Guidance for Topic 2 – Practical 1

Investigating the conditions needed for the action of a protease enzyme

Safety

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Guidance

This straightforward practical, which investigates the effect of pH on the digestion of egg albumin by pepsin, is useful as a starting point for students to design their own enzyme investigations. It gives the opportunity to investigate denaturation of enzymes and the rate of an enzyme-catalysed reaction. It can also be used to revise the Biuret test for protein.

Note that this practical may be used in addition to Practical **2** in this topic, or as an alternative to it. It could instead be used during Topic **6**, *Human physiology*, when studying digestion and absorption.

Apparatus and materials

Each student or pair will need:

- Biuret reagent
- 10 cm^3 egg albumin suspension
- 2 cm^3 1% pepsin solution
- 1 cm^3 boiled 1% pepsin solution
- 1 mol dm^{-3} hydrochloric acid
- six test tubes and rack
- pen to mark test tubes
- water bath at 35–40 °C
- four 2 cm^3 syringes

Setting up the practical

Egg albumin suspension should be made up according to the directions on the dried albumin package, or sprinkle 10 g dried albumin on 1000 cm^3 3% sodium chloride solution, leave to stand for one hour, stir gently and store in the refrigerator. Alternatively, a beaten egg albumin can be mixed with 500 cm^3 water and filtered through glass wool to produce the suspension.

1 mol dm^{-3} hydrochloric acid can be supplied in dropping bottles as only a few drops are needed per group.

Biuret reagent can be bought ready prepared to add directly to the test solution. If this is not available, the Biuret test can be carried out as follows.

- Solution A, 2 mol dm^{-3} NaOH (**corrosive**) – 80 g sodium hydroxide (wear eye protection and gloves) made up to 1000 cm^3 with water
- Solution B, 0.2 mol dm^{-3} Cu_2SO_4 – 5 g copper(II) sulphate-5-water made up to 1000 cm^3 with water

Mix solution A with the test solution in the ratio 1 : 1. Add solution B one drop at a time, shaking well after each addition. A purple or pink colour shows the presence of protein.

Supporting the practical

Students may need reminding about the colour change produced by the Biuret test. It can be helpful to highlight the temperature of the water bath and ask why this temperature has been chosen (see Questions and further work).

Answers to questions

1 Digestion breaks down large insoluble molecules to produce small soluble molecules. In this case protein is digested to amino acids by protease (pepsin).

2 Digestion should occur in tube C, where the enzyme is present and the acid provides the optimum pH for it to work. There may be a little digestion in tube A, where the enzyme is present, but because there is no acid the pH is not optimum, so digestion cannot proceed at a fast rate.

3 Digestion does not occur at all in tubes B and D. Tube B has no enzyme present, and in tube D the boiled enzyme is ineffective.

4 Boiling denatures the protease enzyme, which is a protein, so that it can no longer catalyse the reaction.

5 The temperature of the water bath is approximately human body temperature, the optimum temperature for the enzyme to work.

6 Students should suggest that the pH of the mixture is measured and that a range of different pHs is used. The time taken for the albumin solution to become clear should be noted at each pH (to obtain a rate of enzyme reaction).

Guidance for Topic 2 – Practical 2

Investigating the effect of temperature on enzyme activity

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Guidance

This relatively straightforward practical introduces students to the idea that temperature has an effect on enzyme activity. Just four temperatures are suggested but this can be modified to include a fuller range. The experiment described has no control tube and students should recognise this failure and understand why a control is important.

This practical can be used as the basis for a range of practical work using other enzymes and variables such as substrate concentration or pH.

Note that this practical may be used in addition to Practical 1 in this topic, or as an alternative to it. It could instead be used during Topic **6**, Human physiology, when studying digestion and absorption.

Apparatus and materials

Each student or pair will need:

- 30 cm^3 5% amylase solution
- iodine solution
- 30 cm^3 1% starch solution
- eight test tubes and rack
- pen to mark test tubes
- water baths at 0 °C, 25 °C, 35 °C and 60 °C
- two 5 cm^3 syringes
- dropping pipette
- four stopwatches

Setting up the practical

Amylase can be obtained from a biological supplier; alternatively, students' own saliva can be used. If using saliva as a source of amylase, ensure good hygiene as it is a potential (if unlikely) source of infection. Students should supply saliva for their own investigation only.

Supporting the practical

As they work, students should consider why the tubes are placed in the water baths for 10 minutes before the contents are mixed.

Clearing up

If using saliva as a source of amylase, ensure good hygiene to minimise the small risk of infection. Students should dispose of their saliva after the investigation by rinsing it down a sink and placing the equipment in bleach.

Solutions should be washed down the sink with plenty of water.

Answers to questions

1 In this experiment, the fastest breakdown of starch should occur at 35°C, which suggests that the optimum temperature for amylase is close to this value.

2 No digestion of starch should occur at 0°C or 60 C. At 0 C, the temperature is too cold for a reaction to occur at an observable rate, while at 65 C the enzyme will have been denatured and therefore unable to function.

3 A control tube should contain the substrate but no enzyme, at 35 C. This is important to show that the presence of enzyme is necessary for the digestion of starch.

4 Suggestions should propose a similar investigation using temperatures close to the rough optimum suggested by this experiment. Temperatures at intervals between 25 C and 45 C would provide a good range.

Guidance for Topic 2 – Practical 3

Immobilised enzymes

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Guidance

This practical enables student to learn how immobilised enzymes are prepared and used, on a small scale. It can be used as a starting point to examine how flow rates, size of alginate beads and concentration of substrate affect the activity of the enzyme.

Note that this practical may instead be used during study of Option **B**, *Biotechnology and bioinformatics*.

Apparatus and materials

Each student or pair will need:

- 2 cm^3 lactase (or invertase)
- 30 cm^3 5% lactose found in full fat milk (or sucrose if invertase is used)
- 8 cm^3 2% sodium alginate made in distilled water
- 100 cm^3 2% calcium chloride solution
- two 10 cm^3 syringes
- three 100 cm^3 beakers
- glass rod or spatula
- small sieve
- distilled water bottle
- 10 cm^3 syringe without plunger
- small piece of muslin or nylon gauze
- screw clip
- retort stand
- glucose test sticks (proprietary medical brand such as Clinisticks or Diastix)

Setting up the practical

Full fat milk can be used as a source of lactase. To prepare the 2% sodium alginate made in distilled water, add the alginate slowly to warm distilled water, stirring constantly, and then allow the mixture to cool. The concentration of the enzyme varies with the age and storage of the enzyme, so trials should be carried out to find the most suitable concentration.

Supporting the practical

Students may need help establishing a suitable flow rate. It may be beneficial organise the class so that different groups collect data from different flow rates and then share their results.

Clearing up

Alginate beads and used test sticks can be safely disposed of in normal waste.

Answers to questions

1 Students might suggest producing a range of substrate concentrations by diluting the original lactose or sucrose solution. They might discuss that in a commercial factory it is important to maximise production and reduce costs and wastage. This can be achieved by adjusting concentration and flow rates.

2 The sizes of the alginate beads are likely to be variable due to the method of production. Instead, an automated dispenser could be used to form the alginate beads and eliminate human error in this process. An even bead size ensures that flow through the column is consistent.

Flow rate is dependent on the pressure from the liquid above and will therefore vary as the volume of solution remaining in the column decreases. To overcome this problem, readings of product concentration could be taken at a specific time as the substrate flows through, so that for all replicates of the experiment the flow rate values at that time will be comparable.

Guidance for Topic 2 – Practical 4

Investigating the rate of fermentation in yeast

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Guidance

In this practical, students have the opportunity to investigate how temperature affects the rate of the enzyme-controlled process of fermentation. Students can work individually or in pairs. If time is limited, students can be organised into groups so that a full range of temperatures is investigated by the class.

Apparatus and materials

Each student or pair will need:

- 5% glucose solution (prepared with cooled, boiled water)
- 10% suspension of dried yeast (prepared with cooled, boiled water)
- two syringes or measuring cylinders (5 or 10 cm^3)
- test tubes
- boiling tubes
- bung and delivery tube to fit the boiling tube
- burette or measuring cylinder
- trough of water
- retort stand
- water baths at a range of temperatures (e.g. 15 °C, 25 °C, 35 °C, 45 °C, 55 °C, 65 °C)

Setting up the practical

The suspension of yeast can be prepared approximately 30 minutes in advance to speed up the practical work and enable students to get underway quickly. A 10% suspension is prepared by dissolving 10 g dried yeast in 100 cm^3 of cool, boiled water. 5% glucose is prepared by dissolving 5 g glucose in 100 cm^3 cool, boiled water.

Supporting the practical

The practical is easy to run but it is important that the seal on the bung to the boiling tube is tight, otherwise carbon dioxide will escape rather than being collected and measured.

Answers to questions

1 Fermentation is controlled by enzymes so there is an optimum temperature for the reaction. At this temperature, which is approximately 35–40 °C, the largest volume of carbon dioxide is produced in the set time period. Students should be able to identify inaccuracies in measuring the volume of carbon dioxide in a measuring cylinder and suggest more accurate alternatives. They might also suggest that there could have been inaccuracies in measuring the volumes of the solutions, especially if measuring cylinders were used, rather than syringes. Students may point out that the rate of reaction varies throughout the measurement period, but should note that as long as the same time period is used for each temperature, the volumes of carbon dioxide produced during this time can be used to indicate the relative initial rates of reaction at each temperature.

2 Three measurements are used to calculate an average value to minimise the effect of error. Any significant anomalies should be omitted from these calculations.

3 As water is boiled, dissolved oxygen is removed. Yeast can respire aerobically or anaerobically, so the removal of oxygen favours anaerobic respiration (fermentation).

Guidance for Topic 2 – Practical 5

Investigating chloroplast pigments in nettles using paper chromatography

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Guidance

This practical enables students to gain hands-on experience of chromatography and to investigate the different pigments present in leaves. It can lead to discussion of other types of chromatography and also be modified to investigate the same types of leaves at different times of year. Students should also become aware that only standardised experiments can be used for comparison so R_f values from reference sources must be used carefully.

Apparatus and materials

Each student or pair will need:

- protective gloves
- nettle leaves (or other suitable soft, non-waxy leaves)
- scissors
- pestle and mortar
- pinch of fine white sand
- 10 cm^3 propanone
- filter paper and funnel
- 5 cm^3 chromatography solvent (91% propanone, 9% petroleum ether)
- 100 cm^3 beaker
- 10 cm length of capillary tube
- boiling tube and bung
- test tube rack
- chromatography paper
- pencil

Setting up the practical

The practical should be conducted in a well-ventilated area. Chromatography solvent should be kept in a sealed container until needed and each student or pair supplied with only the very small quantity that is required.

It may help students to know the type of chromatography paper they are using so that they can compare its quality with reference material they may use. The width of the paper chosen should match the boiling tube with which the students are provided.

Supporting the practical

Ensure that students use a pencil to mark the origin (starting point) as ink may run up the chromatogram and spoil the results. Students need to allow the applied pigment to dry between each application so that the spot is not too dispersed. Patience is required here. Encourage them to consider the temperature of the room and type of chromatography paper used so that they can address the questions at the end of the practical worksheet.

Clearing up

Chromatography solvent should be disposed of with care away from any source of sparks or heat.

Answers to questions

1 The tables should have a heading, ruled headed columns and rows, and where appropriate units should be included.

2 Students should be able to identify chlorophylls a and b, xanthophyll and carotene. Other pigments may be present depending on the plant used and the time of year.

3 The pigments named in **2** enable the plant to capture a range of wavelengths of light for photosynthesis. Xanthophyll and carotene are known as accessory pigments. Other pigments are masking pigments, which protect leaves from the effects of excess sunlight.

4 R_f values that students obtain are unlikely to be as accurate as those from reference sources. Students should note the temperature, type of chromatography paper and the solvent used in their work and in reference sources. These will be the reason for some discrepancies and form a good starting point for discussion of reliability of experiments (such as DNA profiling) that are carried out in different laboratories.

Typical R_f values obtained for the pigments are:

chlorophyll a, $R_f = 0.60$

chlorophyll b, $R_f = 0.50$

carotene, $R_f = 0.95$

xanthophyll, $R_f = 0.35$

5 The pigments carotene and chlorophyll a have smaller molecules than chlorophyll b and xanthophyll, and are carried further up the chromatogram.

6 Temperature affects the movement of molecules and the volatility of solvents.

Guidance for Topic 2 – Practical 6

Observing digestion under a microscope

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Guidance

In this practical students should be able to observe large insoluble food molecules such as starch grains and fat globules under the microscope. Diastase (or α amylase) will only affect the starch so that fat and protein samples should remain unchanged. The practical gives the opportunity to revise the three basic food tests, which students need to be familiar with.

Apparatus and materials

Each student or pair will need:

- access to a heated plate
- coverslips
- microscope
- boiling water
- six microscope slides
- 1% diastase solution (or 1% α amylase solution)
- dropping pipette
- iodine solution
- Biuret reagent
- Sudan III solution
- food samples, including:
  - potato, yam or other starchy food
  - egg albumin (soft boiled) or soft cheese
  - full fat milk or cream

Setting up the practical

Iodine solution, Biuret reagent and Sudan III are best supplied in small dropping bottles at the appropriate concentration. The samples of food supplied to the students can be varied according to local availability. Students should be able to smear the foods on the slides to produce a thin layer, which is visible in the microscope but not too thick to prevent the penetration of enzyme or indicator solutions.

Biuret reagent can be bought ready prepared to add directly to the test solution. If this is not available, the Biuret test can be carried out as follows.

- Solution A, 2mol dm^{-3} NaOH (**corrosive**) – 80 g sodium hydroxide (wear eye protection and gloves) made up to 1000 cm^3 with water
- Solution B, 0.2mol dm^{-3} Cu_2SO_4 – 5 g copper(II) sulphate-5-water made up to 1000 cm^3 with water

Mix solution A with the test solution in the ratio 1 : 1. Add solution B one drop at a time, shaking well after each addition. A purple or pink colour shows the presence of protein.

To prepare **Sudan III solution**:

- Wear gloves and eye protection.
- Dissolve 0.5 g Sudan III in 70 cm^3 ethanol (**highly flammable** and **harmful**) and 30 cm^3 wter, using a warm water bath, and filter. (Alternatively, if readily available, 100 cm^3 70% ethanol should be used.)

Supporting the practical

Students may need assistance in producing a thin layer of food on their slides. They may need reminding about how to carry out the tests for protein, starch and fat, so a demonstration at the start of the practical may be helpful.

Answers to questions

1 All the foods, except the yam or potato that was treated with diastase, should look similar to their original condition. Heating may have changed the appearance of egg albumin solution if this is used.

2 The starch grains in potato (or other starchy food) are digested by diastase.

3 The indicators showed the presence of protein, starch and fat at the start and end of the experiment. A change in the indicator provided a visual cue, confirming whether or not digestion had occurred.

4 Digestion converts large insoluble molecules into small soluble molecules that can be absorbed by the small intestine.

Guidance for Topic 2 – Practical 7

Osmoregulation in ciliates

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Guidance

Students may take a little time to identify the contractile vacuoles in the chosen ciliates. Stalked organisms are much easier to see because of their limited mobility. Only a few drops of the different dilutions of salt water are needed. Organisms should be allowed to 'rest' for a period of 2 or 3 minutes between observations to enable them to equilibrate to the new conditions. This should be done away from the heat of the microscope stage and can be achieved by turning off the lamp between readings.

Apparatus and materials

Each student or pair will need:

- culture of ciliates (e.g. *Vorticella* or *Stentor*, stalked ciliates are easiest to observe)
- microscope
- cavity slides
- distilled water
- dilutions of salt water at 0.1%, 0.2%, 0.4% and 0.8% (sea water can be used)
- dropping pipette for each dilution of salt water

Setting up the practical

The ciliates for this practical can be obtained from biological suppliers. Larger specimens are preferable so that students can clearly see the contractile vacuoles.

Supporting the practical

It may help to have reference material available to help students identify the structures within their organisms. Suitable reference material is readily available in textbooks and on the internet.

Answers to questions

1 Students may suggest that contractile vacuoles empty more frequently in lower concentrations of salt solution.

2 Students may suggest that this is because the organisms would have more water to dispose of. They may not consider the concentration of the ciliate's cytoplasm, which will match the concentration of the salt solution when no emptying of the vacuole takes place. Ciliates also use contractile vacuoles to collect excretory waste, such as ammonia, from the cytoplasm. Waste enters by diffusion and active transport. As osmosis draws water from the environment into the cytoplasm, the vacuole moves to the surface and disposes the contents outside the body.

3 Freshwater organisms constantly need to eject water entering their cytoplasm by osmosis, from their freshwater surroundings, to keep the solute concentration in their cells higher than that of the water around them. In sea water, organisms adopt a different osmotic strategy and maintain their solute concentration at the same level as the salt water around them in normal conditions. If marine organisms are put into fresh water or a dilute solution, water will enter their cells by osmosis and they will have to fill and empty their contractile vacuoles more frequently in order to get rid of it.

Guidance for Topic 2 – Practical 8

Measuring the rate of photosynthesis using algae

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Guidance

The purpose of the practical is to allow students to investigate the effect of light intensity on photosynthesis. Algae confined in alginate balls enable the quantity of plant material to be standardised and ensure that the experiment can be conducted on a laboratory scale. Use of hydrogencarbonate indicator provides an opportunity to revise the concept of indicators and a colorimeter is a useful way to introduce light absorbance in a wider context and give an opportunity for ICT.

Note that this practical may instead be used during study of Topic **8**, *Metabolism, cell respiration and photosynthesis (HL)*.

Apparatus and materials

Each student or pair will need:

- culture of algae (*Scenedesmus* sp.)
- 50 cm^3 measuring cylinder or beaker
- 3% sodium alginate solution
- 10 cm^3 beaker
- stirring rod
- 25 cm^3 beaker
- 2% calcium chloride solution
- 10 cm^3 syringe barrel
- retort stand
- distilled water
- small sieve or strainer
- hydrogencarbonate indicator
- stopwatch or clock
- identical lamps (same wattage bulbs)
- light meter
- six translucent glass bottles with lids (McCartney bottles)
- black paper or cloth to cover one McCartney bottle
- heat filters (Perspex screens or cylinders filled with water)
- colorimeter with 550 nm filter (if a colorimeter is not available, a set of sealed bottles containing hydrogencarbonate indicator at different pHs can be used to provide a reference scale)

Setting up the practical

Scenedesmus sp. can be bought from suppliers of microbial cultures, and cultured easily under cool lamps over a period of days before the practical.

Calcium chloride is an **irritant**. Wear eye protection and gloves when making up the solution. Add 2 g calcium chloride to 100 cm^3 distilled water.

To make 3% sodium alginate solution, 3 g sodium alginate is added slowly to 100 cm^3 warm distilled water, stirring constantly. Allow the mixture to cool.

Supporting the practical

Students may need practice in preparing the alginate balls. If colorimeters are not available students will need a reference set of hydrogencarbonate indicator samples for a range of suitable pHs. Hydrogencarbonate should be freshly prepared and stored in closed containers.

Clearing up

Alginate balls can be disposed of in normal refuse. Solutions should be washed away with plenty of water.

Answers to questions

1 Absorbance indicates the degree of pH change in the different bottles and can be correlated with the rate of photosynthesis (and thus removal of carbon dioxide from the solution). At higher light intensities the rate of photosynthesis is greater.

2 Variables:

independent – light intensity

dependent – rate of photosynthesis

controlled – temperature, quantity of algae, volumes of solutions, background light, length of time the experiment is run

3 **a** accuracy – use a pH probe rather than indicator colour change to measure pH, ensure alginate balls are all the same size

b reliability – repeat the experiment, use more than one bottle at each light intensity