Practical 5 - The effect of pH on enzymes

This practical focuses on making measurements and observations, recording and presenting data, analysis, drawing conclusions and evaluating methods. You will also develop other assessed skills throughout the practical.

Intended learning outcomes

By the end of this practical you should be able to:

- Identify dependent and independent variables
- Make a hypothesis and express this in words and graphically
- Experience relevant methods, analysis and conclusion.
- Describe and explain the relationship between ph and enzyme activity
- Evaluate procedures

Safety Information

	You should wear eye protection throughout this practical.
	Hydrogen peroxide is **corrosive**. Avoid contact with eyes or skin. It will bleach skin or clothes.
	Citric acid is **harmful**.

Background information

- Most enzymes have an optimum pH near to 7 (the pH found inside most cells)
- pH is the measurement of the concentration of Hydrogen ions (H^+)
- Hydrogen ions will affect the hydrogen and ionic bonds within the enzyme
- If these bonds are changed the three dimensional shape is changed altering the shape of the active site
- When an enzymes shape is altered it becomes denatured
- Potatoes are a good source of catalase

You will investigate the effect of pH on the enzyme catalase as it breaks down toxic Hydrogen peroxide, a by-product of some biochemical reactions, into water and oxygen.

- Read the information above
- Identify and write down the dependent and independent variables
- Write down a hypothesis
- Draw a sketch graph to show what you think will happen

- Identify any variables that should be controlled and outline how this should be done
- What would be the best method for collecting the oxygen produced?
- A graph of pH against rate of activity will be produced after the practical. Make sure you know how to calculate rate.
- Know what a buffer solution is and what it does.

Method

Preparations and making observations

1. Use a cork borer to cut cylinders of fresh potato tissue. You will require a piece (pieces) between 6 – 7cm in length. Place on a tile and cut into at least 60 discs, each 1mm wide.
2. Place all the discs in a small beaker of water.
3. Set up the equipment as follows. Clamp a boiling tube to a stand and carefully insert the manometer (with fluid) into a rubber bung.
4. Using a syringe or small measuring cylinder place $5cm^3$ of buffer solution pH3 into the boiling tube.
5. Carefully add 10 of the potato discs followed by $5cm^3$ of hydrogen peroxide solution.
6. Place the bung back into the boiling tube as quickly as possible.
7. Start the stop watch and time how long it takes for the manometer fluid to rise by 5cm. (Mark start point on tube and measure 5cm)
8. Carefully agitate the tube to make sure that the potato discs do not stick together.
9. Wash out the boiling tube and repeat the experiment using a different pH buffer and ten new potato discs each time.
10. Make sure you use a clean syringe / measuring cylinder with each different buffer solution.
11. If time allows repeat the procedure for increased reliability.

Write-up

- Record your results in a clear table ensuring units are put in headers.
- If replicate results not done obtain a set of mean readings by using other class members results.
- Calculate the rate of reaction.
- Plot a graph of pH against rate.
- Explain your findings using your knowledge of enzymes.
- Assess the reliability of the results obtained and suggest any modifications you could make to improve the experiment
- How could you measure the volume of gas produced by this method and by altering the method

Practical 5 - Lesson Plan

The effect of pH on enzyme activity

Context

A practical investigation set in the context of 9700 syllabus – The effect of pH on enzyme activity

Key aims of the lesson

This practical is designed to develop the skills of observation, analysis and evaluation.

Intended learning outcomes

By the end of the practical and the write-up the student should be able to

- Experience relevant methods, analysis, conclusions and evaluation.
- Describe and explain the relationship between pH and enzyme activity.

Resources required

White board or flipchart and suitable pens or blackboard and chalk

Practical materials specified on the Technical Information Sheet.

Copies of the student worksheets.

Planned activities

Timings/ minutes	**Teacher/ Student Activities**
End of previous lesson	**Preparation –** 2 page student worksheet given out for students to read in preparation for the practical lesson. To consider identification of the variables, formulate a hypothesis and review previous learning on cell membranes
0 - 3	**Introduction** to the aims, intended outcomes and shape of the lesson – teacher led oral presentation
3 - 5	**Context –** review of enzymes, key points written on board
5 - 8	**Introduction to method –** Teacher briefly outlines method and answers any student questions on procedure. Teacher emphasises safety concerns with cork borer (or sharp knife) and hydrogen peroxide
8 - 45	**Carrying out the practical –** students carry out the practical work..
45 - 50	**Obtain results –** Students enter results into table and clear away apparatus as soon as they have finished

50 - 60	**Drawing together the threads –** Teacher led discussion on the skills that have been developed as well as discussion on results obtained. Practical write up to be completed in following lesson or as homework activity

Useful information

Discussion / evaluation points should include:

- explanation of the shape of the graph
- consistency of the enzyme within the potato, age of potato
- possible problems with the method e.g. Lack of temperature control in reaction tube, loss of gas before bung inserted
- for students unable to obtain a full set of results the following could be used for analysis

pH	Time taken for manometer to move 5cm (minutes)
3	10
4	8
5	6
6	4
7	2
8	5
9	8
10	10

Practical 5 - Technical information

The effect of pH on enzymes

The apparatus and materials required for this practical are listed below.

The amount of apparatus listed is for one student or one group of students if they are to work in groups.

1. fresh potato (each student requires a core (cores) approximately 10cm in length). (More if experiment is to be repeated)
2. 1 boiling tube
3. Single bore rubber bung
4. cork borer
5. white tile
6. scalpel
7. small beaker
8. 2 x 10cm^3 graduated pipette or measuring cylinder or syringe
9. Manometer tube (3mm diameter)
10. Stop watch
11. Forceps
12. 20 volume Hydrogen peroxide
13. Range of buffers (pH 3 – 8).

Additionally each student will require access to a sink and running water.

Commercial buffer tablets are available from most chemical wholesalers, however it is possible to make up buffer solutions in the laboratory. (Details from, Laboratory Manual for Schools. Heinemann. 1977)

Sodium hydrogen phosphate/citric acid buffer – range pH 3.0 – 8.0.

To make up 100cm^3 of buffer use 0.1M Citric Acid & 0.2M Sodium hydrogen phosphate in the following proportions:

pH	Citric acid / cm^3	Sodium hydrogen phosphate / cm^3
3.0	79.45	20.55
4.0	61.45	38.55
5.0	48.50	51.50
6.0	36.85	63.15
7.0	17.65	82.35
8.0	2.75	97.25

Safety Precautions/Risks.

Hydrogen peroxide = C 

Citric acid = H 

A risk assessment should be carried out as a matter of course.