Objectives

B1.9 Explain the effects of temperature, substrate concentration and pH on enzyme activity.

B1.10 *Investigate the factors that affect enzyme activity.*

B1.11 Demonstrate an understanding of rate calculations for enzyme activity.

Maths requirements

1a Recognise and use expressions in decimal form.

1c Use ratios, fractions and percentages.

2b Find arithmetical means.

2c Construct and interpret frequency tables and diagrams, bar charts and histograms.

4a Translate information between graphical and numerical form.

4e Draw and use the slope of a tangent to a curve as a measure of rate of change.

Learning outcomes

8th **SB1.9** Describe the effect of temperature on enzyme activity.

8th **SB1.9** Describe the effect of substrate concentration on enzyme activity.

8th **SB1.9** Describe the effect of pH on enzyme activity.

8th **SB1.9** Explain what is meant by the optimum pH/temperature of an enzyme.

9th **SB1.9** Explain why temperature, substrate concentration and pH affect enzyme activity.

9th **SB1.11** Calculate the rate of enzyme activity from experimental data.

Exploring

1. Enzyme activity (pH and/or substrate concentration) – Core practical

Full instructions for an investigation into the effect of pH on enzyme activity are given on Students' sheet CP2 (pH and Enzyme Activity), which is the core practical for this topic. It uses the same reaction (amylase and starch) as for Exploring 1 in *SB1g Enzyme action*. This investigation could be adapted to look at the effect of substrate concentration at a set temperature and pH (e.g. using 2%, 1.6%, 1.2%, 0.8% and 0.4% starch solutions). The reaction between catalase and hydrogen peroxide can be used to investigate the effect of substrate concentration, the instructions for this additional practical are provided on Worksheet SB1h.2.

For investigations into the effect of pH using starch/amylase, the buffer solutions of different pH can be made as follows:

pH	3	4	5	6	7	8
Volume of 0.2 mol dm^{-3} Na_2HPO_4	20.55	38.55	51.50	63.15	82.35	97.25
Volume of 0.1 mol dm^{-3} citric acid	79.45	61.45	48.50	36.85	17.65	2.75

Alternatively, addition of dilute hydrochloric acid and sodium hydrogen carbonate solution (prior to adding the amylase) and testing with pH paper/ meter will give a reasonable range of pHs to test. Human amylase works best at a pH of about 7.

Before the lesson, check the speed of the reaction using 2 cm^{-3} of starch with 2 cm^{-3} of amylase and 1 cm^3 of buffer at pH 6. It should take about 60 seconds. If the reaction is too fast either reduce the enzyme volume or increase the starch volume. If the reaction is too slow increase the enzyme volume or concentration or reduce the starch volume or concentration.

This practical can be followed using datalogging equipment and a colorimeter or light sensor, though this is not always successful. It is advisable to try this out yourself first.

⚠ Safety

Students should wear eye protection while working with iodine solution, and should avoid splashing the solution. Treat splashes on skin or clothing with 0.1 mol dm^{-3} sodium thiosulfate solution until the brown stain is removed, then wash with water. Students should take care when working with water above 50 °C. If using saliva, students should only work with their own saliva. Contaminated apparatus should be rinsed after the activity and placed in bleach or disinfectant solution for about 30 minutes before washing normally.

Support: Restrict the pHs tested by the students to 4, 6 and 8. This should simplify interpretation and produce a peak at pH 6.

Stretch: Ask students to identify the limitations of the method they have used (in particular the limits created by the number of different pHs to be investigated), and consider how best to overcome these given the restrictions of time and equipment available.

Expected results

The optimum pH for human salivary amylase is pH 7. Other amylases may vary from this value, but there should be one pH that clearly allows greater enzyme activity than the others.

The **ALDS** spreadsheet *SB1h pH and enzymes* may be useful to support this activity (see Explaining 1).

The practical on Worksheet SB1h.2 is an optional practical that supports the core practical ideas. For investigating substrate concentration using catalase/hydrogen peroxide: Set up the apparatus as shown in the diagram on Worksheet SB1h.2. Prepare the potato puree in a food blender by peeling the potatoes, chopping the flesh into cubes and blending to a smooth paste. This should be done as close to the lesson as possible, as catalase activity reduces over 2–3 hours. If needed, add a little water to make the paste less viscous. Prepare the range of concentrations of hydrogen peroxide immediately before the lesson. Keep dilutions in clean brown bottles as the peroxide degrades in light. Discard all unused solution, as contaminants in stock bottles can cause explosions after a while. Make sure students use the 100 cm^3 measuring cylinder for collecting gas from concentrations above 20 vol.

If bubbles from the rubber tubing are too large, insert a glass pipette or glass tubing into the open end of the rubber tubing.

The table for Question 3 will need to be displayed on the board. Some students may need the diagram on the **ALDS** presentation *SB1h Substrate concentration* to help them understand why the rate of reaction changes with substrate concentration.

⚠ Safety

Hydrogen peroxide concentrations of 20 vol and above are IRRITANT. Eye protection and clothes protection should be worn. Rinse splashes of peroxide and potato puree off skin as quickly as possible.

Support: Guide students through calculating the rate of oxygen produced, and the analysis of the collated table, including identification of anomalies and calculation of mean values.

Stretch: Expect students to produce their own diagrams to explain the shape of the curve. They should compare their diagrams with the one on the **ALDS** presentation *SB1h Substrate concentration* to identify any differences.

Expected results

The graph produced from mean values for each concentration should be similar to graph E in *SB1h Enzyme activity* in the Student Book. Rate of reaction should increase in proportion to substrate concentration until all enzyme active sites are filled by substrate molecules. Above this concentration, no further increase in rate of reaction will occur unless more enzyme is added to the mixture.

Course resources

Bio Students' sheet CP2

Equipment

In amylase/starch investigation in the effect of pH, for each pH tested: test tube containing 5 cm^3 freshly made 1% starch suspension (mix 5 g soluble starch with a little cold water, pour into 500 cm^3 of boiling water and stir well, then boil until you have a clear solution), test tube containing 2 cm^3 1% amylase solution (or 0.5% pancreatin solution) or saliva collected by student (see instructions in SB1e Exploring 1), water bath at optimum temperature for the enzyme (e.g. 37 °C), buffer solution at a set pH (see table above), 5 cm^3 syringe or pipette, beaker of water for washing pipette, eye protection, 0.01 mol dm^{-3} iodine solution, well tray (spotting tile).

Optional: if using saliva – hypochlorite (bleach) solution or 1% Virkon solution for disinfection of equipment and benches, small beaker or other container for collecting saliva

In catalase/hydrogen peroxide investigation into substrate concentration: range of concentrations of hydrogen peroxide solution, puréed potato, large syringe, two-holed bung with delivery tube in one hole connected to rubber tubing (see diagram), trough containing water, conical flask, 2 cm^3 syringe, 50 cm^3 or 100 cm^3 measuring cylinder, clamp stand and boss, stop clock or stopwatch, eye protection.