Chapter 5 – Enzymes

Subject content

Content
- Enzymes

Learning outcome

(a) state that large molecules are synthesised from smaller basic units
- glycogen from glucose
- polypeptides and proteins from amino acids
- lipids such as fats from glycerol and fatty acids

(b) explain enzyme action in terms of the 'lock and key' hypothesis

(c) explain the mode of action of enzymes in terms of an active site, enzyme-substrate complex, lowering of activation energy and enzyme specificity

(d) investigate and explain the effects of temperature and pH on the rate of enzyme catalysed reactions

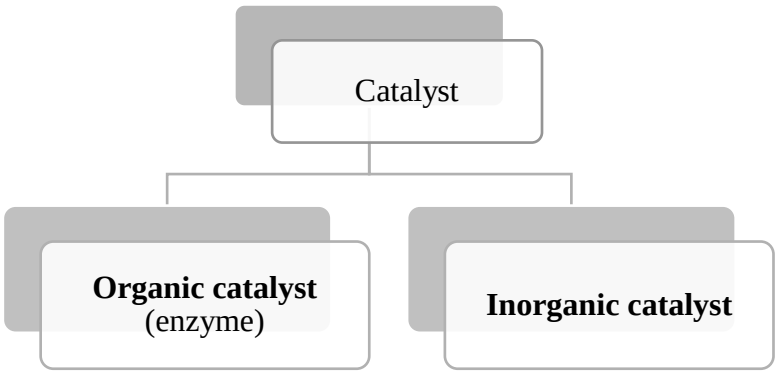
Use the knowledge gained in this section in new situations or to solve related problems.

5.1 Classification and Properties of Enzymes

Catalysts

Catalyst

Substance which speeds up a chemical reaction, without itself being chemically changed at the end of the reaction



Differences

Organic catalyst	Inorganic catalyst
• Globular proteins • Catalyse a specific reaction of a substrate • Synthesised by ribosomes in living cells • More sensitive to temperature & pH • Example: (a) amylase (starch → maltose) (b) maltase (maltose → glucose) (c) sucrase (sucrose → glucose + fructose)	• Mineral ions / small molecules • Catalyse diverse reactions • Not synthesised in living cells • Less sensitive to temperature & pH • Example: (a) manganese(IV) oxide (b) vanadium(V) oxide (c) transition metals

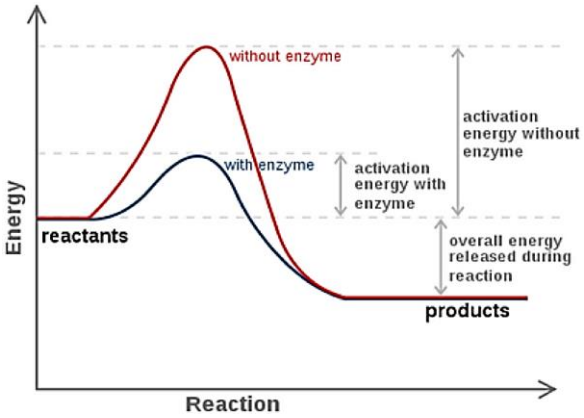
Enzymes

Enzymes

Biological catalysts that are made up of **proteins**
- speed up chemical reactions
- remain chemically unchanged at the end of reaction

Activation energy: energy needed to start a chemical reaction
Provide **alternative pathway** with **lower activation energy**
→ formation of **enzyme-substrate complex**

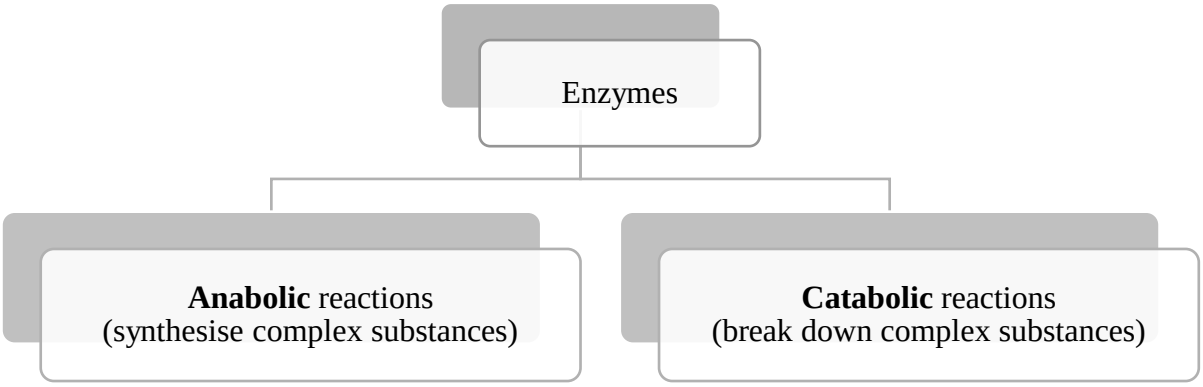
Enzyme-catalysed reaction:



Reactions that enzymes catalyse

Digestion (enzyme-catalysed process)
- Large complex substances → simpler smaller molecules
  1. soluble in water
  2. small (diffuse through CSM)
- Examples:

Digestive enzyme	Digest
(a) amylase	starch → maltose
(b) maltase	maltose → glucose
(c) protease	protein → amino acids
(d) lipase	fats → fatty acids + glycerol



Enzymes build up & break down complex substances

Reaction	Examples
1. **Anabolic**	• amino acids → polypeptides → proteins
2. **Catabolic**	• glucose → carbon dioxide + water • hydrogen peroxide $\xrightarrow{\text{catalase}}$ water + oxygen (toxic) (non-toxic)

- Enzymes catalyse practically all the chemical reactions in an organism
- Enzymes are produced only when they are needed
  → digestive enzymes only produced when there is food to digest

Naming of enzyme: substrate + '**ate**'
→ lipase: enzyme that acts on lipids

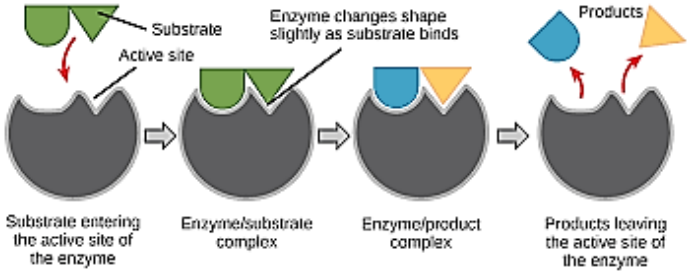
Hydrolases (digestive enzymes): catalyse hydrolytic reactions in the body

Hydrolases	Function
1. **Carbohydrases**	Digest carbohydrates
2. **Proteases**	Digest proteins
3. **Lipases**	Digest lipids

5.2 Characteristics of Enzymes

Characteristics of enzymes

1. Protein in nature
2. Speed up chemical reactions
3. Remain chemically unchanged at end of reaction
4. Required in minute amounts
5. Sensitive to temperature
6. Sensitive to pH
7. Specific in action



Characteristics:

Characteristic	Explanation
1. **Speed up chemical reactions**	• Lower activation energy required for reaction to start
2. **Required in minute amounts**	• Highly efficient molecules • Reusable – remain chemically unchanged at end of reaction • Small amount → catalyse large number of chemical reactions
3. **Highly specific**	• Each chemical reaction is catalysed by a unique enzyme • Specificity of each enzyme: due to shape of active site (only fits certain substrate)
4. **Catalyse reversible reactions**	• Some reactions in living cells: reversible • Formation of carbonic acid: $CO_2 + H_2O \xrightarrow{\text{carbonic anhydrase}} H_2CO_3$

Lock and key hypothesis

Process	Explanation
1. **Substrate enters active site**	1) **Enzyme molecule**: **lock** that has unique **keyhole** (**active site**) 2) **Substrate molecule**: **key** that fits the lock exactly (substance that enzyme acts on)
2. **Enzyme-substrate complex**	Substrate binds to enzyme → enzyme-substrate complex
3. **Chemical reaction occurs**	• Break chemical bonds in substrate molecule • Substrate molecule converted → product molecules
4. **Products leave active site**	• Product molecules: separate from enzyme molecule • Enzyme molecule: unchanged – combine with other substrate molecules

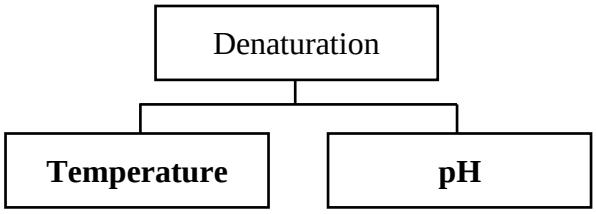
5.3 Factors Affecting Enzymes

Denaturation

Change in the three-dimensional structure of enzyme or any other soluble protein, caused by heat or chemicals (acids & alkalis)

Denaturation (irreversible process)

- Caused by
  1. extreme heat (high **temperature**)
  2. extreme acids, alkalis (extreme **pH**)
- Enzyme loses active site
  - Substrate cannot fit into active site
  - No reaction will occur



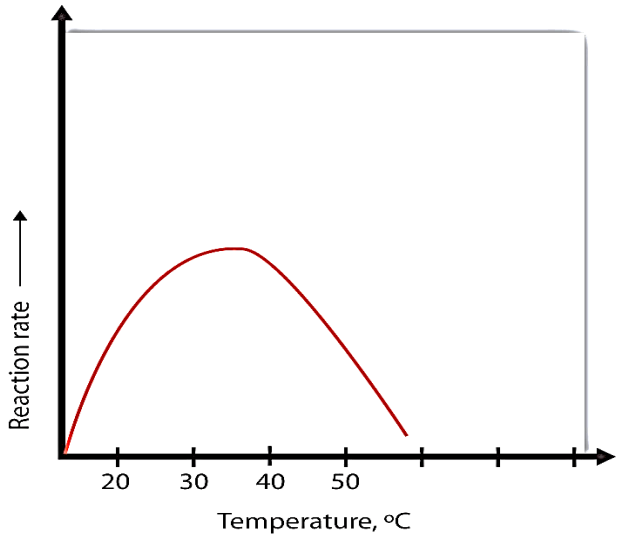
Factors affecting activity of enzyme

1. **Temperature**
2. **pH**

Factor	Explanation	Result
1. **Temperature** (very high)	Rising temperature increases rate of reaction until optimum temperature is reached	Enzyme denatured
2. **pH** (extreme levels)	Decreasing / increasing pH from optimum pH of enzyme decreases enzyme activity	

Temperature

Optimum temperature: temperature at which enzyme is most active (40 ~ 45°C)



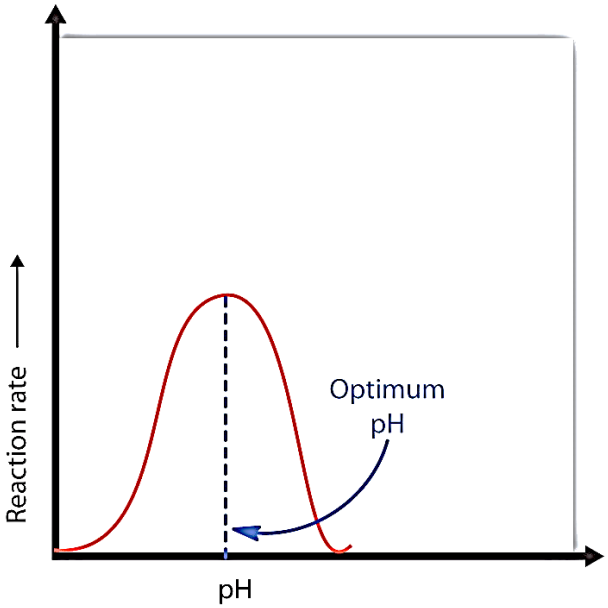
Effect of temperature on rate of enzyme reaction:

Temp.	Rate of reaction	Explanation			
		Kinetic energy of molecules	Chance of substrate molecules colliding with enzyme + fitting into active sites	Rate of formation of enzyme-substrate complex	Enzyme activity
low	low	low	very low	low	• Enzyme is inactive
increase	increase	increases	increases	increases	• Temperature ↑ 10℃, rate of enzyme reaction × 2
optimum	highest	highest	highest	highest	• Enzyme is most active
above optimum	rapid decrease				• High temperature breaks bonds that keep enzyme protein in shape • Active site of enzyme loses its 3D shape Substrate molecule no longer fit into active site • Temperature ↑ rate of denaturation ↑

pH

Optimum pH: pH value at which enzyme is most active

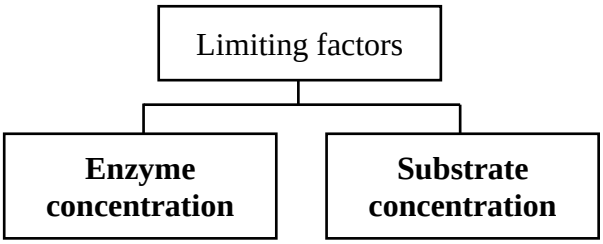
- Affected by acidity or alkalinity of solutions which they act in
- Extreme changes in acidity / alkalinity → denature enzymes



Limiting factors

Limiting factors: affects rate of reaction if quantity changed

1. **Enzyme concentration**
2. **Substrate concentration**



Constant enzyme concentration

Phase (increasing substrate con.)	Explanation
0 → A	• Rate of reaction increases until it reaches point A
A → B	• After point A, increasing the substrate concentration does not increase rate of reaction any further • At any given instant, **all enzyme molecules are being made use of** (enzyme molecules are saturated → amount of products formed per unit time remains the same

Increased enzyme concentration – more enzyme molecules act on substrate molecules, rate of reaction ↑

Phase (increasing substrate con.)	Limiting factor
0 → X	substrate concentration
X → Y	enzyme concentration

Typical questions

Multiple choice questions

1. Which statements are correct for all enzymes? (N2015/P1/Q5)
   1 Enzymes have an optimum pH.
   2 Each enzyme can catalyse a number of different reactions.
   3 Enzymes lower the activation energy needed for a reaction.
   4 Enzymes catalyse only reactions that break down complex substances.
   5 The shape of the active site is permanently altered by excessive heat.
   6 Enzymes work fastest at 35°C.

   A 1, 2 and 3
   B 1, 3 and 5
   C 2, 4 and 6
   D 4, 5 and 6

2. Four test tubes containing identical quantities of lipase and fat were set up in a water-bath at 37°C. The table gives further information about the contents of the test tubes. In which test tube would digestion be most rapid? (N2017/P1/Q4)

	pH	bile
A	5	absent
B	5	present
C	8	absent
D	8	present

3. Four statements about the active site of a human enzyme are given.
   1 The shape of the active site changes when the temperature falls to 10°C and does not return to normal when the temperature returns to 37°C.
   2 The active site of the enzyme is the same shape as the substrate molecule.
   3 The specificity of an enzyme depends on the shape of its active site.
   4 The shape of the active site changes when the enzyme is heated to 60°C and does not return to normal when the temperature returns to 37°C.

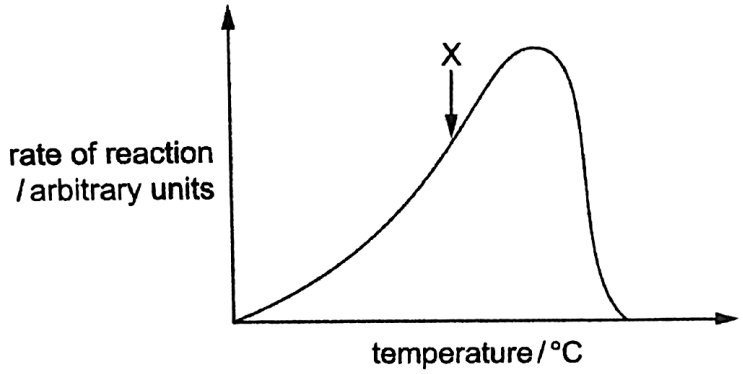
   Which statements are correct? (N2017/P1/Q6)
   A 1, 2 and 3
   B 1 and 4 only
   C 2 and 3 only
   D 3 and 4 only

4. Some statements about the active site are listed.
   1 It accounts for the specificity of the enzyme.
   2 It can be used once only.
   3 It is altered irreversibly by exposure to a high temperature.
   4 It lowers the activation energy needed for chemical reactions.

   Which statements are correct? (N2018/P1/Q5)
   A 1 and 3 only

B 2 and 4 only
C 1, 3 and 4
D 2, 3 and 4

5. In an investigation, 10 cm^3 of a substrate solution was added to a beaker containing a solution of an enzyme. The graph shows the effect of temperature on the rate of the reaction between the substrate and the enzyme.

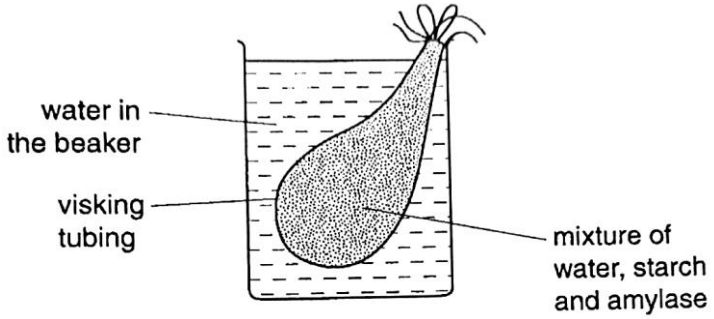


Which process is taking place at point X on the graph? (N2018/P1/Q6)

A The activation energy is increasing.
B The enzyme is starting to denature.
C The number of active sites is increasing.
D The rate of enzyme-substrate collisions is increasing.

Structured questions

1. The figure below shows an apparatus set up to investigate digestion. (N2011/P2/A1)



The apparatus was left at 30°C for four hours.
After this time the water in the beaker was tested and found to contain reducing sugars.

(a) (i) Explain why reducing sugars were found in water in the beaker. [3]

Amylase catalysed the decomposition of starch to reducing sugars inside the visking tubing. The visking tubing is partially permeable so it allows small particles to pass through it. As the reducing sugars are small and have a higher concentration inside the visking tubing than outside, they diffuse out of the visking tubing down a concentration gradient into the water in the beaker. Thus, reducing sugars are found in the water in the beaker.

(ii) The water in the beaker was also tested for starch.
State the result of this test and explain your answer. [2]

The result of this test is negative. Starch is a very large molecule and is unable to pass through the visking tubing. Besides, after four hours, all the starch might have been converted into reducing sugars and none would be left inside the visking tubing.

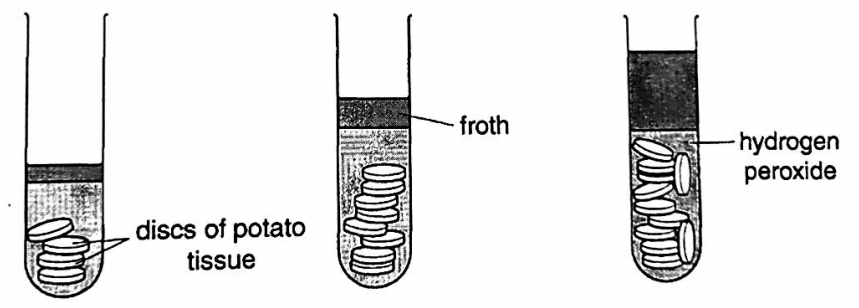
(b) State the property of the visking tubing which made these results possible. [1]

Visking tubing is partially permeable. It only allows certain particles to pass through it.

2. (a) Hydrogen peroxide is a toxic chemical produced in plant and animal cells. (N2012/P2/A6)
The enzyme catalase is also found in plant and animal tissues.
Catalase breaks down hydrogen peroxide to water and oxygen.

$$\text{hydrogen peroxide} \xrightarrow{\text{catalase}} \text{water} + \text{oxygen}$$

The mixture of water and oxygen forms a froth.
An investigation was carried out into the factors affecting the action of catalase. The figure below shows the results of the investigation.



(i) State the factor being investigated. [1]

The factor being investigated is the amount of catalase present in the increasing number of discs of potato tissue.

(ii) State two factors that need to be kept constant in this investigation. [2]

The amount of hydrogen peroxide used for each test tube needs to be kept constant.
The temperature of the mixture in each test tube needs to be kept constant.

(iii) Suggest a suitable control for this investigation. [1]

A test tube containing hydrogen peroxide with no discs of potato tissue.

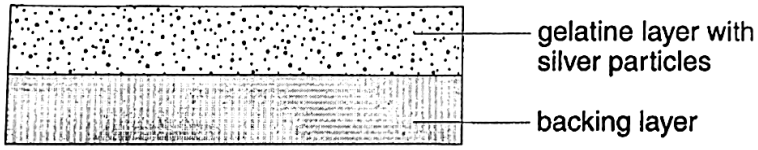
(b) State a conclusion that can be made from the results shown in the figure above. [1]
The higher the amount of catalase present, the higher the rate of breakdown of hydrogen peroxide to water and oxygen and the more froth is produced.

3. (N2013/P2/A4)

(a) State what is meant by the term *activation energy*. [1]

Activation energy is the energy required in order to start a chemical reaction.

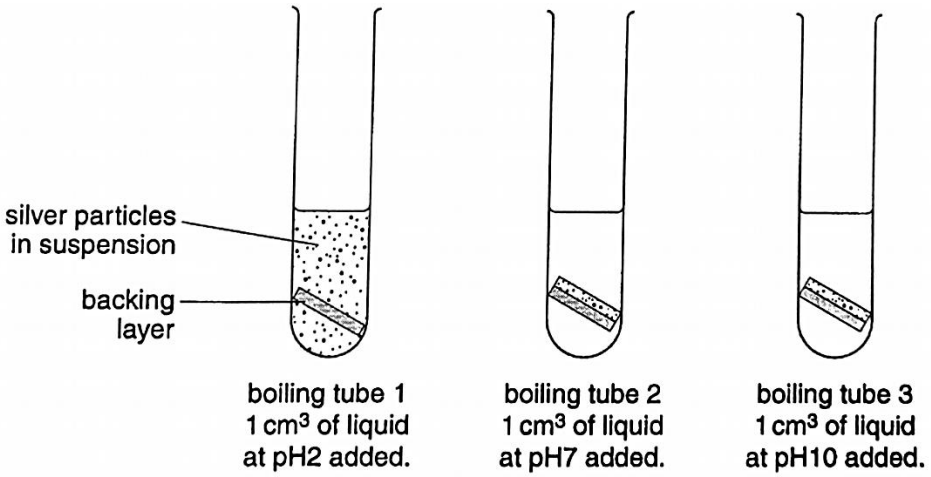
(b) The figure below shows a section of photographic film.
The top layer is made up of silver particles embedded in a later of gelatine which is a type of protein.



In an investigation, a 20 mm length of photographic film was placed into each of three boiling tubes.

- The film was immersed in 20 cm^3 water.
- 1 cm3 of liquid at different pH values was added to the boiling tubes.
- 1 cm3 of protease solution was added to each boiling tube.
- Each boiling tube was shaken gently to mix the contents.
- Each boiling tube was kept at 37°C for 1 hour.

The figure below shows the apparatus and the results of the investigation.



(i) Explain the results in boiling tubes 1, 2 and 3.
boiling tube 1 [2]

pH 2 is the optimum pH for the enzyme protease to work. Protease is a type of biological enzyme that digests proteins. The gelatine layer is decomposed by protease into polypeptides. As a result, the silver particles embedded in the gelatine are released into the water, forming a suspension.

boiling tube 2 and *boiling tube 3* [2]

The enzymatic actin of protease is highly dependent on pH. Protease only works well in acidic pH conditions. At pH 7 (boiling tube 2) and pH 10 (boiling tube 3), protease is denatured. Thus the gelatine layers are not digested by protease. The silver particles remain embedded in the gelatine and the solutions are clear.

(ii) State two factors kept constant during the investigation. [2]

Length of photographic film
Amount of water
Amount of liquid at different pH values
Amount of protease solution
Extent of mixing of contents
Temperature of each boiling tube
Amount of time taken to conduct the experiment

4. (a) Rennin is an enzyme produced by the stomach of young cows.
The action of rennin is to cause liquid milk to clot, forming solid lumps.
In an investigation, mixtures of milk and rennin are kept different temperatures and the time taken for the milk to form lumps are recorded.
The results of the investigation are shown in the table below.

Temperature / °C	Time taken to form lumps / minutes
10	did not form lumps
20	11.0
30	6.0
40	2.5
50	7.0
60	did not form lumps

(i) State the optimum temperature for the action of rennin. [1]

40°C

(ii) Suggest a reason for the result at 60°C. [1]

At 60°C, the rennin was denatured by heat and lost its catalytic function to cause milk to form lumps.

(b) Suggest a possible nutritional advantage to the young cow of the action of rennin. [2]

Since rennin causes liquid milk to clot and form solid lumps, it allows the milk to stay for a longer period of time in the stomach. Other proteases like pepsin would have more time to digest the proteins in the milk to polypeptides. Subsequently, the polypeptides can be further digested into amino acids, which can be absorbed into the bloodstream of the young cow.

5. (N2011/P2/B10 EITHER)

(a) Define the term enzyme. [3]

Enzymes are biological catalysts.
They are proteins and catalyse many different biochemical processes occurring in living cells. They are not changed by the chemical reactions that they speed up. They are denatured at high temperatures.

(b) Sketch a graph to show the effect on the rate of an enzyme-catalysed reaction. Label the axes.

(c) Describe the role of a named enzyme in digestion. [4]

Amylase catalyses the decomposition of starch into maltose. Salivary amylase, produced by the salivary glands in the mouth, works in neutral conditions (around pH 7). In the mouth, a small amount of starch is digested by the salivary amylase. In the stomach, salivary amylase is denatured by the acidic conditions. Thus, no digestion of starch occurs in the stomach. Pancreatic amylase, produced by the pancreas, works in alkaline conditions. In the small intestine, the rest of the starch is digested by the pancreatic amylase into maltose.

6. (N2014/P2/B10 OR)

(a) Describe the properties of enzymes. [6]

Enzymes are biological catalysts. They are proteins and catalyse many different biochemical processes occurring in living cells, without themselves being chemically changed. Enzymes are highly sensitive to temperature changes and are denatured at high temperatures. At temperatures above 40°C, the rate of enzyme activity decreases. The high temperatures destroy the chemical bonds holding the enzymes in their specific three-dimensional shapes. As a result, the active sites of the enzymes are changed. Enzymes can only act on substrates that can fit exactly into their active sites. Thus, the enzymes cannot catalyse the decomposition of the substrates anymore.

(b) Biological washing powders (detergents) contain one or more enzymes.
Suggest the advantages of using biological washing powders compared to those without enzymes. [4]

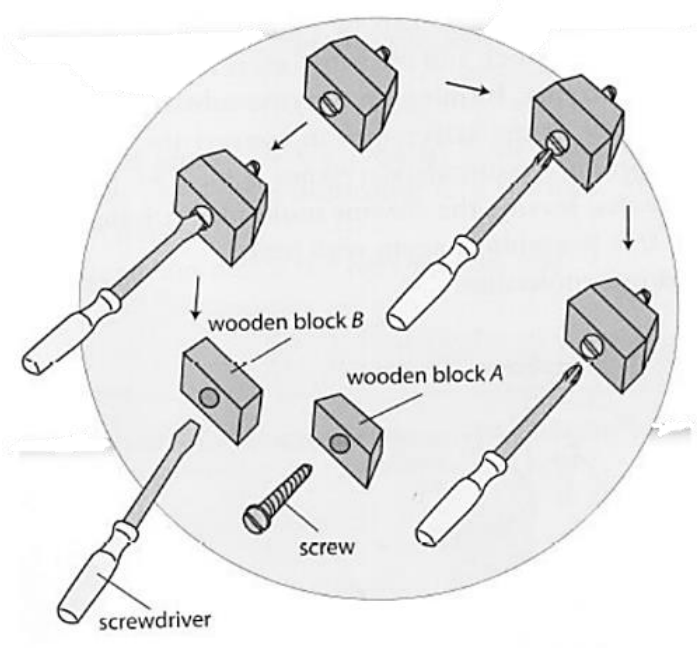
The enzymes in biological washing powder can be used to catalyse the decomposition of food substances such as fats and oils, while some washing powders without enzymes cannot. As the enzymes are not chemically changed at the end of the reaction and can be reused, only a small amount of biological washing powder is needed. Enzymes work well at room temperature. Thus, hot water is not needed and we can save energy by avoiding the need to heat up the water.

7. Describe what happens during an enzyme-catalysed reaction. [5]
(N2019/P2/B9 OR a)

During an enzyme-catalysed reaction, the enzyme provides an alternative pathway with a lower activation energy needed for the reaction to occur.

The substrate binds to the active site, which is complementary in three-dimensional shape to the substrate to form an enzyme-substrate complex. Here, the substrate is like a key while the enzyme is like a lock according to the 'lock-and-key' hypothesis. In the enzyme-substrate complex, bonds in the substrate are broken in catabolic reactions to form new products. Bonds are formed between substrates in anabolic reactions to form a new product. The products then detach from the active site of the enzyme and the enzyme remains chemically unchanged and is free to catalyse a new round of reaction.

8. The diagram below represents an enzyme reaction.



(a) State what each of the following represents in an enzyme reaction. [2]
(i) wooden block A : product molecule
(ii) wooden block B : product molecule
(iii) screwdriver : enzyme

(b) State two ways in which this diagram accurately represents an enzyme action. [2]
The screwdriver remained unchanged after removing the screw, which is similar to an enzyme molecule remaining chemically unchanged after a reaction. [1]
The screwdriver tip fits the screw perfectly, which is similar to the enzyme active site being complementary to the substrate molecule. [1]

(c) Suggest one way in which the diagram does not accurately represent an enzyme action. [1]

Enzyme specificity should be shown by complementarity between substrate molecule and enzyme active site, and not the chemical bond (screw) and the enzyme active site.

The diagram did not show an enzyme's sensitivity to pH or temperature.

9. Substance Y is an extract from a living organism. A student was required to investigate the ability of Y to bring about the hydrolysis (digestion) of starch into reducing sugars. The student set up two water baths, one containing boiling water (100℃) and the other containing water at 36℃.

The student set up the reaction mixtures as follows:

test tube	reagents
1	1 cm^3 of distilled water + 9 cm^3 of starch
2	1 cm^3 of Y (pre-treated at 36℃ for 1 minute) + 9 cm^3 of distilled water
3	1 cm^3 of Y (pre-treated at 100℃ for 1 minute) + 9 cm^3 of starch
4	1 cm^3 of Y (pre-treated at 36℃ for 1 minute) + 9 cm^3 of starch
5	1 cm^3 of Y (pre-treated at 36℃ for 1 minute) + 9 cm^3 of albumin (egg white)
6	1 cm^3 of Y (pre-treated at 36℃ for 1 minute) + 1 cm^3 of sodium hydroxide + 8 cm^3 of starch

After 15 minutes, the student tested the mixture in each test tube for reducing sugar using Benedict's test. Her observations were as follows:

test tube	observation for Benedict's test
1	Mixture remained blue
2	Mixture remained blue
3	Mixture remained blue
4	Brick red precipitate observed
5	Mixture remained blue
6	Mixture remained blue

State and explain three evidences that show that Y contains an enzyme. [6]

Evidence 1: Comparing test tubes 4 and 5, a change in the substrate from starch to albumin resulted in a negative result for the Benedict's test in test tube 5. [1]

This indicates that the enzyme in Y is specific in its action. [1]

Evidence 2: Comparing test tubes 3 and 4, when there was pre-treatment at 100℃, a negative Benedict's test result was observed in test tube 3. [1]

This indicates that the enzyme in Y was denatured at a high temperature above optimum temperature. [1]

Evidence 3: Comparing test tubes 4 and 6, the addition of sodium hydroxide resulted in a negative result for Benedict's test, showing that catalysis of starch hydrolysis is no longer possible. [1]

This indicates that the enzyme in Y was sensitive to pH changes. [1]

10. In an investigation, a cloudy, white gel containing milk protein was prepared. The gel was poured into Petri dishes and allowed to set. Cavities were made in the gel and various liquids were poured into the cavities as shown in Figure A below. (N2009/2A/Q6)

- 1 contained pepsin and hydrochloric acid
- 2 contained pepsin
- 3 contained boiled pepsin
- 4 contained water

Figure B below shows the results after 24 hours.

Fig. A

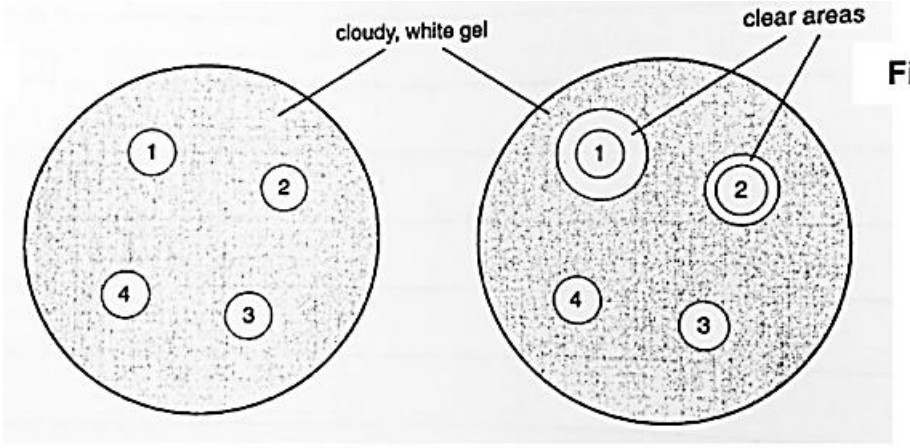


Fig. B

(a) Suggest what caused the clear areas around cavities 1 and 2. [3]

Pepsin molecules diffused into the area around cavities 1 and 2. [1]

The clear areas around cavities 1 and 2 were a result of milk protein being hydrolysed / chemically broken down to polypeptides. [1]

This is due to the hydrolytic action of pepsin. [1]

(b) Explain the difference in the results between cavities 1 and 2. [1]

Pepsin has a faster rate of protein hydrolysis under acidic conditions because it is an enzyme found in the stomach.

Cavity 1, with hydrochloric acid, provided the optimum pH condition for pepsin to function. Hence the clear area around cavity 1 is larger than cavity 2.

(c) Explain why no clear area developed around cavity 3. [1]

Cavity 3 contained boiled pepsin. The boiling process introduced high temperature which resulted in denaturation of pepsin.

(d) State the purpose of cavity 4. [1]

Cavity 4 contains water which acts as a control to show that any clear regions found around the cavities are due to the hydrolysis of protein by pepsin.