

Edexcel Biology

International A-level

CP 04 - Factors affecting enzyme reactions

Flashcards

What are the four factors that affect enzyme activity?

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1. Enzyme concentration
2. Substrate concentration
3. Temperature
4. pH

How is a control set up in a practical measuring enzyme activity?

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Replace the enzyme solution with distilled water or boiled enzyme solution.

How can a colorimeter determine the rate of reaction of trypsin and milk?

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As trypsin digests the proteins in milk, milk becomes more translucent and more light passes through. The decrease in absorbance can be measured by the colorimeter.

Outline the practical procedure used to measure the effect of enzyme concentration on enzyme activity, using trypsin and milk.

Outline the practical procedure used to measure the effect of enzyme concentration on enzyme activity, using trypsin and milk.

1. Dilute stock solution of trypsin with distilled water to produce 4 concentrations.
2. Zero the colorimeter using 2 cm³ of milk suspension and 2 cm³ of distilled water.
3. To another cuvette, add 2 cm³ of milk suspension and 2 cm³ of the stock trypsin solution. Mix, place in the colorimeter and measure absorbance at 15 second intervals for 5 minutes.
4. Rinse the cuvette with distilled water.
5. Repeat step 3 at all trypsin concentrations.

How is the rate of reaction calculated from time?

How is the rate of reaction calculated from time?

Rate of reaction = $1/\text{time}$

What is the effect of enzyme concentration on enzyme activity?

What is the effect of enzyme concentration on enzyme activity?

As the concentration of enzyme increases, **successful collisions to form ES complexes** increase, so the rate of reaction **increases** to an optimum.

Beyond the optimum, the rate plateaus as **substrate concentration** becomes limiting.

How is the effect of substrate concentration on enzyme activity investigated?

How is the effect of substrate concentration on enzyme activity investigated?

Prepare a simple dilution of milk/substrate concentrations.

Add each solution to 1 cm³ of trypsin of a fixed concentration. Record the absorbance immediately and every 15 seconds for 5 minutes.

What is the effect of substrate concentration on enzyme activity?

What is the effect of substrate concentration on enzyme activity?

Enzyme activity **increases** initially as substrate concentration increases, as **substrate concentration is limiting**, and a higher concentration results in **more successful collisions** to form **ES complexes**.

Beyond a certain substrate concentration, enzyme activity **plateaus**, as all the enzyme active sites are saturated and **enzyme concentration is limiting**.

How is the effect of pH on enzyme activity investigated?

How is the effect of pH on enzyme activity investigated?

Add a fixed volumes of buffer solutions with a range of pH values to 1 cm³ of trypsin and 2 cm³ of milk, both of a fixed concentration.

Record the absorbance immediately and every 15 seconds for 5 minutes.

What is the effect of pH on enzyme activity?

What is the effect of pH on enzyme activity?

Enzyme activity is **highest** at the **optimum pH**.

Above or below the optimum pH, enzyme activity decreases as the unsuitable pH **disrupts its tertiary structure** and changes the shape of its **active site**, causing **partial denaturation**.

Complete denaturation may occur at extreme pH values.

How is the effect of temperature on
trypsin activity measured?

How is the effect of temperature on trypsin activity measured?

Prepare water baths with a range of temperatures. Place 2 cm³ of trypsin solution and 2 cm³ of milk in each water bath.

Leave for 5 minutes to allow the solutions to reach the temperature of the water bath.

Mix together. Record the absorbance immediately and every 15 seconds for 5 minutes.

What is the effect of temperature on enzyme activity?

What is the effect of temperature on enzyme activity?

Increasing temperature **increases** enzyme activity to an **optimum**. Enzyme and substrate molecules gain **kinetic energy** and move faster, so there are more **successful collisions to form ES complexes**.

Beyond the optimum temperature, enzyme activity **decreases** as the high temperature **disrupts the tertiary structure** of enzymes and **denatures** them.

State a hazard and safety precaution involved in this practical.

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Students may have allergic reactions to enzymes, so avoid contact with skin and eyes, wear eye protection.