MICROSCALE BIOLOGY

4 microscale experiments for BGE Biology

Supports delivery of
SCN 3-12a, SCN 4-13b & SCN 3-19a;
N5 Cell Biology KA4

Page

1 **Microscale Catalase**

Investigating the effect of hydrogen peroxide concentration on catalase activity.

2 **Microscale Dopa Oxidase**

Investigating the effect of inhibitors on a biological reaction.

3 **Microscale Titration**

Investigating the relative vitamin C content of different fruit juices.

4 **Microscale Amylase**

Investigating the effect of enzyme concentration on amylase activity.

This guide provides suggested aims that can be adapted as appropriate for your learners. The activity boards are available in powerpoint format to allow editing.

INTRODUCTION

Microscale Biology - The advantages

This guide comprises 4 experiments "in miniature". Some of the benefits of microscale work include a reduction in the volume of consumable reagents, limited use of glassware and associated clean-up time and quick replication times. In addition, it promotes a focus on accurate measurements and fine motor skills.

The practical work outlined in this guide is consistent with our vision of providing robust, inexpensive, versatile and reliable experiments that demonstrate the link with living things.

The Experiments

There are four experiments in this guide. Three of the experiments introduce learners to enzymes as biological catalysts that can be used to speed up reactions. One of these enzymes, **catalase**, is often used in industry for a variety of processes and provides an opportunity to address SCN 4-13b. The final experiment looks at the vitamin C content of citrus fruits and serves as a springboard into discussions focused on healthy lifestyles and a balanced diet, within the wider context of the digestive system (SCN 3-12a).

Although independent variables have been suggested for each experiment, these can be adapted and the accompanying activity boards are available in powerpoint format for editing purposes (also in PDF format).

Health & Safety

For each experiment, health and safety guidance is included to support risk assessment within your setting. The lower volumes of all reagents reduces the risks associated with identified hazards.

MICROSCALE CATALASE

A microscale enzyme study

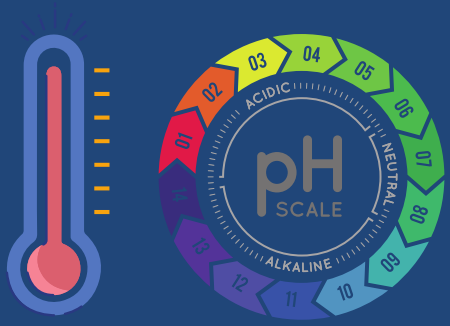
Catalase is a common enzyme found in nearly all living organisms exposed to oxygen. An enzyme is a protein that speeds up biological reactions. Catalase speeds up the break down of a harmful chemical called hydrogen peroxide, which is a by-product of cell metabolism. The products of this reaction are oxygen and water.

hydrogen peroxide (substrate) → catalase (enzyme) → **oxygen + water** (products)

Without catalase, hydrogen peroxide would cause oxidative damage and the production of reactive oxygen species. Consequently, catalase is crucial to protecting the cell from these potentially damaging species.

A number of factors are known to affect enzyme reactions, including:

- temperature
- pH
- substrate concentration



Each of these factors could be investigated using the protocol below.

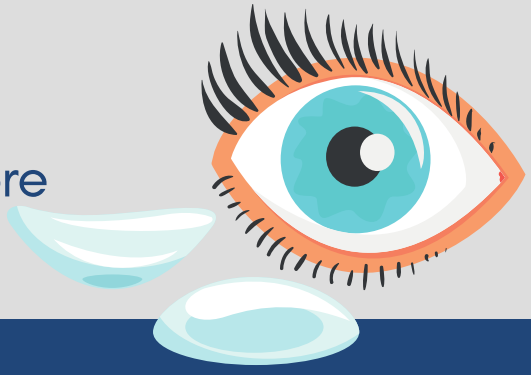
COMMERCIAL USES OF CATALASE

- In the food industry for removing hydrogen peroxide from milk prior to cheese production.

- In food wrappers where it prevents food from oxidising.

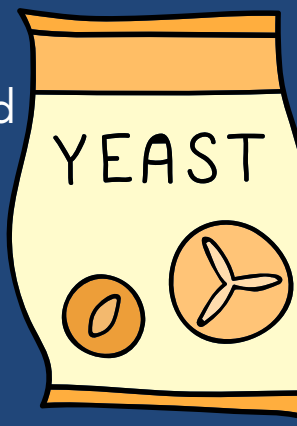
- In the textile industry to remove hydrogen peroxide from fabrics to make sure the material is peroxide-free.

- In contact lens hygiene – a few contact-lens cleaning products disinfect the lens using a hydrogen peroxide solution. A solution containing catalase is then used to decompose the hydrogen peroxide before the lens is used again.



HEALTH & SAFETY

When starting an experiment with a class, you must ensure you are comfortable and familiar with the risk assessment. What are the hazards, the level of risk and control measures that must be put in place? Is the current risk assessment appropriate for your learners? Do you need to make adjustments? This page on the SSERC website provides a Risk Assessment template and information about Dynamic Risk Assessments.



HAZARD	RISK	CONTROL MEASURES
Yeast suspension	Baker's yeast is a Level 1 organism, as outlined in the SSERC Code of Practice, and poses no risk to learners. Can be used in experiments by teachers with no specialist training.	Suspensions of Baker's yeast can be disposed of using good domestic hygiene practices. Page 7 of the Code of Practice provides full guidance, if required.
Hydrogen peroxide SSERC hazardous chemical database	This protocol suggests small volumes of 20vol hydrogen peroxide. At this concentration, a "health hazard" warning is highlighted: • risk of skin burns • eye damage • harmful if inhaled or swallowed	Wear eye protection. Teacher or technician should dilute the hydrogen peroxide to the working concentration(s). For **S1-S2 learners**, the concentration of hydrogen peroxide could be reduced to **1 vol, 5vol and 10vol**.

MATERIALS

This experiment investigates the effect of substrate concentration on enzyme activity. The substrate is hydrogen peroxide and the enzyme is catalase. As oxygen is formed, foam will be observed on the activity boards. The diameter of the foam circle should be measured, using a ruler, as a measure of enzyme activity.

AIM

To investigate the effect of substrate concentration on catalase activity.

INDEPENDENT VARIABLE

Concentration of hydrogen peroxide.

DEPENDENT VARIABLE

Catalase activity, measured indirectly by recording the diameter of the foam circle.

SAMPLE SIZE

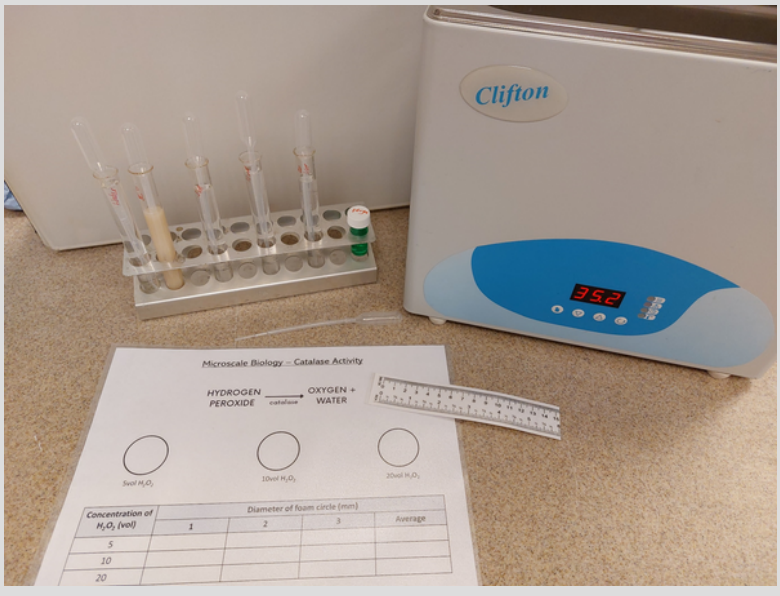
The experiment should be repeated three times at each substrate concentration. This should only be increased if the results are very variable.

SOURCE OF ENZYME

Baker's yeast suspension.

MATERIALS REQUIRED (PER PAIR)

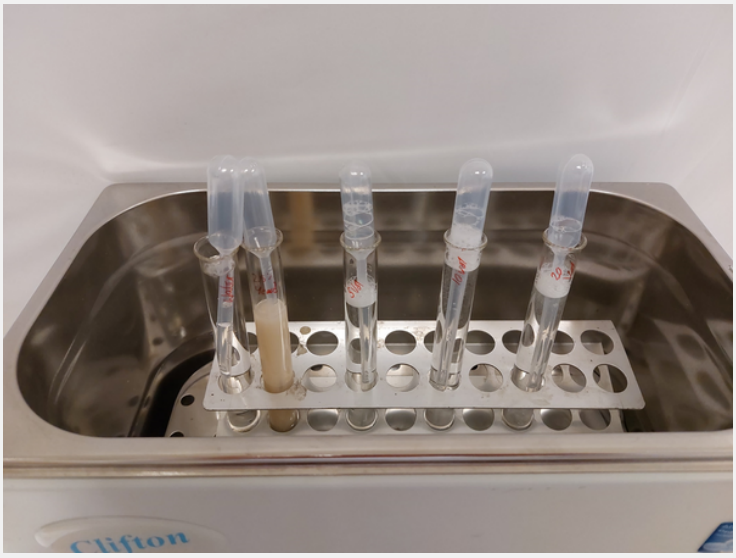
- 2.5% Baker's yeast suspension
- Test tubes containing:
  - 10ml 5vol hydrogen peroxide
  - 10ml 10vol hydrogen peroxide
  - 10ml 20vol hydrogen peroxide
- detergent
- distilled water
- 6x 3ml plastic pipettes
- ruler
- waterbath at 35°C
- stopwatch
- activity board.



METHOD

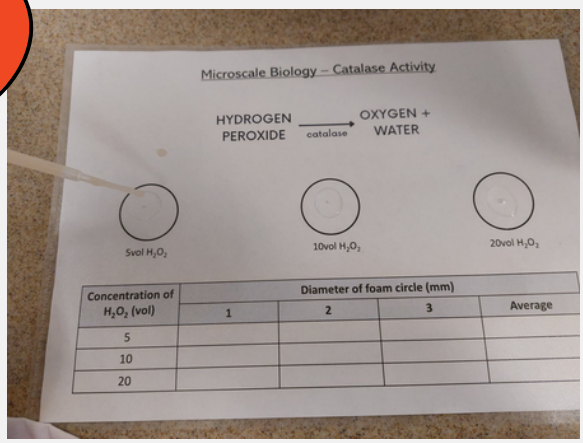
STEP 1

Add 2 drops of detergent to the test tubes of hydrogen peroxide, using a clean pipette. Put the test tubes of hydrogen peroxide and the yeast suspension into the waterbath to equilibrate to the optimum temperature (35°C).



STEP 2

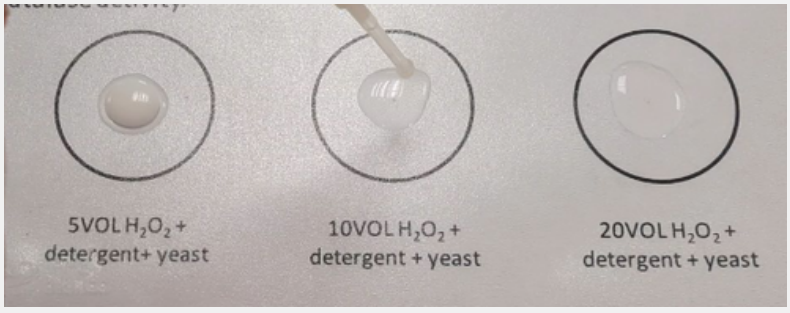
Use the table below to add the reagents to the circles on the activity board. Always add yeast last, as this is the source of the catalase enzyme.



Reagent	Drops of reagent to add to circle		
	5 vol	10 vol	20 vol
H_2O_2 + detergent	5	5	5
Water	3	3	3
2.5% yeast	2	2	2

STEP 3

Leave the reaction for 3 minutes and then measure the diameter (in mm) of the foam circle, using a ruler. Wipe the activity board clean and repeat the experiment a further two times.

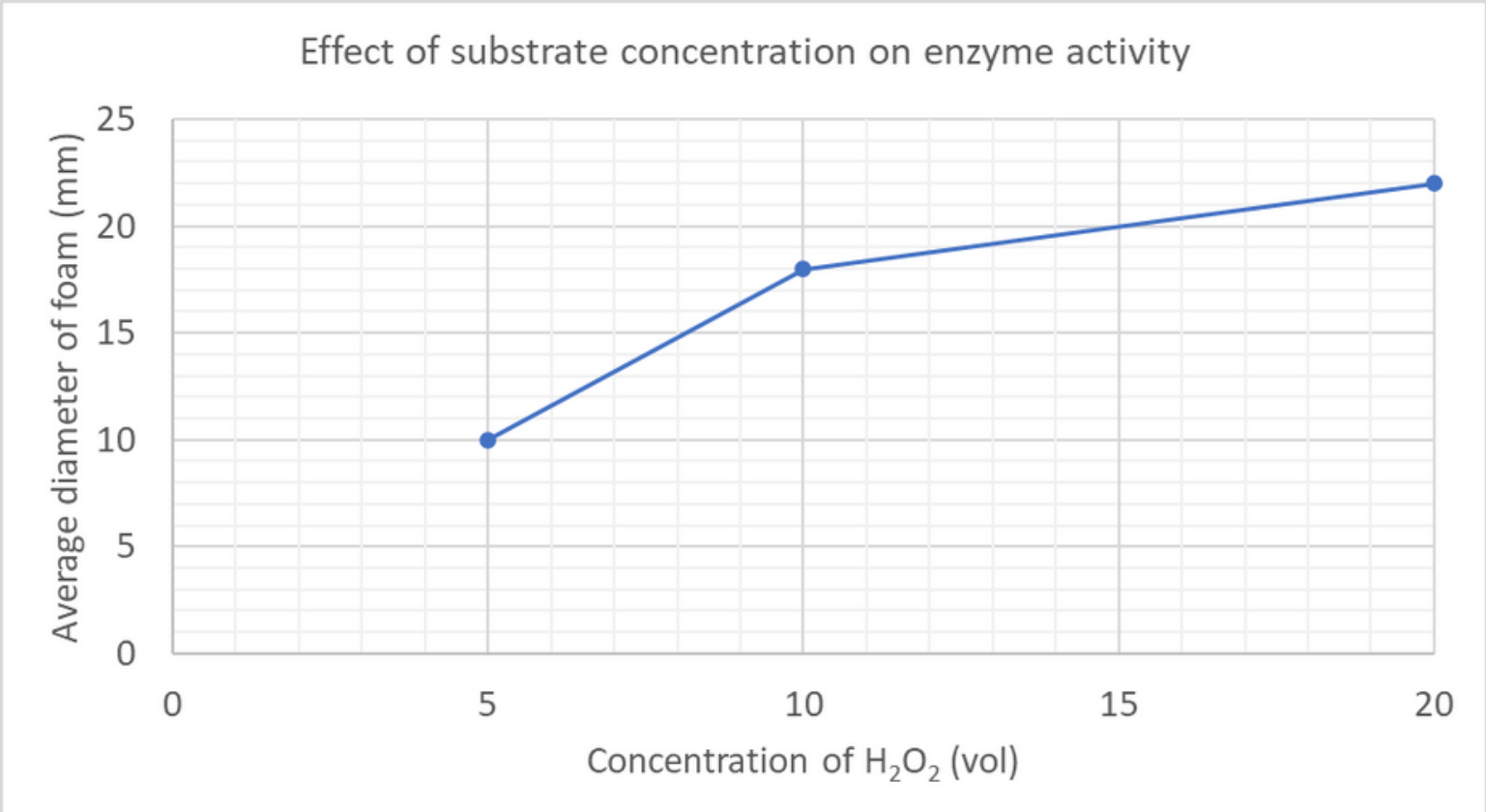


RESULTS

Concentration of H_2O_2 (vol)	Diameter of foam (mm)			
	1	2	3	Average
5				
10				
20				

TYPICAL RESULTS:

Concentration of H_2O_2 (vol)	Diameter of foam (mm)			
	1	2	3	Average
5	10	11	10	10
10	17	17	19	18
20	22	21	24	22



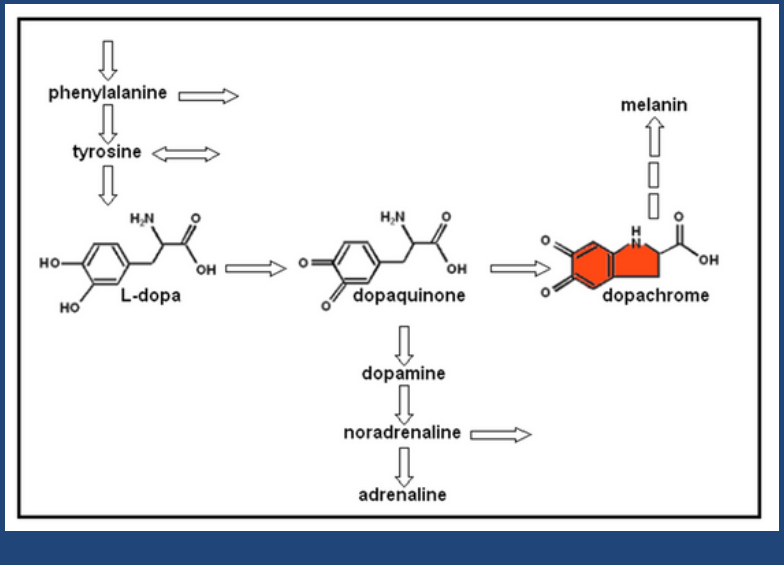
Learners should now reflect on their aim to form a conclusion. Another useful exercise at this point is to evaluate the method.

MICROSCALE DOPA OXIDASE

A microscale enzyme study

In the BGE, learners are often introduced to catalase as the classic example of an enzyme. However, dopa oxidase is a fantastic alternative – it is quick and simple to extract from bananas, reinforcing the idea that enzymes are found in all living things. No specialist equipment is required for this experiment (although a centrifuge can be used to make the enzyme extraction "cleaner") and the product is formed very quickly.

In this experiment, dopa oxidase (enzyme) converts L-dopa (substrate) to a product called dopaquinone. This is then sponentaously converted to a coloured product called dopachrome. Dopachrome is orange/red in colour and so its production can be monitored visually.



Dopa oxidase is an interesting enzyme to study because its activity is linked to other reactions including:

- the breakdown of phenylalanine, leading to wider discussions about phenylketonuria (PKU).
- the production of dopamine, an important neurotransmitter.
- the production of melanin, involved in pigmentation.

HEALTH & SAFETY

When starting an experiment with a class, you must ensure you are comfortable and familiar with the risk assessment. What are the hazards, the level of risk and control measures that must be put in place? Is the current risk assessment appropriate for your learners? Do you need to make adjustments? This page on the SSERC website provides a Risk Assessment template and information about Dynamic Risk Assessments.

HAZARD	RISK	CONTROL MEASURES
Dopa oxidase	Since this enzyme has been extracted from a natural source (rather than a powder), there is no risk associated with it.	No control measures required.
L-dopa	At the concentration and volume used in this protocol, L-dopa presents no hazards.	No control measures required.
Copper sulfate SSERC hazardous chemical database	At the concentration and volume used in this protocol, copper sulfate presents no hazards.	No control measures required.

MATERIALS

This experiment investigates the effect of inhibitors on dopa oxidase activity. Depending on the stage of your learners, this protocol can be adapted to investigate alternative independent variables, e.g. substrate or enzyme concentration or the effect of pH by including a range of buffers (replacing water with buffer). The dependent variable is enzyme activity, measured indirectly by a qualitative assessment of the colour observed after 3 minutes.

AIM

To investigate the effect of inhibitors of dopa oxidase activity.

INDEPENDENT VARIABLE

Type of inhibitor.

DEPENDENT VARIABLE

Dopa oxidase activity, measured indirectly by recording the colour of the product formed.

SAMPLE SIZE

The experiment should be repeated three times. This should only be increased if the results are variable.

SOURCE OF ENZYME

Banana.

MATERIALS REQUIRED (PER PAIR)

Stage 1 - extract the enzyme

- Banana
- plastic bag
- distilled water
- 2x 3ml plastic pipettes
- scissors
- 2x microfuge tubes (optional)
- micro-centrifuge (optional)
- bijou bottle
- marker pen

Stage 2 - the microscale assay

- Enzyme from step 1
- 10 mmol/L L-dopa
- Beaker of green tea (add 1 green tea bag to 50ml boiling water and leave for 2 hours)
- 10 mmol/L copper sulfate
- distilled water
- 5x 3ml plastic pipettes
- Activity board

METHOD

STAGE 1

STEP 1

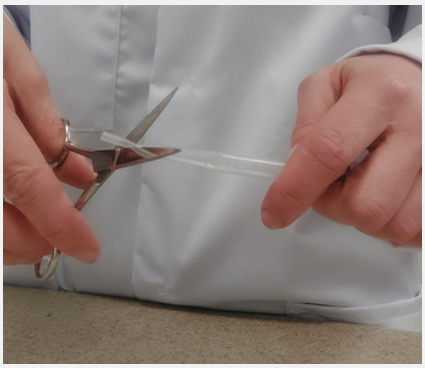
Break off a small piece of banana and put it into the plastic bag.

STEP 2

Add 5ml cold distilled water to the bag and mash the banana.

STEP 3

Using the scissors, trim the end off the pipette to make step 4 easier. At this point, you can proceed to step 4 and carry out **centrifugation** or you can remove some of the liquid from the bag and pipette into the bijou bottle, labelling the sample "enzyme".



STEP 4 - CENTRIFUGATION (OPTIONAL)

Using the pipette, divide the banana mixture between 2 microfuge tubes.

STEP 5 - CENTRIFUGATION (OPTIONAL)

Centrifuge the tubes for 2 minutes, balancing the tubes in volume **and** by placing them opposite each other in the rotor.

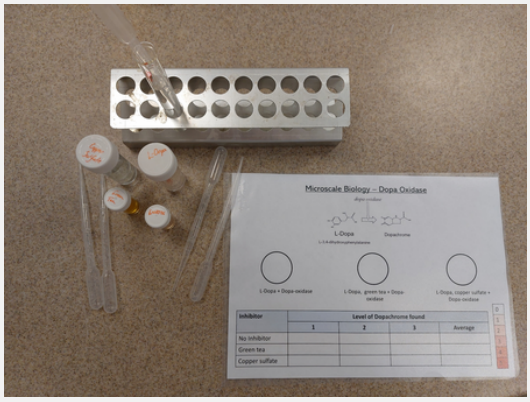
STEP 6

Use a pipette to draw off the supernatant into a clean bijour bottle. Label the bottle "enzyme".

STAGE 2

STEP 7

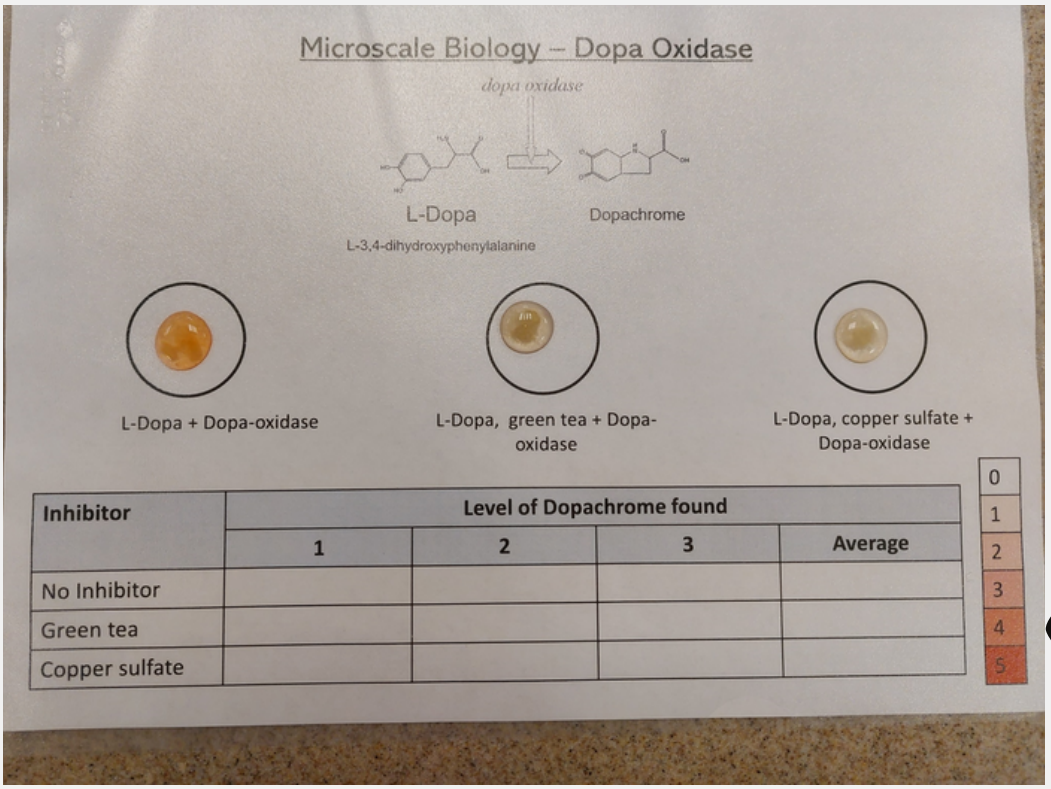
Use the reaction table below to add each reagent to the activity board. Do this in the order listed in the table. Volumes are in "drops".
Start the stopwatch after enzyme addition - leave for 3 minutes.



Reagent	Drops of reagent to add to circle		
	Control	Green Tea	Copper sulfate
L-Dopa	3	3	3
Green tea	-	3	-
Copper sulfate	-	-	3
Water	3	-	-
Enzyme	4	4	4

STEP 8

The appearance of the red colour indicates product formation. use the colour chart to provide a qualitative measure of reaction rate.



Inhibitor	Colour rating after 3 minutes
None	
Green Tea	
Copper sulfate	

0 1 2 3 4 5

STEP 9

Clean the activity board with water and repeat the experiment a further two times. Form a conclusion in response to the aim and evaluate the method.

Did green tea inhibit the reaction? What does this mean?

Discuss what is meant by a positive and negative control.

MICROSCALE TITRATION

Comparing the nutritional content of fruit using a simple titration

For CfE Third Level Science benchmark SCN3-13a, learners should explore the structure and function of organ systems, including the digestive system. As part of wider discussions of health and wellbeing and a balanced diet, it would be appropriate to consider the role of vitamins and minerals. This experiment introduces BGE learners to a simple titration that aims to establish the relative vitamin C content of citrus fruits.

In this experiment, vitamin C (or ascorbic acid) is oxidised to produce dehydroascorbic acid when iodine is titrated into the solution. As this happens, iodine is reduced to iodide ions. This continues until all the ascorbic acid has been oxidised.

- After this point, the iodine reacts with a starch indicator and a blue-black complex is formed.

The more vitamin C present in the solution, the more iodine it takes to reach the "end-point" of the titration.

HEALTH & SAFETY

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HAZARD	RISK	CONTROL MEASURES
0.005 mol/L Iodine solution SSERC Chemical Database	At this concentration, iodine poses no health risk. However, iodine can stain skin, clothing and surfaces. Care should be taken to avoid spills.	No control measures required.

MATERIALS

This experiment investigates the relative vitamin C content of citrus fruits. using a simple, microscale titration. The protocol can be adapted to include a range of vegetables or fruit juices. A further layer of complexity could be included by performing a "standard" titration of vitamin C solutions of known concentration. This could then be used to estimate the vitamin C concentration of each juice sample.

AIM

To compare the vitamin C content of citrus fruits.

INDEPENDENT VARIABLE

Type of fruit juice.

DEPENDENT VARIABLE

Relative vitamin C content, determined by the number of drops of iodine required to reach the end-point of the titration.

SAMPLE SIZE

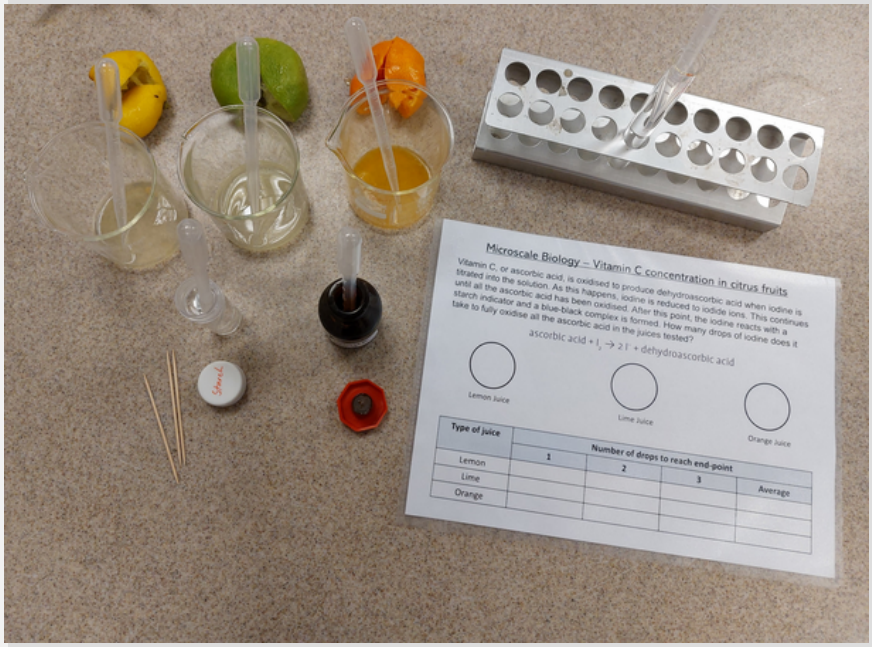
The titration should be repeated three times. Alternatively, it can be repeated until "concordant" results are achieved.

INDICATOR REQUIRED

0.5% starch solution

MATERIALS REQUIRED (PER PAIR)

- 0.005 mol/L iodine solution
- 0.5% starch solution
- Selection of fruit (e.g. lime, lemon, orange)
- knife
- 3x beakers
- muslin
- filter funnel
- 5x 3ml plastic pipettes
- 3x cocktail sticks
- activity board



METHOD

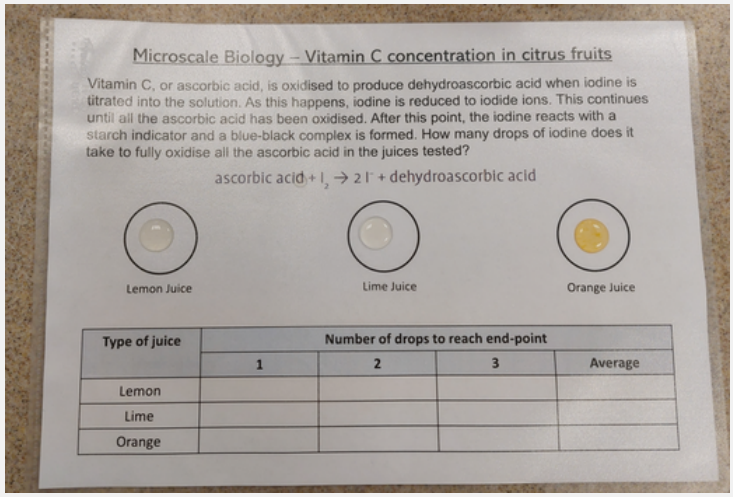
STEP 1

Cut the fruit in half and squeeze the juice into a beaker. Pass through muslin, sitting within a filter funnel, to remove any pulp.

Repeat this process for each of the fruits. It is best to use fruits that have a light colour juice - this makes it easier to identify the end-point in the titration.

STEP 2

Use the reaction table below to add each reagent to the activity board. Do this in the order listed in the table. Volumes are in "drops".



Microscale Biology – Vitamin C concentration in citrus fruits

Vitamin C, or ascorbic acid, is oxidised to produce dehydroascorbic acid when iodine is titrated into the solution. As this happens, iodine is reduced to iodide ions. This continues until all the ascorbic acid has been oxidised. After this point, the iodine reacts with a starch indicator and a blue-black complex is formed. How many drops of iodine does it take to fully oxidise all the ascorbic acid in the juices tested?

ascorbic acid + I_2 → $2I^-$ + dehydroascorbic acid

Lemon Juice | Lime Juice | Orange Juice

Type of juice	Number of drops to reach end-point			
	1	2	3	Average
Lemon				
Lime				
Orange				

Reagent	Number of drops added to reaction circle
Juice	9
Starch indicator	1

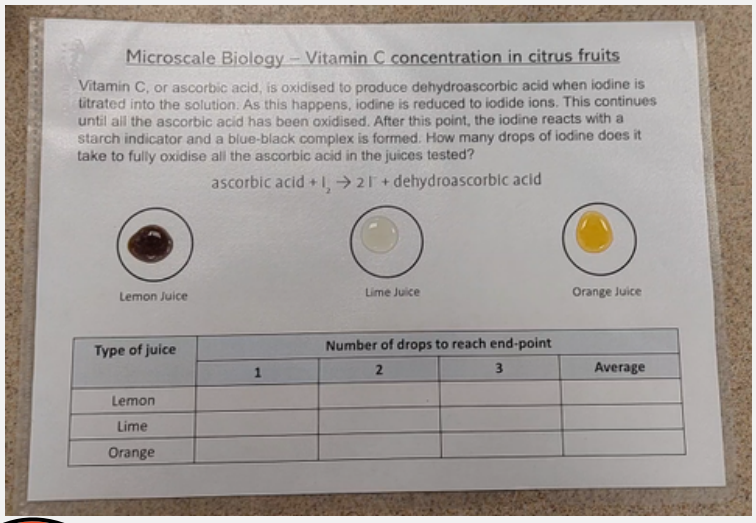
STEP 3

Starting with the first juice in the left-hand circle, add iodine drop-wise to the reactants. After each drop, mix with a clean cocktail stick. If the blue-black complex disappears, continue adding iodine. When the blue-black complex remains after mixing, record the total number of drops of iodine that were added.

STEP 4

Repeat Step 3 with the remaining juices, using a fresh cocktail stick each time.

Repeat each titration a further two times. Alternatively, your teacher might wish for you to repeat this until you achieve "concordant results".



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ascorbic acid + I_2 → $2I^-$ + dehydroascorbic acid

Lemon Juice | Lime Juice | Orange Juice

Type of juice	Number of drops to reach end-point			
	1	2	3	Average
Lemon				
Lime				
Orange				

Type of juice	Number of drops of iodine required to reach end-point			
	1	2	3	Average
Lemon				
Lime				
Orange				

STEP 5

Clean the activity board with water. Form a conclusion in response to the aim and evaluate the method.

Which citrus fruit contains the most vitamin C?

What was the purpose of the starch solution that was combined with the fruit juice?

Alternative ideas:

- Test the effect of cooking processes on the vitamin C content
- Compare the vitamin C content of fresh fruit juices with packaged fruit juices.

TECHNICAL NOTES

- **To prepare 1L iodine solution (0.005 mol/L):** Combine 2 g of potassium iodide and 1.3 g of iodine in a small beaker. Dissolve in a few drops of distilled water. Transfer contents to a 1L volumetric flask, washing down to rinse all traces of the solution. Make up to 1L with distilled water.
- **To prepare 50cm^3 starch indicator (0.5%):** In a beaker, add 0.25g soluble starch to a small volume of distilled water. Stir to form a paste. Add distilled water to a final volume of 50cm^3 and mix over heat until dissolved. Cool before use.
- **To prepare standard ascorbic acid solutions**: Use a vitamin C tablet (1000mg/ml) dissolved in 200cm^3 water. This gives a stock solution of 5 mg/ml. Use this stock to produce your series of working solutions.
- **Sources of vitamin C:** This protocol has used lime, lemon and pineapple juice squeezed from fresh fruit and then passed through muslin. As noted above, packaged fruit juice could also be compared. Vegetables could be blended with water and used in this protocol. However, the colour of the resulting "juice" should be considered – lighter coloured juices are easier when detecting the endpoint blue-black colour.

MICROSCALE AMYLASE

Supports delivery of N5 Cell Biology KA4

Amylase is a protein molecule called an enzyme. It is formed from a chain of amino acids, arranged in a particular order, that folds to create a unique shape that includes an active site. In humans, amylase is found in the pancreas and salivary glands. It is an example of a degradation enzyme: it speeds up the breakdown of starch (a large carbohydrate molecule) into maltose (a smaller molecule made up of 2 glucose molecules joined together).

starch (substrate) —amylase (enzyme)→ **maltose** (product)

In this experiment, amylase will be collected from saliva. This is safe but each person should work only with their own saliva sample and be responsible for placing used materials in disinfectant at the end of the lesson.

In this protocol, the concentration of enzyme will be altered. However, it can be adapted to investigate the effect of substrate concentration, pH or any inhibitors that might affect the rate of reaction.

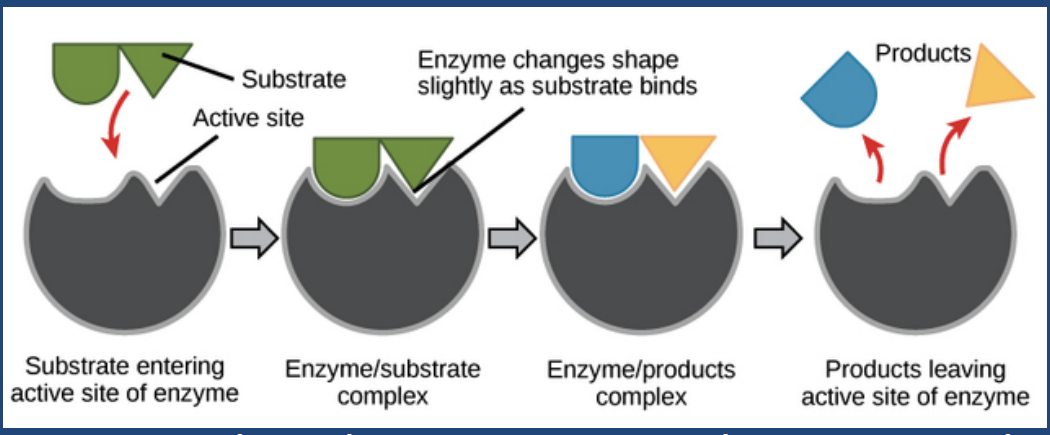


Figure: This image shows a substrate (green, e.g. starch) binding to the active site of an enzyme (grey, e.g. amylase) resulting in the release of small products (blue and yellow, e.g. maltose).

HEALTH & SAFETY

An example risk assessment for this protocol can be downloaded from here. This should be reviewed and evaluated in light of your own setting, e.g. age & stage of learners, etc.

MATERIALS & METHOD

This experiment investigates the effect of enzyme concentration on amylase activity. To reduce the risk to technicians preparing powdered alpha-amylase, salivary amylase will be collected from pupils by swirling a sip of water around their mouth for a few seconds and then transferring the contents to a cup. When this amylase is combined with a starch suspension, the breakdown of starch can be monitored using iodine, which will turn blue-black in the presence of starch. As the concentration of starch decreases, the colour intensity of this blue-black complex will decrease, providing an indirect assessment of the enzyme reaction.

AIM

To investigate the effect of enzyme concentration on amylase activity.

INDEPENDENT VARIABLE

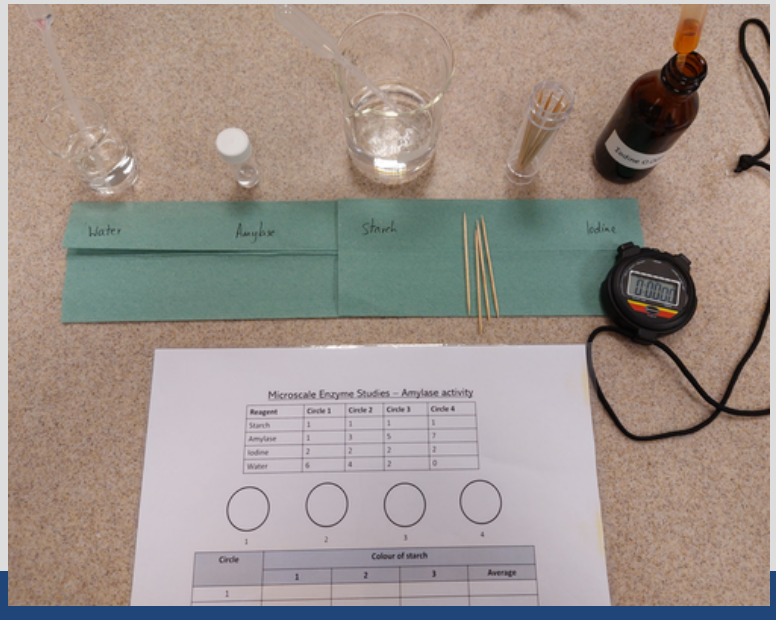
Amylase concentration

DEPENDENT VARIABLE

Enzyme activity, qualitatively determined using the colour of the solution following incubation.

MATERIALS REQUIRED (PER PAIR)

- 0.5% starch suspension
- distilled water
- 0.005 mol/L iodine
- bijou bottle
- cocktail sticks
- marker pen
- cup with small volume of drinking water
- 4x 1ml plastic pipettes
- beaker of water to rinse out pipettes
- activity board (in a polypocket)



STEP 1

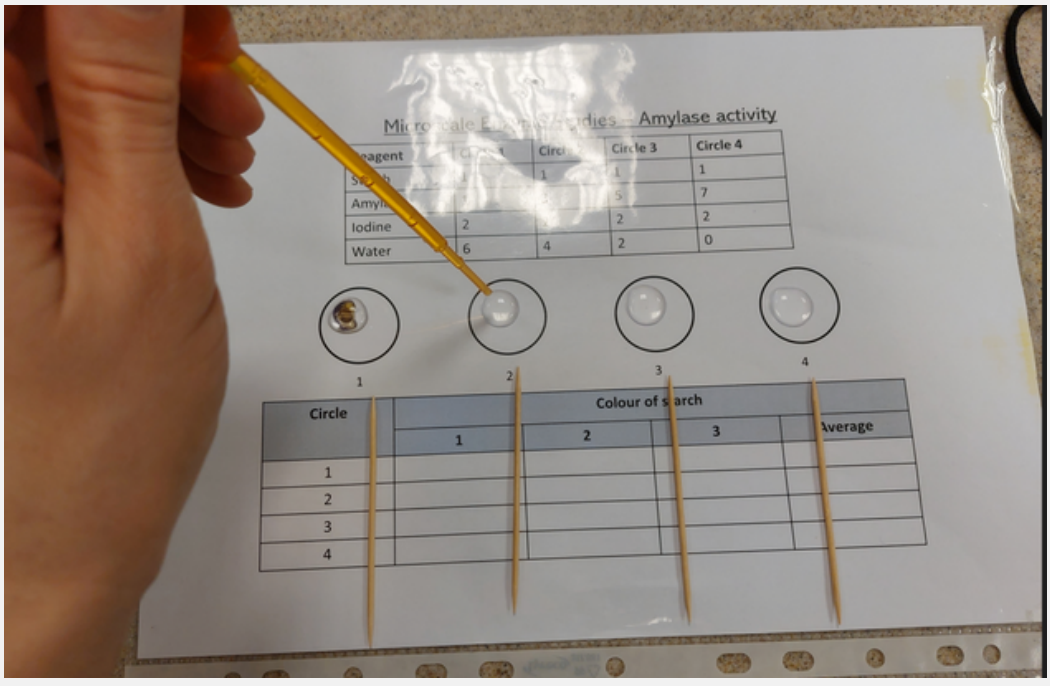
Collect a cup containing a small volume of drinking water. Swirl the water around your mouth for 20s and then transfer the contents back into the cup. Decant the contents of the cup to a bijou, labelled "amylase".

STEP 2

Use the table below to add the correct number of drops of each reagent to the reaction circles. To each reaction circle, add:

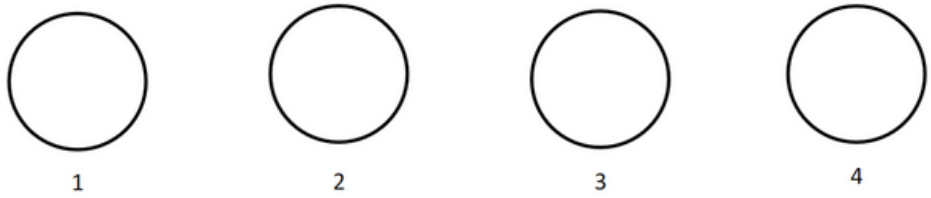
- Water first
- Then amylase
- Then starch
- Wait for 30s
- Then add iodine and mix using a cocktail stick.

Reagent	Circle 1	Circle 2	Circle 3	Circle 4
Water	7	5	3	1
Amylase	1	3	5	7
Starch	1	1	1	1
Iodine	1	1	1	1

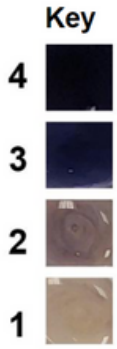


STEP 3

Use the colour chart on the activity board to qualitatively assess the quantity of starch remaining in the sample. The higher the number (the darker the colour), the more starch present, indicating a lower rate of reaction.



Circle	Colour of starch			
	1	2	3	Average
1				
2				
3				
4				



TROUBLESHOOTING

As this protocol involves individual learners using their own saliva as a source of amylase, variability can be expected. If the reaction proceeds too quickly, reduce the incubation time of 30s prior to iodine addition (Image 1). If the reaction proceeds too slowly, increase the incubation time to 60s prior to iodine addition (Image 2).

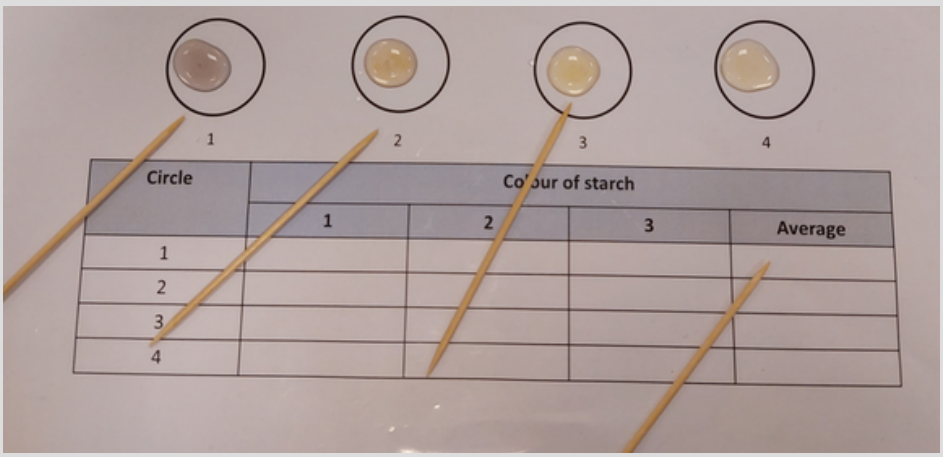
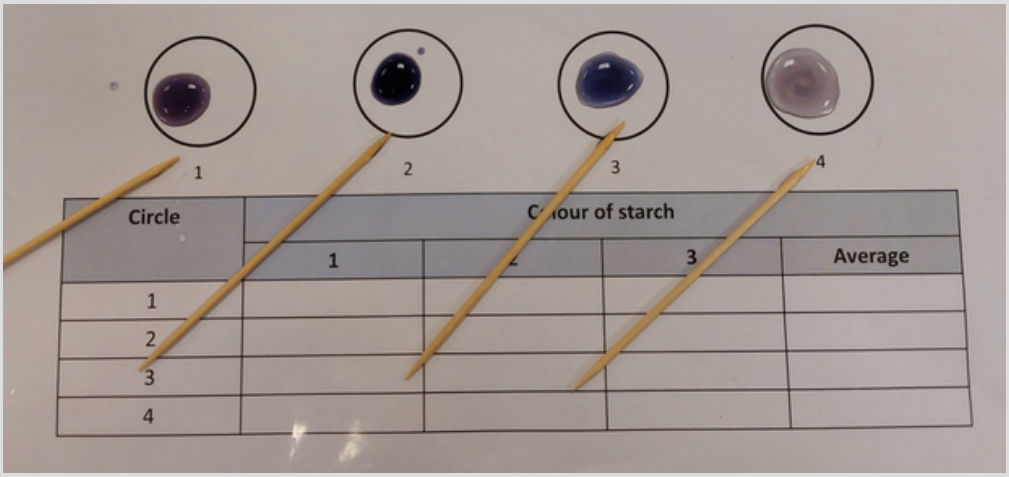
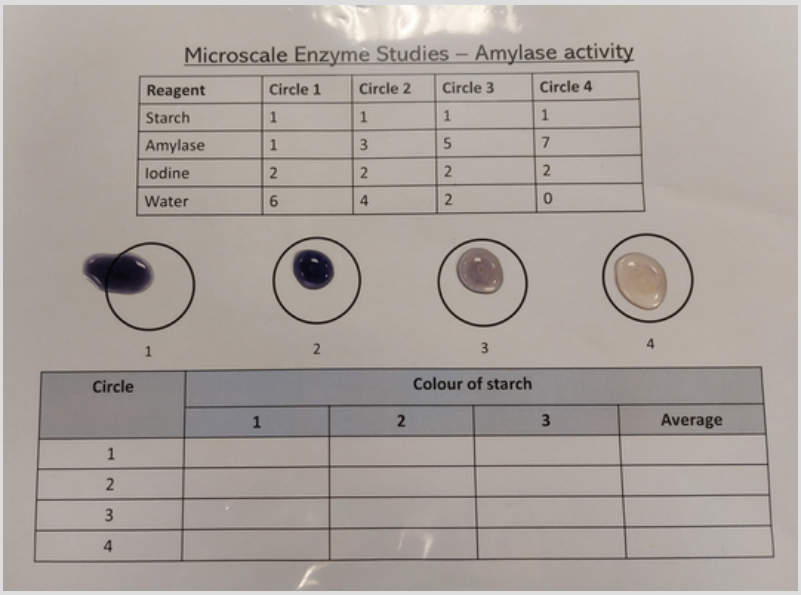


Image 1: Reaction has proceeded too quickly and most starch has broken down. Reduce incubation time.

Image 2: The reaction has proceeded too slowly and the starch has not broken down. Increase incubation time.



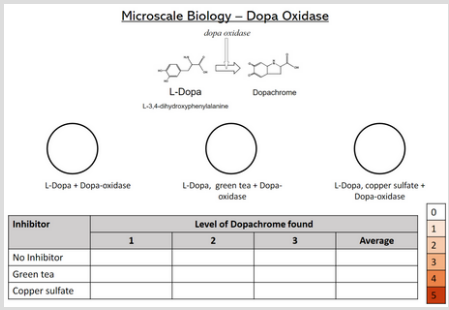


Goldilock zone achieved!

INDEPENDENT VARIABLES

This microscale assay can be easily adapted to monitor the effect of substrate concentration and incubation time. The effect of pH on this reaction could be monitored by substituting water with a pH buffer. Temperature is less easily investigated robustly using this methodology.

SUPPLIER INFORMATION



All activities

- Activity boards - available here as a powerpoint file to allow for editing.

Microscale Catalase activity

- Yeast suspension - made using Allison's Baker's yeast (shown opposite).

Microscale Dopa Oxidase activity

- Microcentrifuge - SSERC have used the Sprout Plus Mini Centrifuge (£262.50, Philip Harris; Product code: B8R09549). Timstar supply the Piccolo Microcentrifuge (CE90620) - £206.18.
- L-Dopa - this can be expensive through traditional suppliers. However, there are alternative sources online and in some health food shops.

L-dopa sourced online

Philip Harris "Sprout" Microcentrifuge

CONTACT

Any questions about this protocol? Contact Annie McRobbie (annie.mcrobbie@sserc.scot)