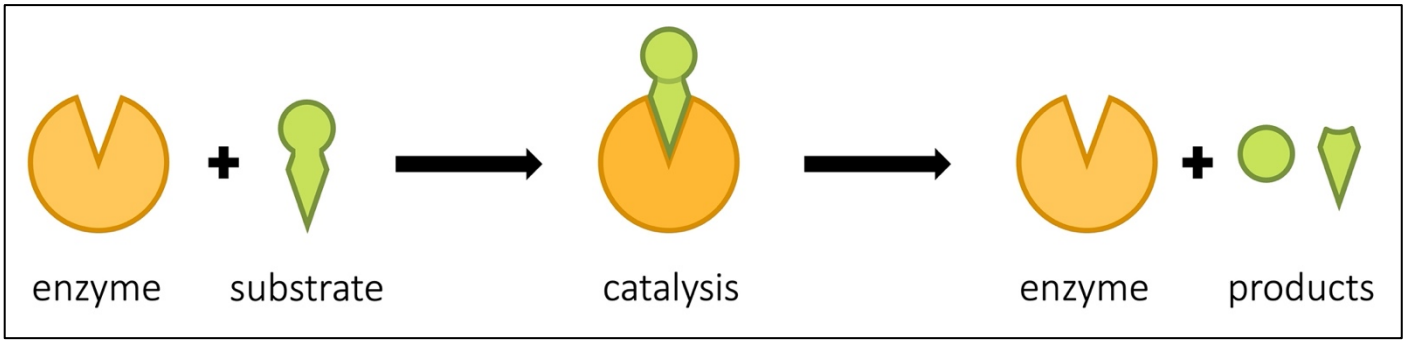
Factors Affecting Enzyme Activity

Introduction

Enzymes are globular proteins that act as catalysts for biological reactions. An enzyme will speed up a reaction rate without being consumed. All biochemical reactions are catalysed by enzymes. An enzyme works by binding to a specific reactant (called the substrate) via a complementary indentation called the active site. According to the induced fit model, the active site undergoes a conformation change to better adhere to the substrate and this stresses the substrate's bonds, catalysing its conversion into a product. Enzymes are also very specific – they only act on one substrate (or a class of related molecules). Typically, only one substrate will fit into the active site.



EXPERIMENT 1: Amylase and Starch

Amylase is an enzyme found in both saliva and the digestive juices of the intestines. It catalyses the hydrolysis of starch (a polysaccharide) into shorter subunits (dextrins or maltose). The extent of the hydrolysis depends on how long the amylase is allowed to react. If the starch is hydrolysed completely, the resulting product is glucose (a monosaccharide).

Iodine forms a blue-black complex when reacted with starch but does **not** react with glucose. If iodine is added to a glucose solution, the only colour seen is the brown-yellow colour of the iodine. Hence, the activity of amylase can be measured according to the colour of a starch solution once iodine is added. If the solution turns blue-black, the amylase activity is low (starch has not been hydrolysed); whereas if the solution turns brownish-yellow, the amylase activity is high (all the starch has been hydrolysed).

Methodology

1. Set up three test tubes in a test tube rack (label 1 – 3).
2. In tube 1, add 4ml of 1% starch solution and 4ml of amylase solution.
3. In tube 2, add 4ml of 1% starch solution and 4ml of deionised water.
4. In tube 3, add 4 ml of deionised water and 4 ml of amylase solution.
5. Place the test tube rack in a 37ºC water bath for 10 minutes.
6. Remove tubes from water bath, add three drops of iodine solution to each tube and mix.
7. Transfer contents to a cuvette and measure the absorbance of green light with a colourimeter.
8. Record your observations.

Results

	Tube 1	Tube 2	Tube 3
Qualitative Observations			
Colourimeter Reading			

Discussion

1. Tube 1 measures the effect of amylase on starch hydrolysis. What is the role of tubes 2 and 3?

2. A colourimeter measures absorbency by shining a light of a particular wavelength on a solution and measuring how much of that wavelength passes through (high value = more absorbance). Suggest why the absorbency of green light was measured via colourimetry in this experiment.

3. Colourimeters have lower accuracy when values are greater than 2. Suggest how the method could be modified to improve the reliability of the data if recorded values were greater than 2.

EXPERIMENT 2: Catalase and Hydrogen Peroxide

Hydrogen peroxide is produced as a waste product in every cell of the human body. It is a by-product of many normal chemical reactions. If the cells did not break down the hydrogen peroxide, they would die. In cells, the enzyme catalase works on its substrate hydrogen peroxide converting it to water and oxygen.

When paper discs are soaked in catalase and then immersed in a solution of hydrogen peroxide, the catalase-soaked disc begins to produce oxygen gas which causes the disc to float. The time taken for the disc to resurface can be used to indicate the rate of activity (high activity = less time).

Methodology

1. To a 200ml beaker, add 100ml of 1% hydrogen peroxide solution (beaker 1).
2. To a second 200ml beaker, add 100ml of 0.5% hydrogen peroxide solution (beaker 2).
3. Using a hole puncher, punch 6 paper discs from filter paper.
4. Using a pipette, add 1ml of 0.05% catalase solution to a well of a multi-well plate.
5. Using forceps, submerge a paper disc in the catalase solution for 10 seconds, then drop the disc on the surface of the hydrogen peroxide in beaker 1.
6. Begin timing as soon as the disc touches the surface and measure how long it takes (in seconds) for the disc to resurface.
7. Repeat steps 5 and 6 for beaker 2.
8. Conduct three trials in total for each beaker and record your observations.

Results

	Time taken for disc to resurface (seconds)				
	Trial 1	Trial 2	Trial 3	Average	Standard Deviation
1% H_2O_2					
0.5% H_2O_2					

Discussion

1. Which set of data is more precise? How can you tell?

2. The precision of a data set is impacted by *random errors* (uncontrolled variables). Suggest two random errors that may have affected the precision of the results.

3. The accuracy of a data set is impacted by *systematic errors*. Suggest one potential systematic error that may have affected the accuracy of the results.

EXPERIMENT 3: Self-Design Experiment

Your task is to design an experiment using one of the two methodologies outlined above, to test the effect of a factor that affects enzyme activity. This could include temperature, pH, substrate concentration or an inhibitor (silver nitrate can inhibit catalase activity, while white kidney beans contain amylase inhibitors).

You may work in small groups (three students maximum) to collect the data. Five different independent variable conditions should be tested (allows for generation of a reliable trend line) and six trials should be undertaken (allows for the calculation of standard deviation and the removal of any outliers).

Following the collection of data, each student must complete an individual experimental report. This report should conform to the grading requirements for the internal assessment and should therefore include the following sections:

- Research Question
- Introduction
- Hypothesis
- Variables
- Material / Method
- Results
- Discussion
- Evaluation
- Conclusion

This report will be graded, and feedback provided to help develop your report writing skills. The better the standard of your report, the better the quality of the feedback provided.

Experiment Development Form

Select your basic methodology (circle appropriate choice):

Amylase and Starch Catalase and Hydrogen Peroxide

Select your independent variable (tick the appropriate box):

☐ Temperature ☐ Substrate Levels ☐ White kidney beans (amylase)

☐ pH ☐ Silver Nitrate (Catalase) ☐ Other (check with teacher)

Identify the specific range of conditions you will be testing for your independent variable (five):

Predict the outcome of your experiment (i.e. write a hypothesis):

Outline how the design will be modified to test your independent variable (i.e. basic methodology):