Edexcel Biology GCSE

CP 02: pH and Enzyme activity

Practical notes

Effect of pH on the rate of activity of amylase enzyme

Aim

Use continuous sampling with iodine solution to determine the time taken for starch (the substrate) to be completely digested, hence calculate the rate of enzyme activity at different pH values.

Equipment

- test tubes
- a test tube rack
- water baths (electrical or Bunsen burners and beakers)
- spotting tiles
- a 5 cm^3 measuring cylinder
- syringes
- a glass rod
- a stopwatch
- starch solution
- amylase solution
- buffered solutions
- iodine solution
- thermometer

Method

1. On a tile, label each well with the time (from 0 onwards) and add a drop of iodine solution to each well.
2. Add 2 cm^3 of each buffer solution using a syringe (ranging from pH 3.0 to 7.0) into each labelled test tube.
3. Immerse the starch solution, amylase solution, and the test tubes of buffer solution in a water bath at 25°C.
4. Allow a few minutes for the temperature to equilibrate.
5. Use a syringe to add 2 cm^3 of amylase into a test tube of buffer solution.
6. Use a syringe to add 2 cm^3 of starch into the same test tube and start timing immediately.
7. Use the glass rod to transfer a drop of the mixture to the well labelled ‘0’ on the tile.
8. Repeat step 6 every 30 seconds, rinsing the glass rod in between every test, until the iodine solution remains brown and does not turn blue-black i.e. all the starch has been digested.
9. Repeat steps 1-8 twice more and take a mean time required for iodine solution to remain brown.
10. Calculate the rate of enzyme reaction by using 1/ time taken for iodine solution to remain brown.
11. Repeat steps 2-8 for buffer solutions with different pH values.
12. Plot a graph of the rate of enzyme reaction against pH.

Controlled variables

- Temperature
- Volume and concentration of starch solution
- Volume and concentration of amylase solution

- Time interval between testing

	Time taken for amylase to completely break down all the starch / s				
pH	1	2	3	Mean time taken / s	Rate of reaction / s^{-1}

Sources of error

The time interval between the testing each sample may be too long to accurately find the time taken for all the starch to be broken down.

Risk assessment

Avoid contact with iodine solution which is an irritant.
Be careful using hot water
If using a Bunsen burner tie long hair back and wear goggles.