INVESTIGATING THE EFFECT OF TEMPERATURE ON ENZYME ACTIVITY

Purpose

- To investigate the effect of temperature on the initial rate of reaction of an enzyme-controlled reaction.
- To calculate Q_{10} for an enzyme-controlled reaction.

SAFETY

Hydrogen peroxide is an irritant and dangerous if swallowed.

Wear eye protection and avoid contact with skin or clothes.

Write a risk assessment including any safety precautions. Discuss this with your teacher before starting. See CLEAPSS Student Safety Sheet 57 for further information.

Initial rate of reaction and Q_{10}

In this activity you will plan and carry out an investigation into the effect of temperature on the initial rate of an enzyme-catalysed reaction, which can be quantified by calculating the temperature coefficient for the reaction. This is known as the Q_{10}. It indicates how much the rate of reaction increases with a 10 °C increase in temperature. You will be focusing on the enzyme catalase, which is found in almost all living tissues. Catalase breaks down hydrogen peroxide (H_2O_2), a toxic metabolic by-product, into water and oxygen. Collecting and measuring the volume of oxygen gas produced is relatively easy and can be used to work out the rate of reaction.

Scientific questions and information research

Research relevant information and state what you are going to investigate.

Before you start planning your experiment you should decide what effect you think temperature will have on the rate of breakdown of hydrogen peroxide catalysed by the enzyme catalase. Write down your idea as a question or hypothesis that you can answer or test and support your idea with biological knowledge. If you present your idea as a question make a prediction about the answer to the question. To help you decide on what you are going to investigate and how you will carry out the practical work you might need to research the background science and methods people have used to investigate similar problems. You will need to consider how to calculate initial rates of reaction and also Q_{10}.

Planning and experimental design

Design an experiment to test your idea or hypothesis and write a detailed plan to use during the investigation. Your experiment will need to give you sufficient data to determine initial rate of reaction at different temperatures. Your experimental data should also allow you to calculate Q_{10} for the reaction. If possible, trial the reaction and the equipment before you embark on detailed planning, as this will allow you to be more realistic, and spot any design flaws. Trialling can help establish volumes, concentrations, timings and practical set-ups to use in your method.

THE FOLLOWING EQUIPMENT WILL BE AVAILABLE

- Soaked peas
- Hydrogen peroxide solution
- Distilled water
- Crushed ice
- 10 cm^3 syringes
- Rubber tubing
- Screw clip or three-way tap
- Test tubes
- Thermometers
- Stopclocks
- Delivery tubes with bungs to fit the boiling tubes or test tubes
- Access to a balance
- Glass rods
- Forceps
- Water baths at a range of temperatures

• Boiling tubes • 100 and 250 cm^3 beakers • Graduated pipettes • Syringes • Measuring cylinders	• Pestle and mortar • Clamp stand with boss and clamp • Other basic laboratory equipment, i.e. Bunsen burners, test tube racks, etc. • You may have access to a gas syringe
You do not have to use all of the equipment listed. You should get your plan checked by your teacher/lecturer before starting the practical work, but it can be modified during the investigation.	

Make sure your plan:

- includes apparatus and a method that will validly answer your question or test your hypothesis
- identifies the independent and dependent variables
- identifies any other variables that may affect the outcome of the experiment and, where possible, controls or allows for them
- includes the range of values you will use for the independent variable and the range you might expect to find for the dependent variable
- has a fully explained control, if appropriate
- includes replicates, if appropriate, and an explanation of why these are necessary
- says exactly what measurements you will make and how they will be made to ensure valid, accurate and precise results are obtained
- says how you will process your data, including how you will calculate initial rate of reaction and how you will use this to calculate Q_{10}
- provides information about any possible sources of error and how these can be minimised
- includes your risk assessment that identifies any safety issues and describes how any risks will be reduced.

Have your plan checked by your teacher/lecturer before you start the experiment.

Performing the experiment

Either use the plan you have created after it has been checked by your teacher/lecturer or use a method supplied by your teacher/lecturer.

Analysis and interpretation of data

Present your results in the most appropriate way in order to show how the initial rate of reaction varies with temperature.

Calculate a Q_{10} value for the enzyme catalase (remember that you can only do this up to the optimum temperature; beyond this the Q_{10} relationship does not hold).

Conclusion and evaluation

Explain any trends or patterns with evidence from your data. Discuss your findings with reference to your question or problem.

State a clear conclusion to your work, summarising what you have found. Support your statements with evidence from your results and relevant biological knowledge.

Comment on any systematic or random errors in the data.

Evaluate your experimental apparatus and methods, commenting on the accuracy and precision of your results.

Propose any changes to the procedure that would improve the quality of the results.

Suggest further experimental work you could carry out to extend your understanding of enzyme activity and temperature.

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Review the students' risk assessments and discuss any safety considerations.

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Ensure eye protection is worn and contact with skin or clothes is avoided.

Notes on the procedure

In this core practical activity students plan and carry out an investigation into the effect of temperature on the rate of an enzyme-catalysed reaction using catalase from peas. Catalase breaks down hydrogen peroxide (H_2O_2) to form water and oxygen. It is a convenient enzyme to use, as it is relatively easy to collect and measure the volume of oxygen gas produced. Students may be familiar with catalase from Topic 2.

The Student Sheet is a planning sheet. The Developing Practical Skills Support provides a framework for the steps in completing an investigation. This can be used to guide students through the process. Once the investigation has been completed, students could use the Developing Practical Skills Self-evaluation Sheet to reflect on what they have done in this practical. Students will not necessarily address all the practical skills in every practical activity. However, this activity lends itself very well to most aspects of using an investigative approach, in particular the selection of the appropriate measurements and analysis and interpretation of the results obtained.

It is a good idea to show students the type of equipment available and also ensure that they have observed the production of oxygen when peas are added to H_2O_2. Mashing peas with distilled water releases the catalase and allows a more quantitative approach to be used in the experiment. A class discussion about what should be included in the practical plan would be useful. Students may need to be given guidance to select a sensible range of temperatures and this range should allow calculation of Q_{10}.

It would be good if students completed the practical work that they have planned. Students could work in pairs, with groups combining results to provide repeats at each temperature to give a class set of data for analysis. The student sheet does not refer to statistical analysis, instead focusing on calculating initial rate of reaction and Q_{10}. If students have suggested a hypothesis to test, they can produce a null hypothesis and test it statistically using a Spearman's rank correlation test, see Maths and Stats Support Sheets 9 and 12.

There are several methods of measuring the rate of this reaction. The apparatus for one method is shown in Figure 1 below.

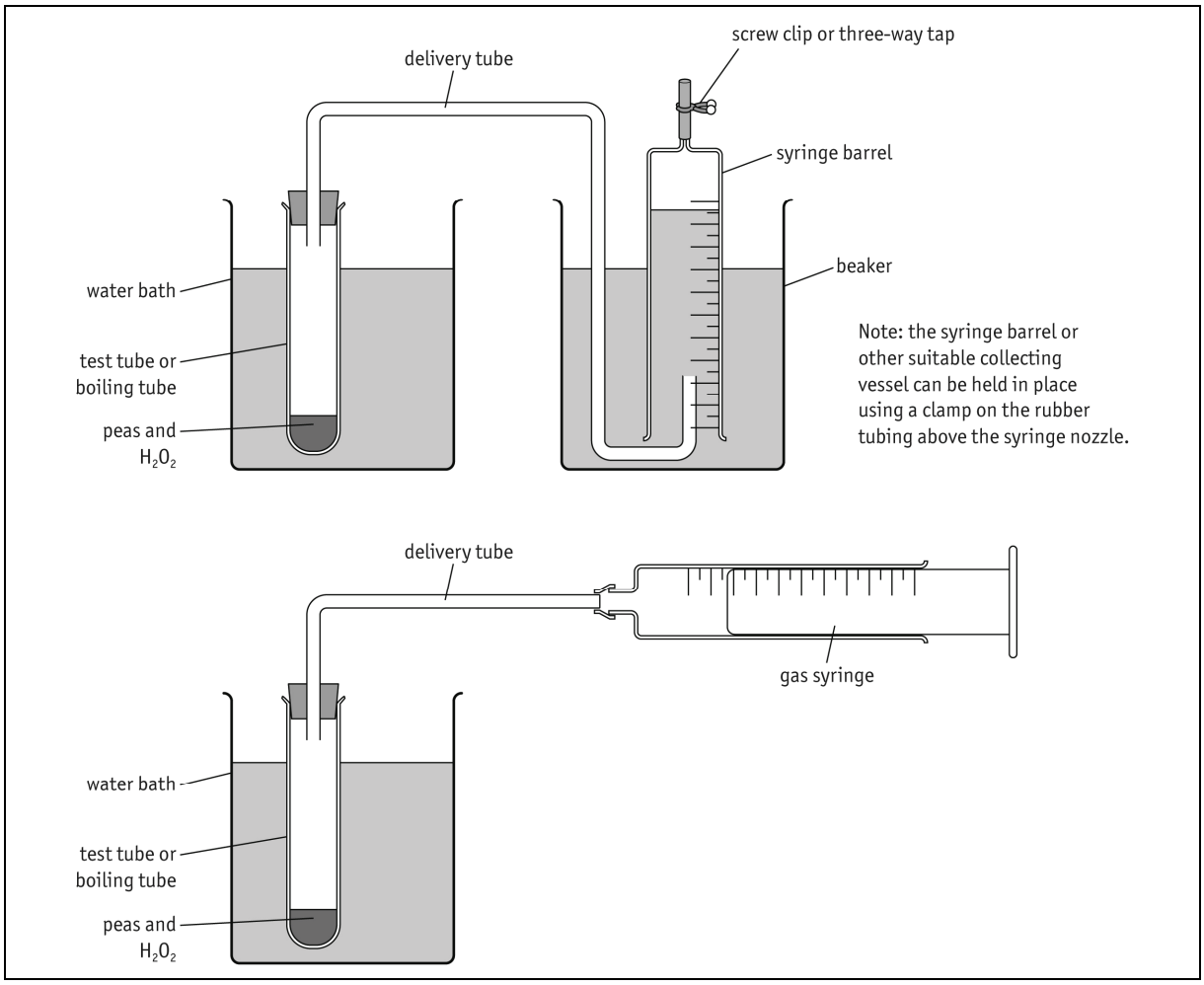

Figure 1 Examples of apparatus used to measure the volume of gas evolved.

It would be good practice for students to spend some time trying out the reaction and the equipment before they embark on detailed planning, as this will allow them to be more realistic about the volume of gas given off and the likely speed of the reaction.

The method employed would involve the following steps.

1 Set up the apparatus as shown. Note that the collecting syringe should not be over the end of the delivery tube when the boiling tube is first attached to the bung.
2 Grind a known mass of peas in a known volume of distilled water.
3 Measure a known quantity of the 'pea solution' made in step 2 into the boiling tube.
4 Add 5 cm^3 of hydrogen peroxide to the pea solution.
5 Reconnect the boiling tube to the apparatus making sure the bung has a gas-tight fit.
6 Place the syringe over the end of the delivery tube.
7 Start timing a collection period as soon as the first bubbles of gas enter the collecting syringe. Take measurements of the volume of oxygen produced at regular intervals.
8 Repeat the procedure at a range of different temperatures. Students could pool results to obtain replicate readings at each temperature.

Presentation of data/development of maths skills

For help with presentation of data and use of graphs to calculate initial rates of reaction refer students to the Maths and Stats Support section in the SNAB Online resources.

At each temperature students should plot a graph of volume of oxygen produced against time. The initial rate of reaction can be determined from these graphs by taking the gradient of the graph at the start of the reaction after time zero. A second graph of initial rate of reaction versus temperature can then be plotted. From this, students can calculate Q_{10} up to the optimum temperature for the enzyme using:

$$Q_{10} = \frac{\text{Rate of reaction at temperature } T + 10\,^{\circ}\text{C}}{\text{Rate of reaction at temperature } T}$$

They should expect a value of about 2, suggesting doubling of initial rate of reaction for a 10 °C rise in temperature.

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20 vol. hydrogen peroxide is an irritant. Wear eye protection and avoid contact with skin. See CLEAPSS Student Safety Sheet 57 for further information.

100 vol. hydrogen peroxide is corrosive. Keep in a dark bottle in a cool place.

General note

In this activity, students plan and carry out an experiment investigating the effect of temperature on rate of reaction. Students may request other apparatus, but the list below will cover most needs. A sample set-up is shown in Figure 1 on page 2. Other set-ups are also possible.

Requirements per student or group of students	Notes
Soaked peas	
30 cm^3 hydrogen peroxide solution (20 vol.)	Students will need about 5 cm^3 for each temperature and each repeat they run. The H_2O_2 decomposes rapidly and needs to be made up fresh by dilution of 100 vol. hydrogen peroxide. If the reaction is too vigorous, dilute the hydrogen peroxide more. Ensure you get fresh supplies each year if you do not use it on other occasions.
Distilled water	
10 cm^3 syringes	
Rubber tubing and screw clip attached or three-way tap	See Figure 1.
Test tubes and boiling tubes	
100 and 250 cm^3 beakers	
Crushed ice	For low temperature water baths.
Graduated pipettes	
Syringes	
Measuring cylinders	
Gas syringe	If available, this can be used rather than collecting by displacement of water.
Thermometers	
Stopclocks	
Delivery tubes with bungs to fit the boiling tubes or test tubes	See Figure 1.
Access to a balance	
Glass rods	
Forceps	
Water baths at a range of temperatures	
Pestle and mortar	
Clamp stand with boss and clamp	
Other basic laboratory equipment, e.g. Bunsen burners, test tube racks, etc.	
Eye protection	

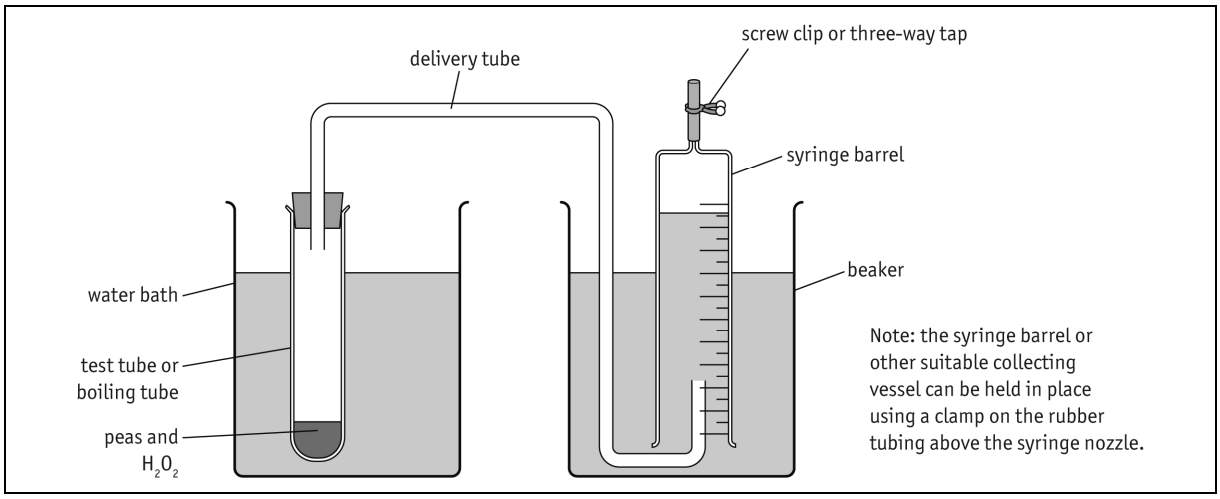

Figure 1 An example of apparatus set up to measure the volume of gas evolved.

If students use the set-up in Figure 1 and complete the experiment once at five different temperatures, they will require the equipment listed below.

Requirements per student or group of students	Notes
Soaked peas	
25 cm^3 hydrogen peroxide solution (20 vol.)	Students will need about 5 cm^3 for each temperature and each repeat they run. The H_2O_2 decomposes rapidly and needs to be made up fresh by dilution of 100 vol. hydrogen peroxide. If the reaction is too vigorous, dilute the hydrogen peroxide more. Ensure you get fresh supplies each year if you do not use it on other occasions.
Distilled water	
10 cm^3 syringe	
Rubber tubing and screw clip attached or three-way tap	See Figure 1.
5 boiling tubes or test tubes	One for each temperature.
Delivery tubes with bungs to fit the boiling or test tube	See Figure 1.
2 × 250 cm^3 beakers	
Crushed ice	For low temperature water baths.
Hot water	For high temperature water baths. Heated using Bunsen burner or kettle.
2 graduated pipettes, syringes or small 5 or 10 cm^3 measuring cylinder	For measuring pea extract and hydrogen peroxide.
Thermometer	
Stopclock	
Access to a balance	
Pestle and mortar	
Eye protection	