ml calcium chloride to the small beaker below the syringe barrel.



2. Gently shake the yeast to resuspend the solution. In a beaker, add 3ml yeast suspension to 3ml sodium alginate solution. Mix well using a stirring rod.

Stage 1: Making Immobilised Yeast

3 Pour the yeast/alginate mixture into the syringe barrel and gently swirl the beaker of calcium chloride. As the mixture drops into the calcium chloride solution, each drop will form a bead.



Stage 2: Dehydrogenase Assay

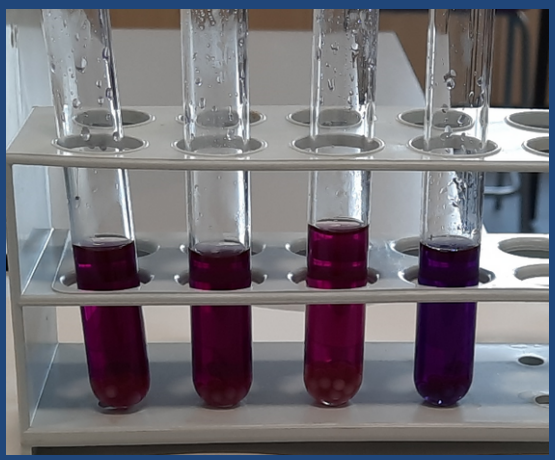
Aim: To investigate the effect of substrate concentration on dehydrogenase activity in yeast.

Materials per group
- 1 test tube rack + 4 test tubes
- marker pen
- stop watch
- safety goggles
- 1 Pasteur pipette

Materials per class
- Immobilised yeast beads (live & dead)
- 2.5%, 5%, 10% glucose solution
- Resazurin dye (1 tablet dissolved in 25ml water)
- water bath at 35°C
- Distilled water

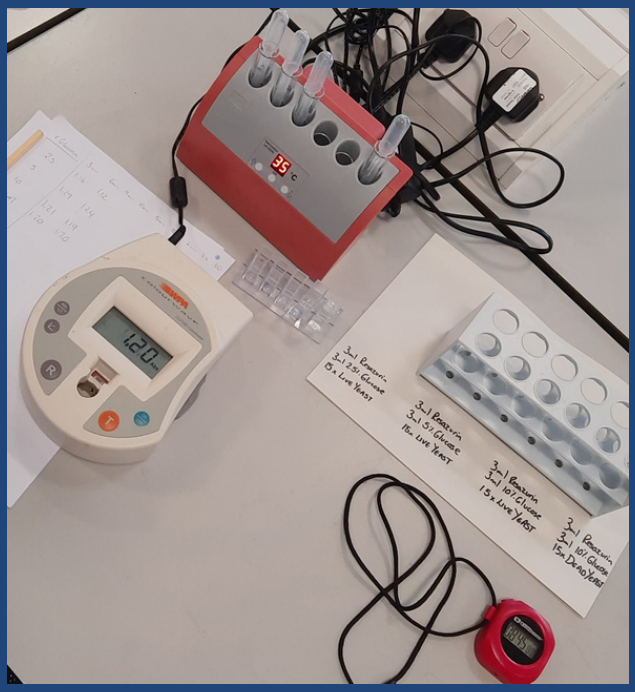
1 Add 3cm^3 resazurin dye, 3cm^3 appropriate glucose solution and 15 immobilised yeast balls to each test tube.

Gentle shake the tubes.



Incubate the test tubes in a water bath at 35°C.

2 Set the colorimeter filter to 590nm. Blank the colorimeter using the appropriate glucose solution.



3 Using a Pasteur pipette, remove samples from the test tubes every 3 minutes and take colorimetric reactions for 30-45 minutes. Pour the solution back into the test tubes after measuring the absorbance.

YouTube

Recording Results

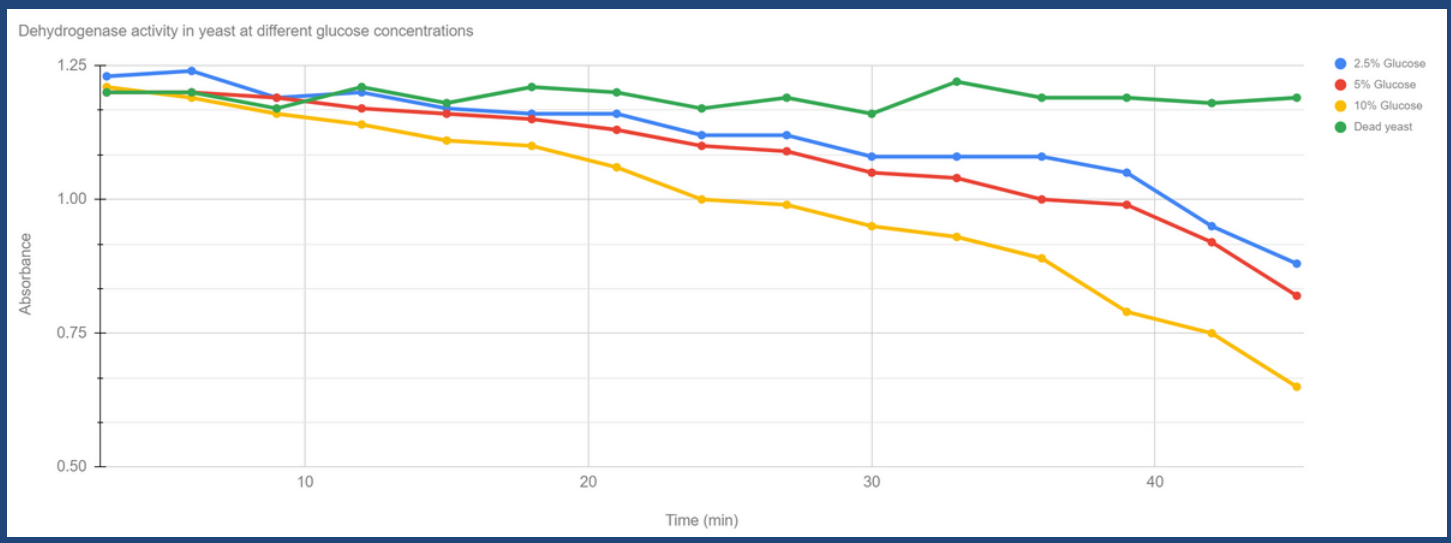
Prior to results analysis, students should be encouraged to reflect on suitable sample size for this experiment. Two repeats (or sharing of data with two groups) would be appropriate, depending on the degree of variation in the results obtained.

This investigation yields quantitative data. Students should now consider the most appropriate presentation format for this type of data. Prompt questions are included in the Pupil Guide.

While paper-based graphs are an excellent and appropriate option, there is also the opportunity to support their digital intelligence by incorporating graph building using Google Sheets or Microsoft Excel. Skills 4.0

A set of sample results are included below.

Glucose concentration (%)	Absorbance of light through solution at each time interval (min)															change in absorbance in 45 min
	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	
2.5	1.23	1.24	1.19	1.2	1.17	1.16	1.16	1.12	1.12	1.08	1.08	1.08	1.05	0.95	0.88	0.35
5	1.2	1.2	1.19	1.17	1.16	1.15	1.13	1.1	1.09	1.05	1.04	1	0.99	0.92	0.82	0.38
10	1.21	1.19	1.16	1.14	1.11	1.1	1.06	1.02	0.99	0.95	0.93	0.89	0.79	0.75	0.65	0.56
control	1.2	1.2	1.17	1.21	1.18	1.21	1.2	1.17	1.19	1.16	1.22	1.19	1.19	1.18	1.19	0.01



Analysing Results to form a Conclusion

Students should reflect on their aim and form a conclusion supported by their data.

Evaluating the Investigation

Students should now reflect on the concepts of accuracy, precision, validity and reliability. The experimental method should be evaluated with respect to these terms, and suggestions made as to how the method could be adapted. The results should also be evaluated - with respect to their variability and reliability.

Teaching Suggestions & Wider Links

Challenge pupil creativity by prompting them to imagine new ways of addressing the problem. What other ways could we measure respiration rate? What tools would we need? What would be the advantages and disadvantages of these alternatives approaches?

Skills 4.0

Idea generation

Imagination

DYW | Developing the Young Workforce

Invite pupils to see the big picture. Why are we interested in measuring respiration rate? Why are we using yeast to do this experiment?

Ethics

Skills 4.0

Sense Making

An investigation of respiration in yeast can promote wider discussions of its impact on biotechnology, including the sustainable production of bioethanol. Pupils could be introduced to companies including the biorefinery, Industrial Biotechnology Innovation Centre (IBioIC), in Grangemouth.

Learning for Sustainability SCOTLAND