ENZYME CONCENTRATIONS AND ENZYME ACTIVITY: PLANNING SHEET

Purpose

- To investigate how enzyme concentration can affect the initial rate of reaction.
- To develop practical skills.

SAFETY

Wear eye protection, lab coats and disposable gloves.

All enzymes are potential allergens and skin contact should be avoided. Asthma sufferers may be particularly sensitive, so alert your teacher.

Hydrogen peroxide is corrosive. Use with great care avoiding contact with eyes, skin and clothing.

Use the scalpel/craft knife with care, cutting on a secure surface.

Reducing concentration

If someone's pancreatic duct becomes blocked it reduces or prevents the release of pancreatic enzymes into the small intestine. The aim of this activity is to investigate the effect of a reduction in enzyme concentration on the initial rate of reaction. The pancreas releases several enzymes, including proteases, which could be used to investigate the effect of enzyme concentration on initial rate of reaction. Other enzymes, including catalase, could be used to investigate the effect of enzyme concentration on initial rate of reaction. Catalase is not released by the pancreas: it occurs in most cells to break down toxic hydrogen peroxide, the by-product of various biochemical reactions.

Why do we measure the initial rate of reaction?

At the start of an enzyme experiment in the lab there will be a fixed amount of substrate in the test tube and no product. As the reaction proceeds, the amount of substrate decreases and the amount of product increases. Therefore the chance of a substrate molecule colliding with an enzyme goes down, so the rate of reaction is slower than at the start. For this reason, when carrying out enzyme catalysed reactions, it is the initial rate of the reaction that is the most valid measurement to take; it will give the rate of the reaction under the desired conditions.

1 Scientific questions and information research

Milk powder contains a white protein called casein. A white suspension of milk powder clears on the addition of the enzyme trypsin. Hydrogen peroxide is broken down by the enzyme catalase, forming water and oxygen gas.

Research relevant information and decide what you think the relationship will be between the enzyme concentration and the initial rate of reaction. Make sure that you understand and explain why we are only interested in the initial breakdown of the substrate. Write down your idea as a hypothesis that you can test. Use scientific ideas to support your prediction.

2 Planning and experimental design

You are provided with the following equipment:

- Standard acidified protease solution or a cylinder of potato tissue (a source of catalase).
- Milk powder or hydrogen peroxide solution (the substrate).
- Standard laboratory glassware and apparatus including a ruler, stopclock and thermometer.
- A colorimeter and cuvette.

NB: Casein will hydrolyse in acid conditions without addition of the enzyme.

Plan an experiment that will test your hypothesis. Make sure your plan:

- includes a hypothesis about enzyme concentration and the breakdown of substrate, with a scientific explanation to support your ideas
- includes a procedure that uses suitable apparatus to produce measurements that will validly test your hypothesis
- includes a method that allows you to assess the initial rate of reaction
- identifies the dependent and independent variables and, where possible, controls or allows for other variables
- has a control and replicates, and that you have explained why these are necessary
- says exactly what measurements you will make and how they will be made
- says how you will make sure the results are valid, accurate, precise and repeatable
- identifies any possible sources of error
- includes a risk assessment with any safety precautions you will take.

Refer to the Practical Skills support sheet for guidance on planning an experiment.

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

On completion of the experiment make sure you have presented your results in the most appropriate way, and identified and explained any trends or patterns in your results, supporting your statements with evidence from your data. Also, using biological knowledge, you should have commented on any variation and possible errors within the data, and proposed changes to your procedure that would improve the experimental results.

The effect of substrate concentration

Having successfully completed the practical work to determine the effect of enzyme concentration, modify your experimental procedure to show how you would investigate the effect of substrate concentration on initial rate of enzyme reaction.

Statement on the practical materials in SNAB Online

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Purpose

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- To develop practical skills.

SAFETY

Ensure eye protection, lab coats and disposable gloves are worn throughout.

All enzymes are potential allergens and skin contact should be avoided. If enzyme solutions are made up from solids this should not be done by students and precautions should be taken to avoid raising dust. Asthma sufferers may be particularly sensitive.

Hydrogen peroxide is corrosive. Directly supervise its use ensuring it is handled with care, avoiding contact with the skin, eyes and clothing.

Ensure scalpels/craft knives are used with care on secure surfaces.

Notes on the procedure

Students should be given the opportunity to plan this experiment themselves. A planning sheet is provided. The experimental work is placed in the context of the reduced enzyme secretions from the pancreatic duct, which occurs with cystic fibrosis (CF). The use of a protease enzyme would strengthen this link, but there are different enzymes and methods that can be used in this experiment. Students will require some guidance before they start planning, regarding the type of enzyme and substrate to use and a method of assessing the initial rate of reaction. Students could be shown the type of equipment available and a class discussion about what should be included in the practical plan is appropriate. 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.

Two possible methods (A and B) are given below. The methods provided are *not* fully comprehensive, but provide a starting point if required. Students need to measure initial rate of reaction. This is done by measuring the slope of the time-course graph at each concentration and plotting a summary graph of initial rate against enzyme concentration.

Either individually or in pairs students could complete an agreed procedure for one of the concentrations and then share results to complete the summary graph.

Some centres have reported very good results for the dried milk experiment, while for others the dried milk powder did not break down. It is always best to check the enzyme activity in advance.

In the ICT support there is a datalogging sheet on monitoring an enzyme-catalysed reaction.

The Core Practical requires investigation of enzyme and substrate concentration. Having completed the practical investigating enzyme concentration, students can plan how to investigate substrate concentration, which would use a similar procedure with the enzyme concentration remaining the same but varying the substrate concentration. If time is available students could complete this in addition to completing the planning activity.

Student method A

You need:	
• Milk powder solution • Test tubes, flat-bottomed tubes or conical flasks • Test tube holder • Stopclock	• Standard protease solution 1% • 5 cm^3 pipettes, syringe or measuring cylinder • Glassware for diluting enzymes

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What do you think will be the effect of reducing the concentration of the protease enzyme on the initial rate of breakdown of the protein found in milk powder? Use scientific ideas to support your idea (hypothesis).

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Procedure

1 Pipette 2 cm^3 of protein solution into a cuvette.

2 Pipette 2 cm^3 of the protease solution into the cuvette. Mix thoroughly and immediately put this cuvette into the colorimeter and start the stopclock.

3 Measure absorbance at suitable time intervals for 5 minutes or until there is little change in reaction.

4 Discard the content of the cuvette and rinse with distilled water.

5 Plot a graph of absorbance against time. Use the graph to determine the initial rate of reaction. This is the initial gradient of the graph and should be the steepest part. Calculate the initial rate by dividing the change in the y-axis by the change in the x-axis values and use whatever units you have plotted on your y- and x-axes.

6 Repeat steps 1 to 5 of the experiment using a range of different enzyme concentrations, ensuring that other conditions are unchanged. Plot a separate absorbance against time graph for each enzyme concentration and calculate an initial rate of reaction from each one.

7 Present your results in the most appropriate way.

8 Identify any trends in your results.

9 Explain any trends or patterns, supporting your statements with evidence from your data and using biological knowledge.

10 State a clear conclusion to your work, summarising what you have found out and comment on the validity of your conclusion.

Comment on the accuracy and precision of your results. Suggest any modifications to your procedure that would improve the experiment.

The effect of substrate concentration

Having successfully completed the practical work to determine the effect of enzyme concentration, modify your experimental procedure to show how you would investigate the effect of substrate concentration on initial rate of enzyme reaction.

Student method B

You need	
• Cylinders of potato tissue • Hydrogen peroxide solution • Buffer solution pH 7.2 • Distilled water • Boiling tube • Bung and delivery tube • 250 cm^3 beakers • Small beaker • 10 cm^3 syringe barrel • 2 × 10 cm^3 syringes or graduated pipettes • Short piece of rubber tubing	• Screw clip • Cork borer • Measuring cylinder • Thermometer • Stopclock • Glass rod • Scalpel or craft knife • White tile • Forceps • Water bath

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Catalase is an enzyme that occurs in most plant and animal cells. It catalyses the reaction:

$2H_2O_2 \rightarrow 2H_2O + O_2$

What do you think will be the effect of reducing the concentration of catalase on the initial rate of breakdown of the substrate, hydrogen peroxide? Use scientific ideas to support your idea (hypothesis).

The initial rate of reaction can be measured by determining the volume of oxygen gas produced in a unit of time using the apparatus shown in Figure 1. Potato tissue provides a source of catalase.

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Use the scalpel/craft knife with care, cutting on a secure surface.

Procedure

1 Set up the apparatus as shown in Figure 1, with the collecting tube filled with water and the screw clip closed.

2 Cut 10 discs of potato, each 0.2 mm thick. Place these in the boiling tube with 5 cm^3 of buffer solution.

3 Add 5 cm^3 of hydrogen peroxide solution to the potato discs. Immediately place the bung and delivery tube firmly into the boiling tube. Place the other end of the delivery tube under the collecting tube.

4 Start a stopclock as soon as the first bubble of oxygen enters the collecting tube from the delivery tube. Collect any gas produced at suitable time intervals for 5 minutes or until there is little change in the volume. Shake the boiling tube gently throughout the reaction period to keep the contents well mixed. Measure the volume of oxygen produced by raising the collecting tube so that the water level in the tube is level with the surrounding water level in the beaker. Wash out the boiling tube thoroughly.

5 Plot a graph of volume of gas produced against time. Use the graph to determine the initial rate of reaction. This is the initial gradient of the graph and should be the steepest part. Calculate the initial rate by dividing the change in the y axis by the change in the x axis values and use whatever units you have plotted on your x and y axes.

6 Repeat steps 1 to 5 of the experiment using a range of numbers of potato discs, ensuring that other conditions are unchanged. Open the screw clip to refill the collecting tube and then tighten again. Plot a separate volume of gas produced against time graph for each enzyme concentration and calculate an initial rate of reaction from each one.

7 Present your results in the most appropriate way.

8 Identify any trends in your results.

9 Explain any trends or patterns, supporting your statements with evidence from your data and using biological knowledge.

10 State a clear conclusion to your work, summarising what you have found out and comment on the validity of your conclusion.

11 Comment on the accuracy and precision of your results. Suggest any modifications to your procedure that would improve the experiment.

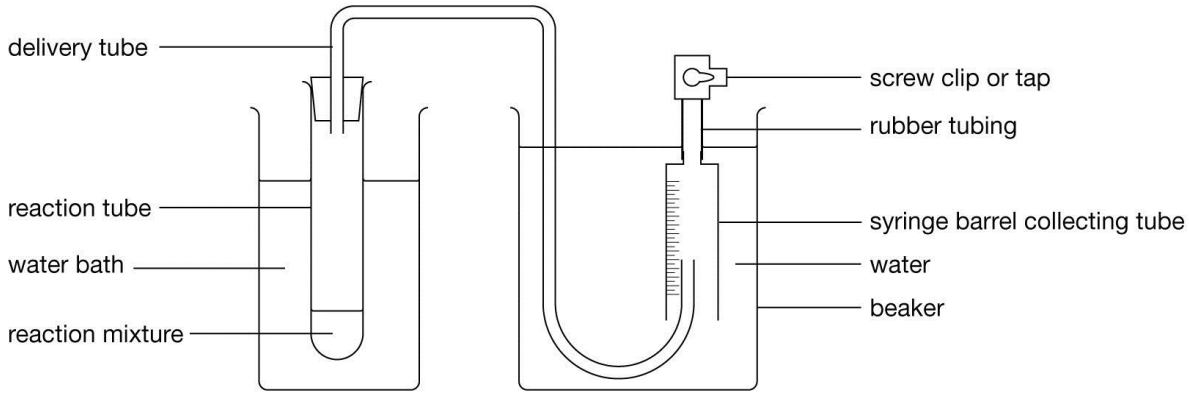

Figure 1 Apparatus for investigating catalase activity.

The effect of substrate concentration

Having successfully completed the practical work to determine the effect of enzyme concentration, modify your experimental procedure to show how you would investigate the effect of substrate concentration on initial rate of enzyme reaction.

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- To investigate how enzyme concentration can affect the rate of reaction.
- To develop practical skills.

SAFETY

Wear eye protection, lab coats and disposable gloves.

All enzymes are potential allergens and skin contact should be avoided. Enzyme powders are irritants and potential allergens. If enzyme solutions are made up from solids this should not be done by students and precautions should be taken to avoid raising dust. Avoid inhalation of powder and wear eye protection and gloves when handling powders. Rinse with lots of water if you come in contact with the enzymes. All spills should be moistened and wiped up immediately. Asthma sufferers may be particularly sensitive. Hydrogen peroxide is corrosive; use with great care and avoid contact with skin, eyes and clothing.

The requirements for this practical will depend on whether the students undertake the planning themselves or are guided. Two basic experimental procedures are provided as a starting point and possible requirements are detailed below. Note that the requirements are given per student per concentration investigated. Students are likely to want to investigate five concentrations each.

Procedure A: Using milk and trypsin

Requirements per student or group of students	Notes
For *each concentration* students investigate, they will need:	
5 cm^3 casein or milk powder suspension (5%)	To make milk suspension use 5 g milk powder in 100 cm^3 water. Marvel® has been found to be the best milk powder to use: it is almost fat-free.
5 ml trypsin solution	Mix 0.5 g trypsin powder in 100 cm^3 water. Add enough alkali (for example, dilute sodium hydroxide) while mixing it up to produce a pH of 9. If making up enzyme solutions *do not* heat to dissolve. Students will also need to dilute this standard solution to give 0.1%, 0.2%, 0.3% and 0.4% solutions.
Test tubes, flat-bottomed tubes, or conical flasks	
Test tube holder	
Stopclock	
Two 5 cm^3 pipettes syringes or measuring cylinders	
50 cm^3 beaker	
Eye protection	

Procedure B: Using catalase and hydrogen peroxide

Requirements per student or group of students	Notes
Cylinder of potato tissue	Students can cut these for themselves using a cork borer and white tile.
Hydrogen peroxide solution	20 volume.
Buffer solution pH 7.2	
Distilled water	
Boiling tube	
Bung and delivery tube	
250 cm^3 beakers	
Small beaker	
10 cm^3 syringe barrel	To collect the oxygen evolved, a small measuring cylinder could be used as an alternative, but the syringe barrel with a rubber tube and screw clip allows the collecting tube to be filled with water very easily by loosening the screw clip.
2 10 cm^3 syringes or graduated pipettes	
Short piece of rubber tubing	
Screw clip	
Measuring cylinder	
Thermometer	
Stopclock	
Glass rod	
Cork borer	To cut cylinders of potato.
Scalpel or craft knife	
White tile	
Forceps	
Water bath	Beaker of water to maintain the reaction tube at a constant temperature would be adequate.

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