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.

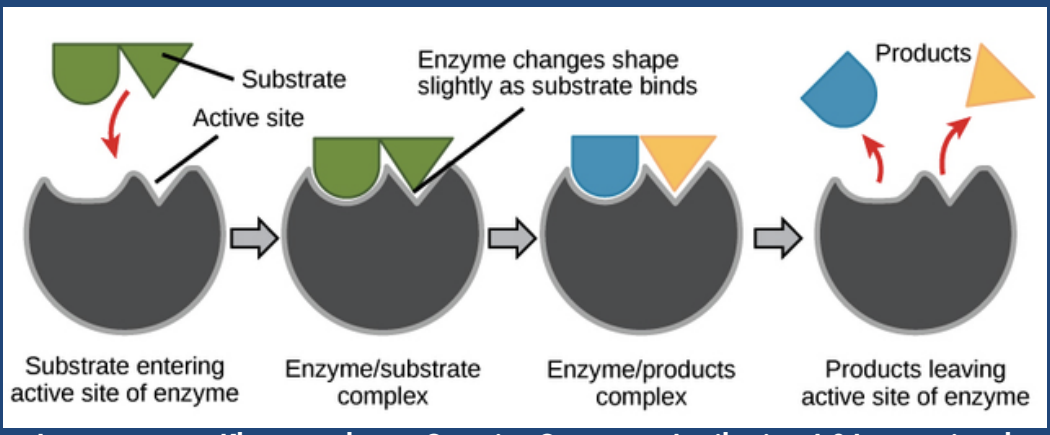


Figure: This image shows a substrate (green, e.g. starch) binding to the active site of an enzyme (grey, e.g. amylase) resulting in the release of small products (blue and yellow, e.g. maltose).

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

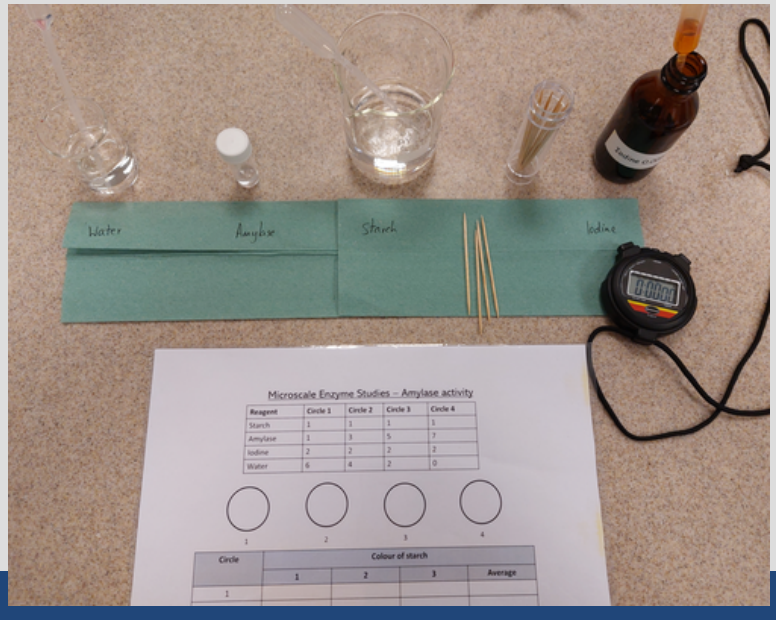
Amylase concentration

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

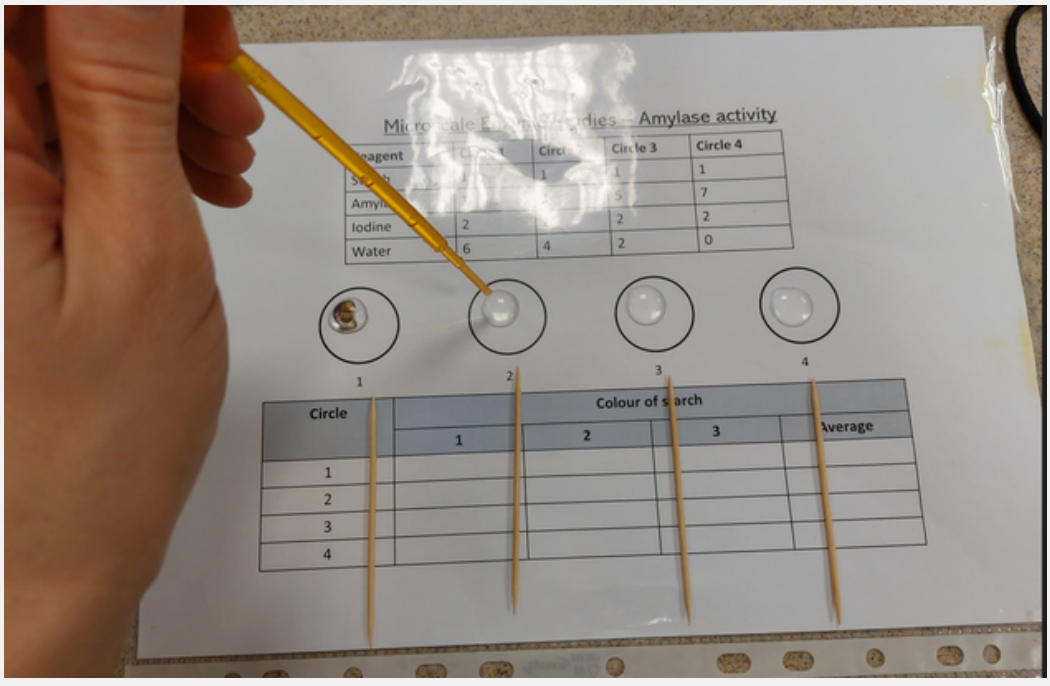
Collect a cup containing a small volume of drinking water. Swirl the water around your mouth for 20s and then transfer the contents back into the cup. Decant the contents of the cup to a bijou, labelled "amylase".

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:

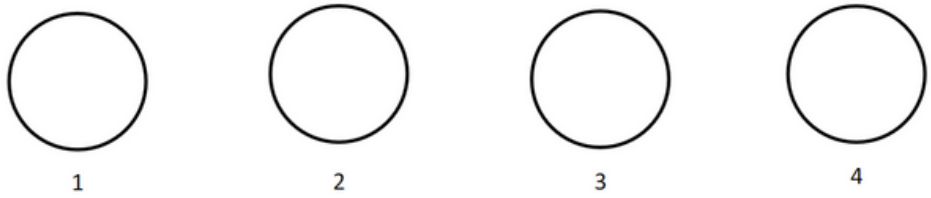
- Water first
- Then amylase
- Then starch
- Wait for 30s
- Then add iodine and mix using a cocktail stick.

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

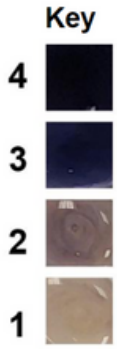


STEP 3

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).

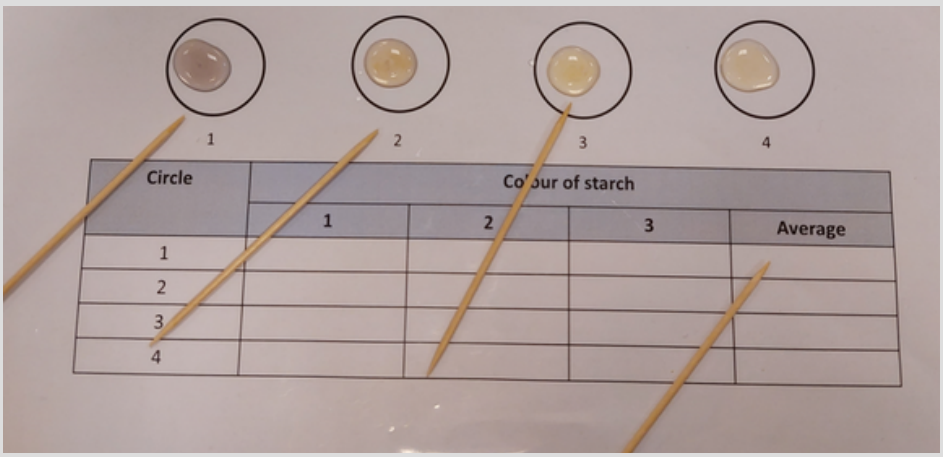
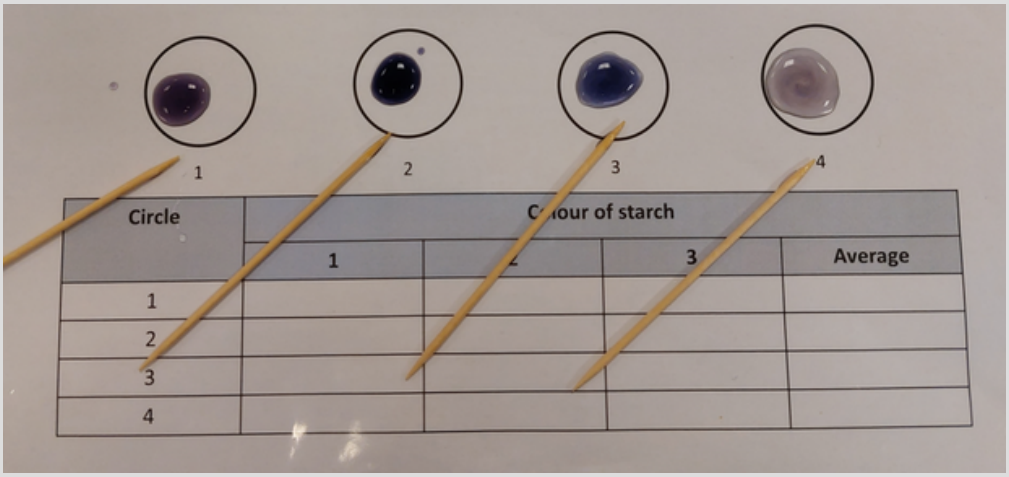
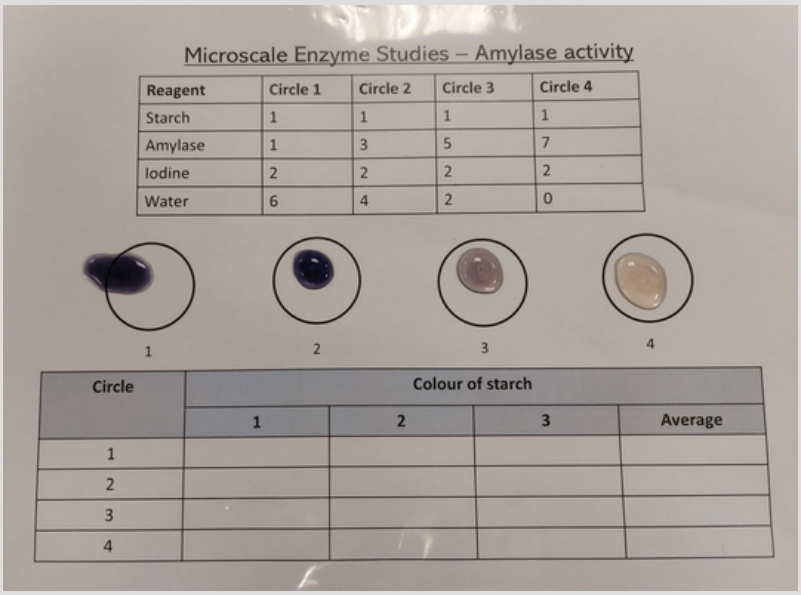


Image 1: Reaction has proceeded too quickly and most starch has broken down. Reduce incubation time.

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.