WJEC Wales Biology A Level

SP 1.4: Investigation into the effect of enzyme concentration on enzyme activity

Practical notes

Introduction

The rate of an enzyme-controlled reaction is influenced by different **factors** including temperature, pH, enzyme concentration and substrate concentration. The effect of each of these factors can be determined by changing **one variable only**, and observing its effect on the rate of reaction.

In this practical, we will determine the effect of **enzyme concentration** on the rate of protein breakdown by the enzyme **Trypsin**.

Equipment

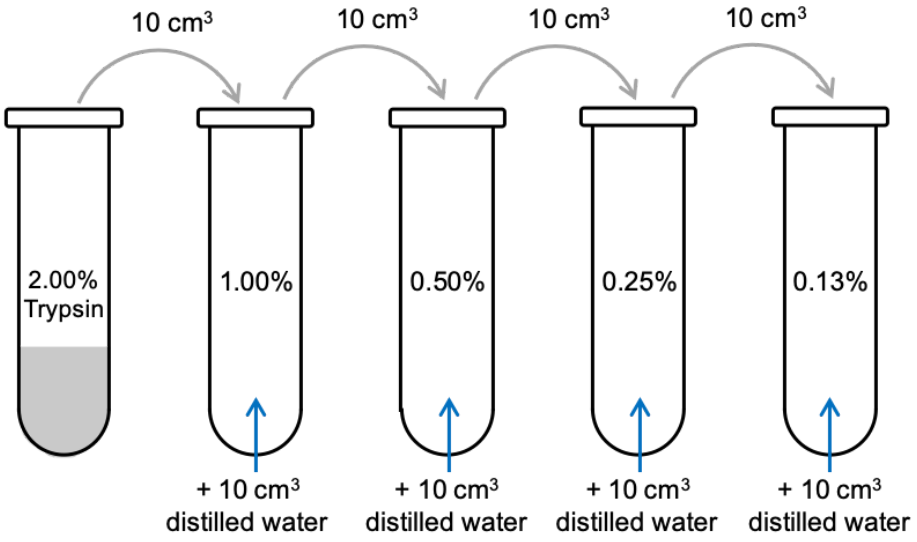
- 2% Trypsin solution
- 2% milk powder solution
- Distilled water
- 5 cm^3 syringe
- 10 cm^3 measuring cylinder
- 9× boiling tubes
- Stopwatch
- Permanent marker pen
- Observation sheet

Risk assessment

Hazard	Risk	Precaution	Emergency
Broken glass	Cuts	Keep glassware away from the edge of the desk	Dispose of broken glassware carefully; elevate cuts and apply pressure; do not remove glass from cuts; seek medical assistance
2% Trypsin solution	Irritation to eyes	Avoid contact with eyes; wear safety goggles	Flood eye(s) with tap water; seek medical assistance
	Irritation to skin	Wear gloves when handling Trypsin solution	Remove contaminated clothing; run the affected area under cold water; seek medical assistance
2% Milk powder solution	Allergic reaction	Do not ingest milk powder solution	Seek medical assistance

Method

1. Using a **serial dilution**, the 2% Trypsin solution and **distilled water**, prepare 1.00, 0.50, 0.25 and 0.13% Trypsin solution:



2. Set up five boiling tubes containing **2 cm^3 2% milk powder solution** solution. Label each with a different concentration of Trypsin solution (i.e. 2.00, 1.00. 0.50, 0.25 and 0.13%).

3. Take the boiling tube labelled 2% Trypsin and position an **observation sheet** (a piece of paper with written text) behind it.

4. Using a 5 cm^3 syringe, add **2 cm^3** 2% Trypsin into the boiling tube and start the stopwatch.

5. The milk powder solution begins **opaque**. As the reaction progresses, the protein is broken down and the solution becomes **clearer**. The endpoint of the reaction can be identified when the letters on the sheet become **visible**. Record this in a suitable table (see below).

6. Repeat steps 3 to 5 for the other Trypsin solution concentrations.

7. **Repeat** the method a further two times to obtain **three repeats** for each concentration.

Variables

Independent variable

The variable that is **changed**
i.e. the concentration of Trypsin solution.

Dependent variable

The variable being **measured** whose value depends on the independent variable
i.e. the time taken for the writing to become visible.

Controlled variables

The variables that are kept **constant** during the experiment:

- Volume of 2% milk powder solution
  5 cm^3 syringe used to measure 2 cm^3 of 2% milk powder solution
- Volume of Trypsin solution
  5 cm^3 syringe used to measure 2 cm^3 of Trypsin solution
- Concentration of milk powder solution
  2% milk powder solution used throughout
- Temperature and pH
  Room temperature and pH of the solution remains relatively stable
- Same individual used to identify the point at which the writing becomes visible

Results

The initial rate of reaction is proportional to the reciprocal of the time taken for the reaction to be complete i.e. **1/t**. This allows values for the rate of reaction to be calculated for each concentration.

Trypsin concentration (%)	Time taken for the writing to become visible (s)			Mean time (s)	Rate or 1/t (s^{-1})
	Repeat 1	Repeat 2	Repeat 3		
2.00					
1.00					

0.50					
0.25					
0.13					

A **graph** of rate of reaction against Trypsin concentration can be plotted and the trend observed.

Conclusion

As the concentration of Trypsin increases there are a greater number of **active sites** available. More **enzyme-substrate complexes** form so the **rate of reaction increases**.

The rate of reaction eventually **plateaus** as another factor (e.g. substrate concentration) becomes **limiting**.