GCSE

Rewarding Learning

CCEA GCSE TEACHER GUIDANCE

Biology Practical Manual

Unit 3: Practical Skills

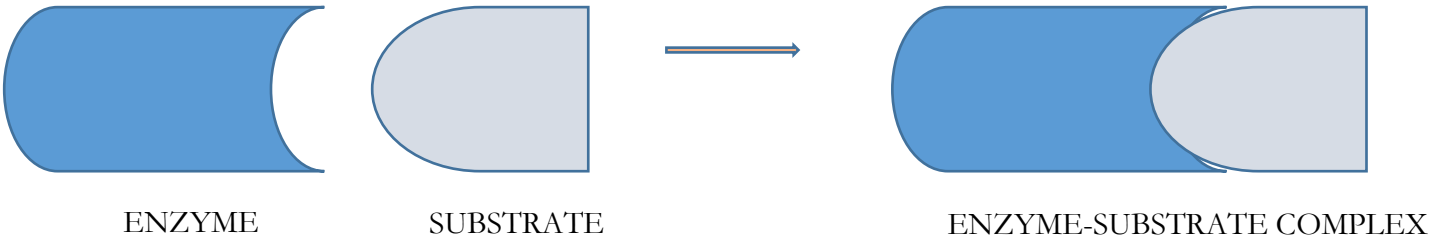
1.4 Investigate the effect of temperature on the action of an enzyme

For first teaching from September 2017

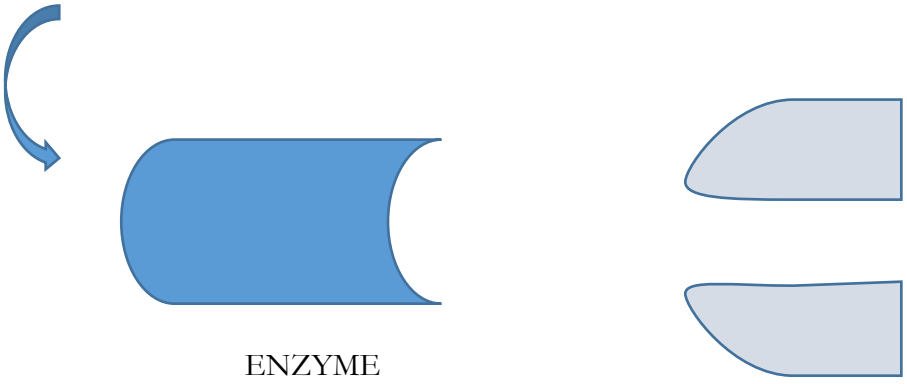
Practical 1.4

Investigate the effect of temperature on the action of an enzyme

An enzyme is a biological catalyst. It will speed up a chemical reaction within the body without being used up. Enzymes are also proteins that are folded into complex shapes. This allows smaller molecules to fit into them at a particular place called an active site. Because of their complementary shapes each enzyme is specific to a particular substrate. They fit together to form an enzyme-substrate complex. The enzyme can then break down the substrate into smaller products or combine two smaller substrates into one larger product.



ENZYME SUBSTRATE ENZYME-SUBSTRATE COMPLEX



ENZYME PRODUCTS

If the temperature changes, then the shape of the active site on the enzyme will change, the enzyme is denatured. This will result in the substrate no longer fitting, so the reaction will proceed more slowly.

The following investigations will compare the rate of enzyme reactions at different temperatures.

Two possible investigations are described:

Investigation 1

Apparatus

- 2 x electric waterbaths
- 2 test tubes
- test tube rack
- thermometer
- measuring cylinder or syringe
- lipase
- milk
- phenolthalein indicator (Phenolphthalein is pink in alkaline solutions of about pH10. When the pH drops below 8.3 it changes to colourless. An alkaline solution of milk is prepared by adding sodium carbonatine. Milk contains fat and the lipase will break this fat down to form fatty acids (low pH) and glycerol.)
- sodium carbonate solution (0.05mol/dm^3)

Method

1. Set the temperature of the first waterbath to 30°C and the second to 60°C.
2. Place a beaker of lipase in each waterbath to come to temperature.
3. Add 5 drops of indicator to test tube 1.
4. Measure 5cm^3 of milk (using syringe or measuring cylinder).
5. Pour the milk into test tube 1.
6. Measure 7cm^3 of sodium carbonate solution and pour into test tube 1.
7. Place the test tube in the water bath (a test tube rack can be used).
8. Use the thermometer to check when test tube 1 has come to temperature and then add 1cm^3 of lipase from the beaker to test tube 1.
9. Record how long it takes for the colour of test tube 1 to change.
10. Repeat steps 3-9 for test tube 2 using the second waterbath.
11. Repeat at each temperature.
12. The investigation can be repeated at another temperature, perhaps one in between 30°C and 60°C

Result

Record results in a suitable table.

A sample table is shown.

Test tube	**Temperature/°C**	**Colour at start**	**Time taken to change to colourless/seconds**
1	**30**		
2	**60**		

Conclusion

The milk with lipase and indicator will change from pink to colourless as the fat is broken down and the concentration of fatty acids increases. The **time** taken for this reaction to occur is affected by **temperature**.

The colour change will happen faster at the lower temperature because the lipase is able to work and speed up the breakdown of milk fats.

The colour change was slower at 60°C because the lipase has been denatured and its shape is no longer complementary to the fats.

Investigation 2 – Alternative

This alternative method also investigates the action of lipase at different temperatures. However, instead of using an indicator and recording the time taken for it to change colour, students will record the pH after a period of time. This will allow them to determine whether the lipase has broken the fats down to fatty acids. The more fatty acids present the lower the pH.

Apparatus

1. 2 electric waterbaths
2. 2 test tubes and racks
3. thermometer
4. lipase solution
5. milk
6. pH meter

Method

1. Set up two waterbaths, one at 30°C and the other at 60°C.
2. Place a beaker of lipase in each waterbath to allow them to come to temperature.
3. Add 5cm^3 of milk to each test tube.
4. Add 1cm^3 of lipase at 30°C to test tube 1.
5. Add 1cm^3 of lipase at 60°C to test tube 2.
6. Place each test tube in the correct water bath.
7. Record the pH of each test tube every 10 minutes.

Results

Record results in a suitable table.

A sample table is shown.

TIME (minutes)	pH of solution at 30°C	pH of solution at 60°C
0		
10		
20		
30		
40		
50		

Conclusion

The pH gets lower as the fat in the milk is broken down to fatty acids (and glycerol). If the solution reaches a low (acidic) pH quickly then the lipase is working to speed up the breakdown of fat.