Factors That Affect Enzyme Action (3.2): Cambridge (CIE) A Level Biology 9700 Exam Questions by Topic

22 questions · 54 marks · about 65 minutes. Answer in the spaces, then check the mark schemes at the end.

Question 1 · Paper 2 · February/March 2023 (22) · Q6(b) · 2 marks

(b) The enzyme collagenase breaks down collagen. Collagenase has several important medical uses, such as in the treatment of burnt skin.

Scientists investigated the effect of pH on the activity of collagenase at 37 °C.

The results of their investigation are shown in Fig. 6.1.

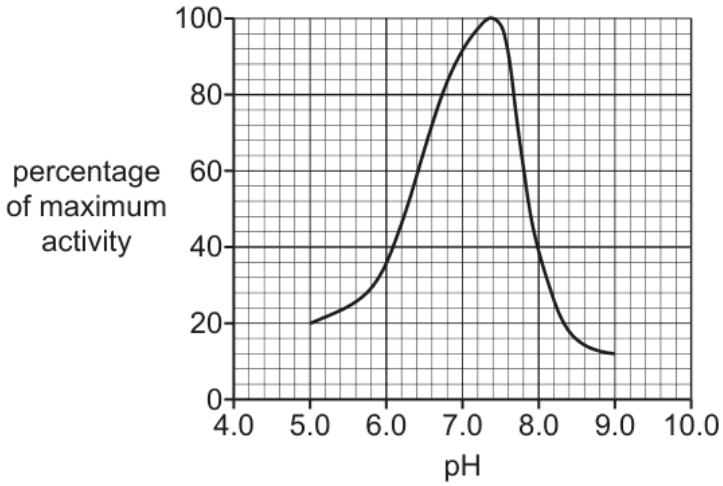


Fig. 6.1

Explain why the activity of collagenase is lower at pH 8.0 than at the optimum pH.

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Question 2 · information from earlier in the question

Keratinases are used to degrade the large quantities of waste feathers from chickens and turkeys that are processed in the food industry. The products of feather degradation can be used in animal feed.

Scientists investigated whether three different keratinases, K12, A22 and P3, were suitable as industrial enzymes. These enzymes were extracted from three different soil bacteria.

The effects of temperature and pH on the activity of each keratinase were investigated.

The results are shown in Fig. 2.1 and Fig. 2.2.

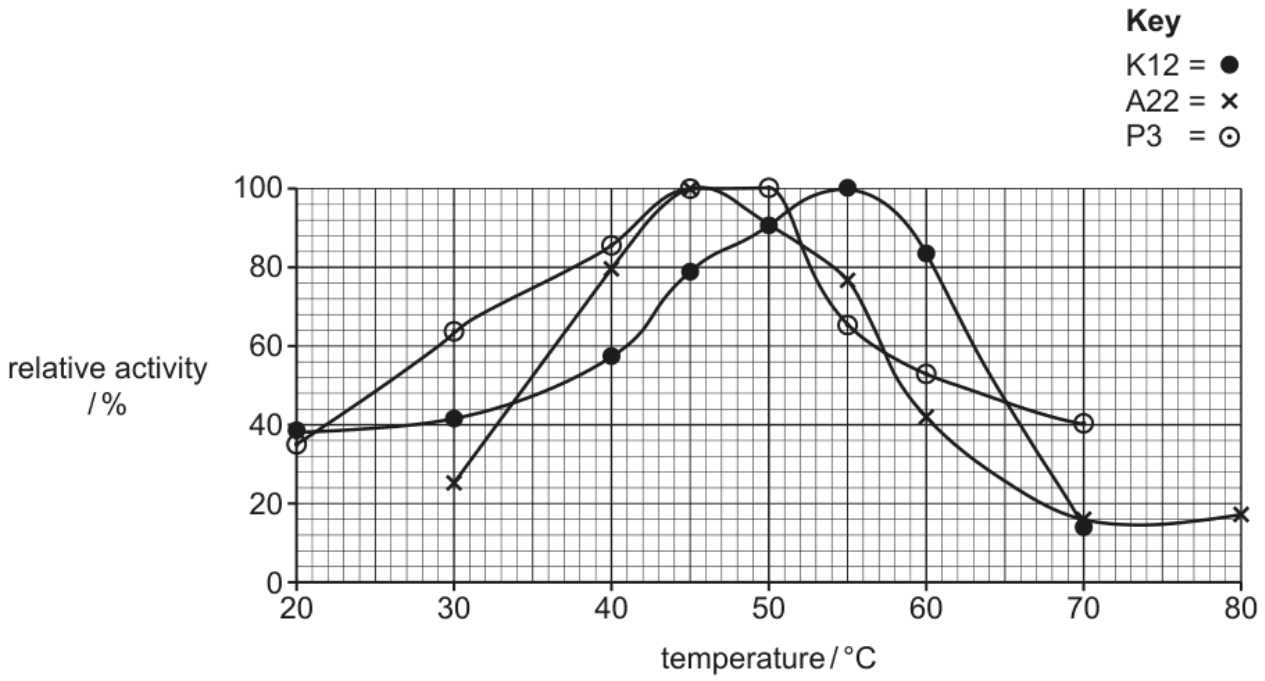


Fig. 2.1

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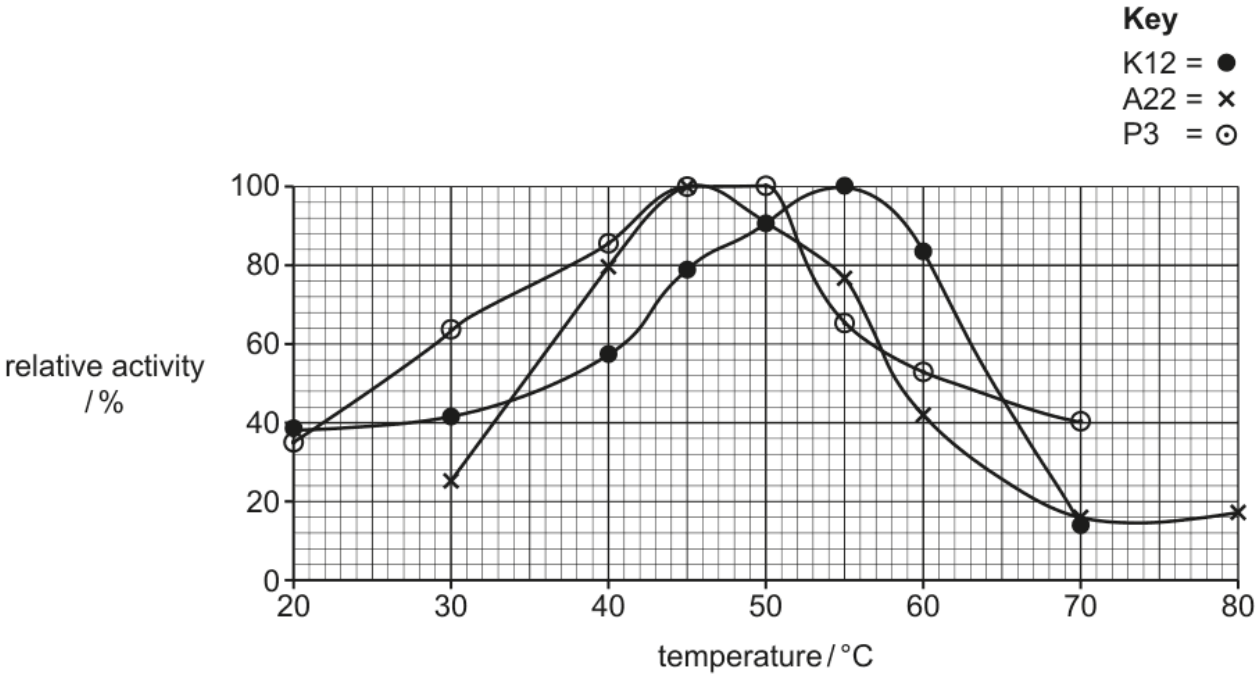


Fig. 2.1

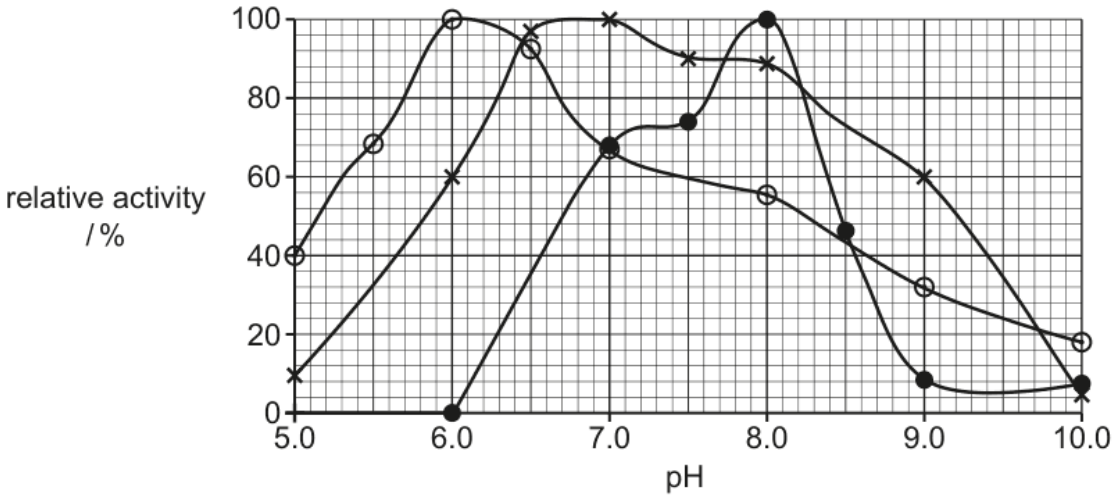


Fig. 2.2

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Question 2 · Paper 2 · October/November 2023 (22) · Q2(e) · 3 marks

(e) Some detergents contain proteases to remove stains from clothes. These enzymes have a high relative activity in alkaline conditions.

The scientists reported that K12 and A22 could be suitable for use in the detergent industry.

With reference to Fig. 2.1 and Fig. 2.2, discuss the advantages **and** disadvantages of using K12 and A22 in the detergent industry.

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Question 3 · information from earlier in the question

2 Starch molecules are the main storage molecules in many types of cereal grain, such as the grain of the barley plant.

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Question 3 · Paper 2 · February/March 2021 (22) · Q2(d)(i) · 3 marks

(d) Fig. 2.3 is a graph showing how the activity of α-amylase extracted from barley seeds changes as the temperature increases from 10 °C to 66 °C.

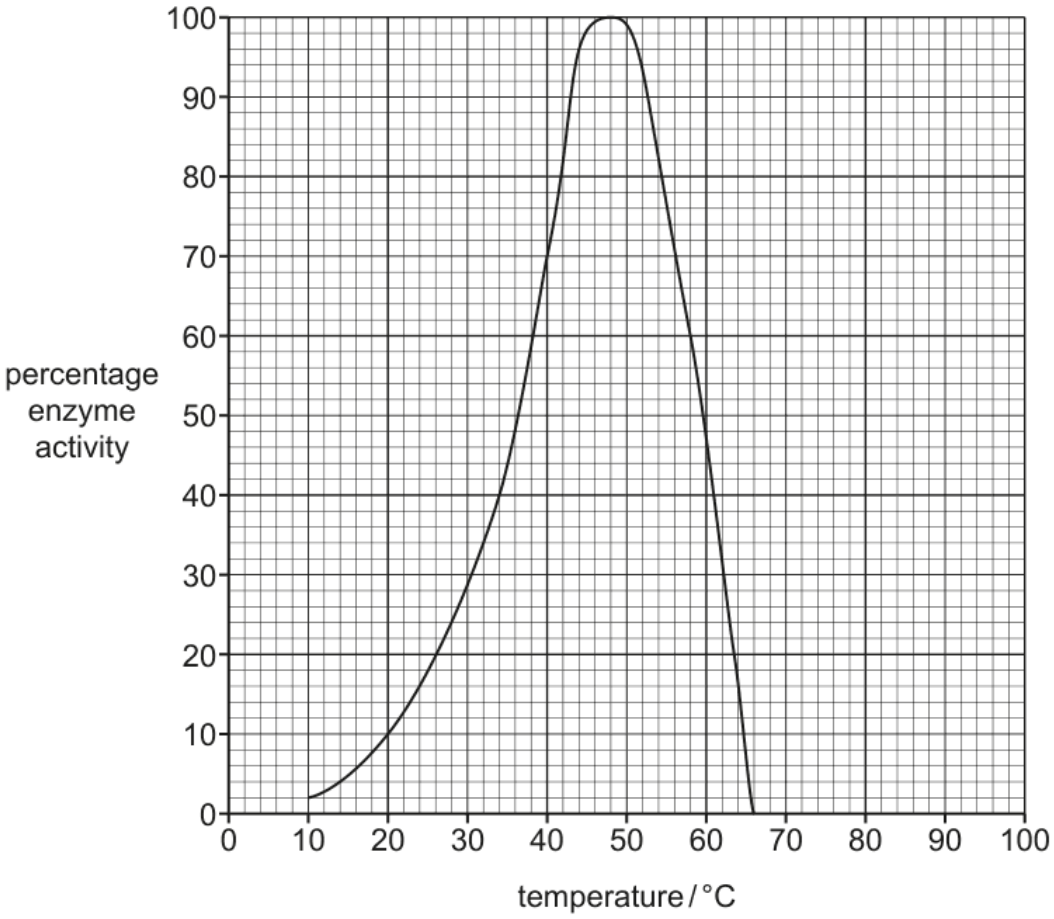


Fig. 2.3

(i) Explain the effect of temperature on the activity of α-amylase extracted from barley seeds, as shown in Fig. 2.3.

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Question 4 · Paper 2 · February/March 2021 (22) · Q2(d)(ii) · 2 marks

(ii) Sketch on Fig. 2.3 the curve that would be obtained using α-amylase enzyme that is heat stable. [2]

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Question 5 · information from earlier in the question

3 During the germination of barley seeds, amylase is produced.

Question 5 · Paper 4 (A level) · October/November 2021 (42) · Q3(c)(i) · 3 marks

(c) In the malting process, germination is stopped before the concentration of sugars in the germinating barley seeds exceeds a concentration that causes shoot or root growth.

Drying the germinating barley seeds at 50 °C is one method used to stop malting.

(i) Explain how this method would stop malting.

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Question 6 · information from earlier in the question

6 Telomerase is an enzyme that is important in maintaining the telomeres that are located at the ends of chromosomes.

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(d) An investigation was carried out into the effect of substrate concentration on the activity of telomerase.

The results are shown in Fig. 6.1.

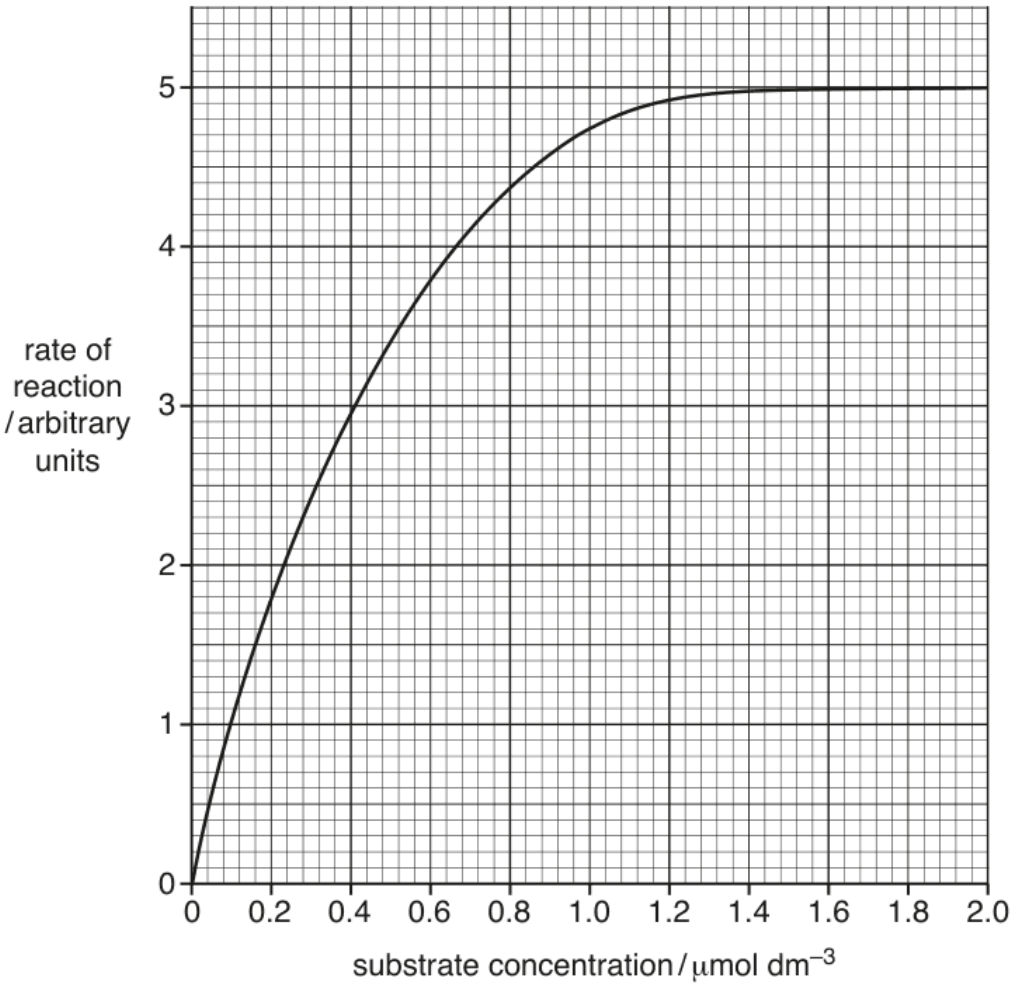


Fig. 6.1

With reference to Fig. 6.1, explain the difference in the rates of reaction obtained for telomerase at a substrate concentration of 0.2 μmol dm^{-3} compared with a substrate concentration of 1.6 μmol dm^{-3}.

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(b) When bananas are peeled, the exposed tissue gradually turns brown in the presence of oxygen in the air. This is due to an enzyme called catechol oxidase, which acts on the substrate catechol. Catechol and catechol oxidase are present in the banana tissue.

The overall reaction is shown in Fig. 3.2.

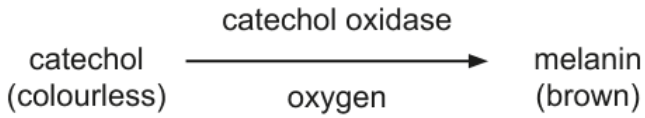


Fig. 3.2

A student investigated how the concentration of catechol oxidase affects the rate of this reaction. All other variables were kept constant throughout the investigation.

For each concentration of catechol oxidase used, the student mixed catechol oxidase solution with catechol and recorded the time taken for the mixture to reach a standard brown colour.

The rate of reaction, **R**, for each concentration of catechol oxidase used was then calculated using the formula:

$$\mathbf{R} = \frac{1}{\text{time to reach standard brown colour in minutes}}$$

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(ii) Fig. 3.3 is a graph showing the results of the investigation.

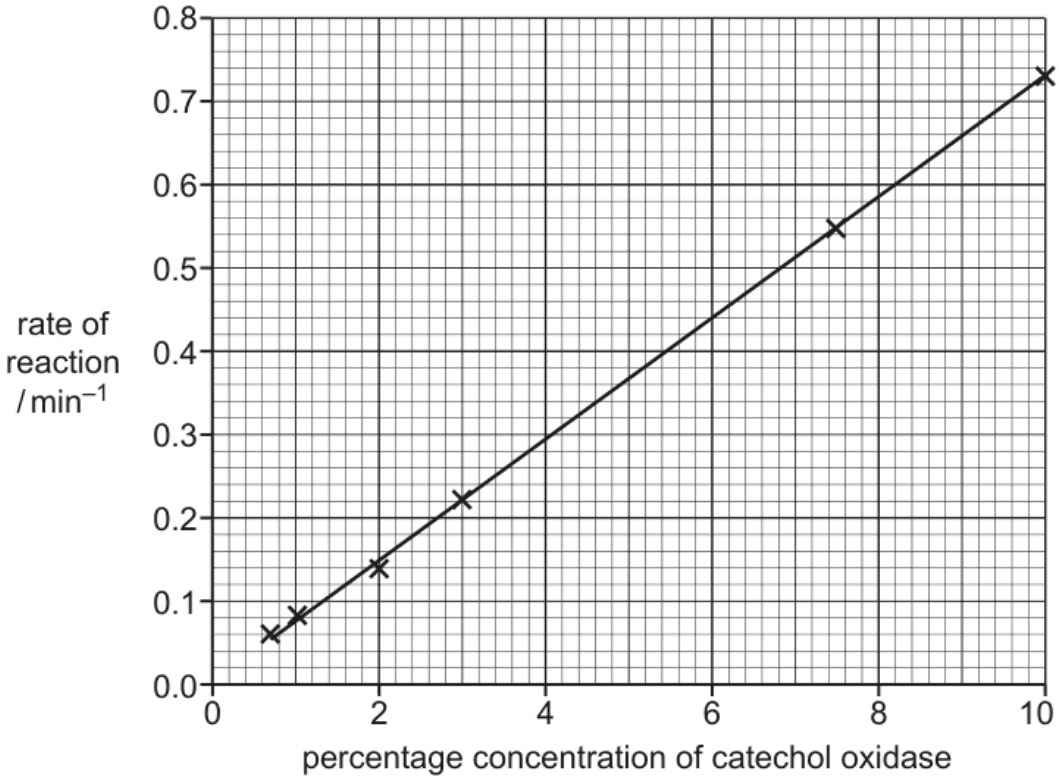


Fig. 3.3

State how the results shown in Fig. 3.3 show that substrate was in excess at all concentrations of catechol oxidase tested.

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(c) The student carried out a further experiment to investigate how the concentration of catechol affects the initial rate of reaction. All other variables were kept constant throughout this investigation.

Fig. 3.4 is a graph showing the effect of varying the concentration of catechol on the initial rate of reaction.

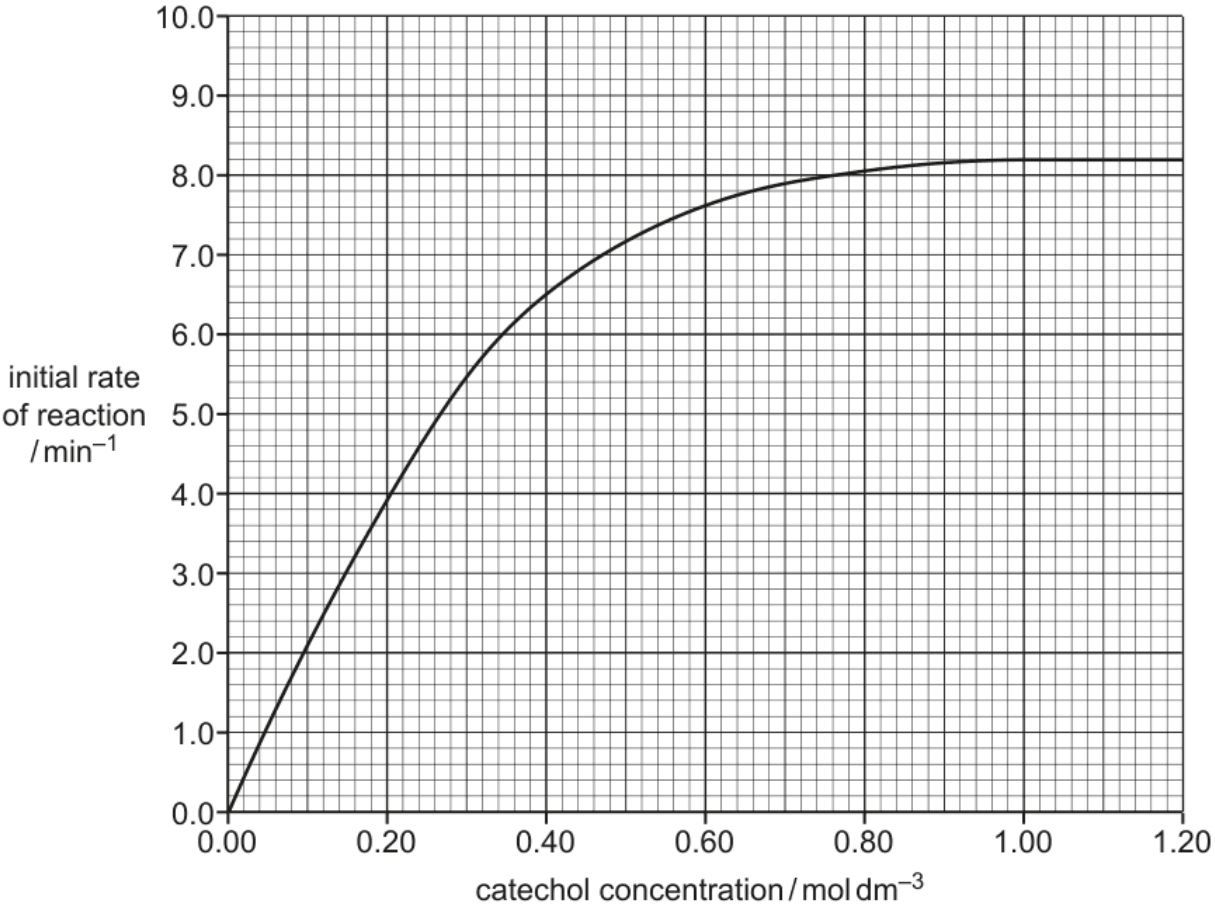


Fig. 3.4

(i) Explain the shape of the curve shown in Fig. 3.4.

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Question 9 · Paper 2 · February/March 2022 (22) · Q3(c)(ii) · 1 mark

(ii) Use Fig. 3.4 to calculate the value of the Michaelis–Menten constant (K_m) for the reaction between catechol oxidase and catechol.

K_m = ... $mol\,dm^{-3}$ [1]

Question 10 · Paper 2 · February/March 2022 (22) · Q3(c)(iii) · 1 mark

(iii) Methylcatechol has a similar shape to catechol. Catechol oxidase can also use methylcatechol as a substrate.

The K_m value for the reaction using methylcatechol as the substrate was found to be much lower than the K_m value for the reaction using catechol as the substrate, when the reactions were carried out under the same conditions.

State what these K_m values indicate about the relationship between the enzyme and the two substrates.

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(b) Scientists searching for a suitable enzyme to use in an industrial process isolated the bacterium *Vibrio parahaemolyticus* from the mouth of the Mediterranean eel, *Muraena helena*.

The scientists discovered an enzyme in the bacterium that was suitable for the industrial process. The scientists named the enzyme VpSP37.

The scientists investigated how the rate of reaction catalysed by VpSP37 is affected by the concentration of its substrate. The results of the investigation are shown in Fig. 3.4.

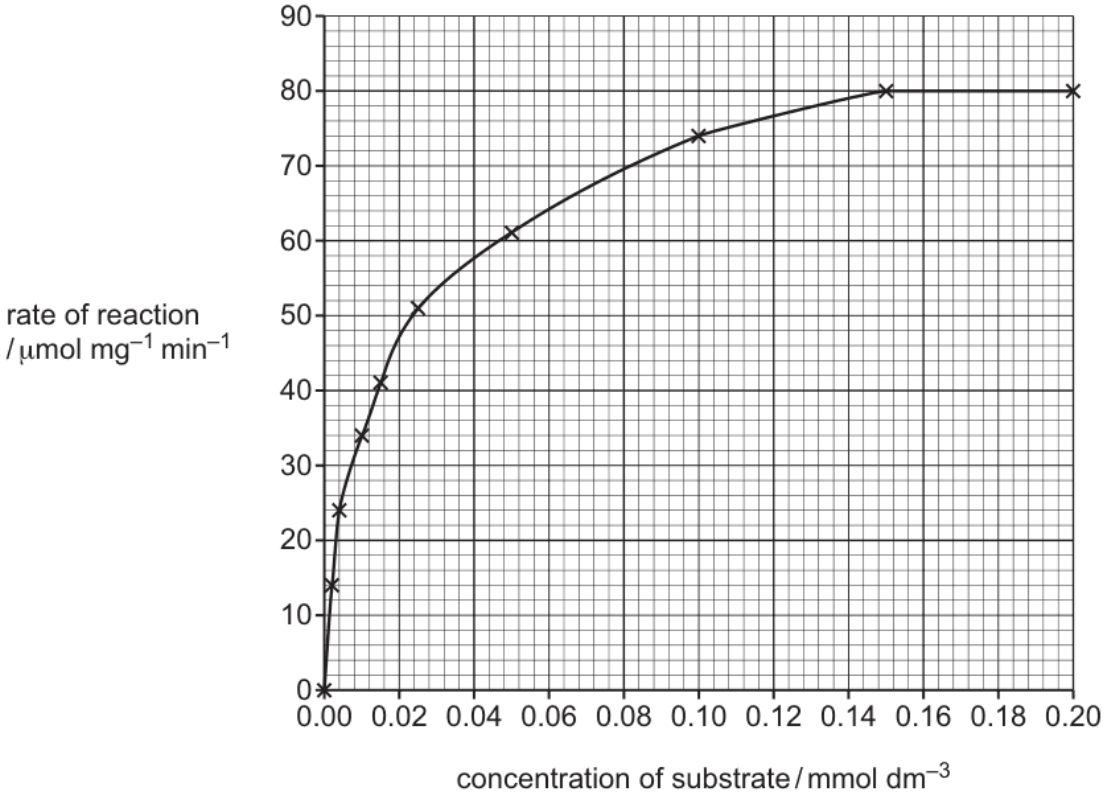


Fig. 3.4

(i) Calculate the Michaelis–Menten constant, K_m, for the enzyme VpSP37 using the information in Fig. 3.4.

Show your working.

K_m = ... [2]

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Question 12 · Paper 2 · May/June 2025 (21) · Q3(b)(ii) · 2 marks

(ii) The scientists discovered other enzymes that were suitable for the industrial process. These enzymes had higher K_m values than VpSP37.

Explain the advantage of using the enzyme VpSP37 in the industrial process rather than one of these other enzymes with higher K_m values.

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Question 13 · Paper 2 · October/November 2023 (21) · Q6(b)(i) · 2 marks

(b) RNA aptamers are short, single-stranded RNA molecules that can be used to study some infectious diseases.

Scientists studying an infectious disease in animals investigated the effect of RNA aptamers on the activity of RNA polymerase that is produced by the pathogen.

The aptamers bind to a specific region of the RNA polymerase.

Table 6.1 shows the effect of two aptamers, F47 and F52, on the activity of RNA polymerase produced by the pathogen.

Table 6.1

aptamer present	V_{max} of RNA polymerase / arbitrary units	K_m of RNA polymerase / arbitrary units
none	1664	346
F47	1072	508
F52	1467	523

(i) With reference to Table 6.1, compare the effect of the aptamers on the affinity of RNA polymerase for its substrate.

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Question 14 · Paper 2 · October/November 2023 (21) · Q6(b)(ii) · 3 marks

(ii) With reference to Table 6.1, suggest explanations for the effect of the presence of an aptamer on the rate of transcription catalysed by the RNA polymerase.

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Question 15 · Paper 2 · February/March 2025 (22) · Q5(c) · 3 marks

(c) RNA polymerase is composed of several polypeptides that move together and change shape as the enzyme performs its functions.

The death cap mushroom, *Amanita phalloides*, produces a toxin called alpha-amanitin, which binds to RNA polymerase at a site other than the active site. Alpha-amanitin reduces the activity of RNA polymerase.

Use the information provided to suggest how alpha-amanitin reduces the activity of RNA polymerase.

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Question 16 · information from earlier in the question

(c) Hydrolytic enzymes, known as collagenases, are secreted by cells in an inactive form.

Cells also secrete inhibitors of collagenases. The activity of the enzymes and inhibitors is regulated so that the development and maintenance of the extracellular matrix is controlled.

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Question 16 · Paper 2 · October/November 2022 (22) · Q5(c)(ii) · 2 marks

(ii) Synthetic inhibitors have been trialled as potential treatment for diseases caused by a lack of regulation of collagenase activity.

Research involves investigating the mechanism of action of an inhibitor.

State the effect that a **non-competitive** inhibitor will have on the maximum rate of reaction, V_{max}, and the Michaelis–Menten constant, K_m, of collagenase.

V_{max} .....

K_m .....

[2]

Question 17 · Paper 2 · May/June 2025 (22) · Q6(a) · 2 marks

6 Horseradish peroxidase (HRP) is an enzyme that can be extracted from the roots of the horseradish plant, *Armoracia rusticana*. HRP is used extensively in industry and technology.

In the reaction catalysed by HRP, hydrogen peroxide (H_2O_2) is used to oxidise an organic substrate. This is summarised in Fig. 6.1.

$$\text{organic substrate + hydrogen peroxide} \xrightarrow{\text{HRP}} \text{oxidised substrate + water}$$

Fig. 6.1

(a) Inhibitors can have an effect on V_{max}, the maximum rate of reaction, and K_m, the Michaelis–Menten constant, of HRP.

Fig. 6.2 shows the effect of substrate concentration on the rate of reaction of HRP.

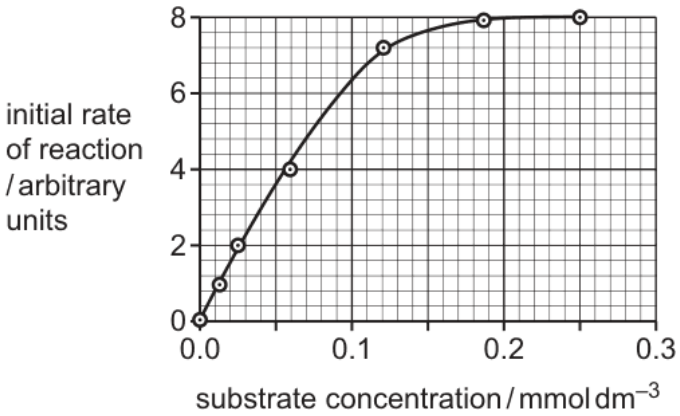


Fig. 6.2

Complete Fig. 6.2 by drawing a curve to show how the presence of a non-competitive inhibitor will affect the rate of reaction of HRP.

Use the curve **you have drawn** to obtain an estimate of K_m.

K_m = ... [2]

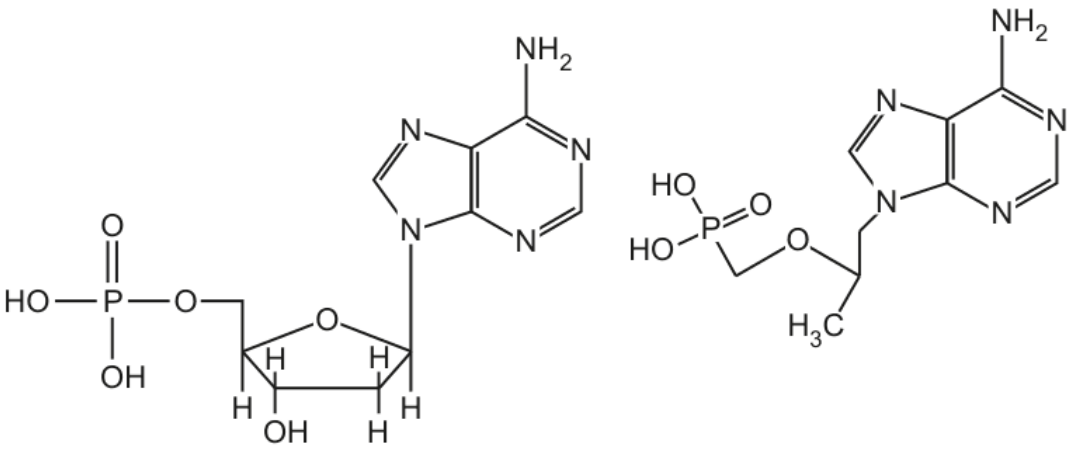
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(b) HIV has a nucleic acid core of RNA. The virus also contains the enzyme reverse transcriptase.

After HIV enters T-lymphocytes, reverse transcriptase catalyses the formation of DNA using activated DNA nucleotides with the viral RNA as a template.

Some drugs, such as tenofovir, have been developed to inhibit the action of reverse transcriptase.

The structure of tenofovir is similar to the structure of deoxyribose adenosine monophosphate, as shown in Fig. 3.2.



deoxyribose adenosine monophosphate

tenofovir

Fig. 3.2

After tenofovir is absorbed into cells it is phosphorylated twice and can be used by reverse transcriptase in the synthesis of DNA.

When a tenofovir molecule is added to the DNA strand being synthesised, the process stops.

Suggest the mechanism of action of tenofovir to prevent the synthesis of DNA by reverse transcriptase. Use the information in Fig. 3.2 in your answer.

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(b) Acetyl-coA combines with oxaloacetate in the Krebs cycle to form citrate. This reaction is catalysed by the enzyme citrate synthase.

Acetyl-coA has a similar shape to succinyl-coA, one of the compounds made in a later part of the Krebs cycle.

An experiment was carried out to investigate the effect of increasing the concentration of acetyl-coA on the activity of citrate synthase. The experiment was repeated, this time adding a solution of succinyl-coA.

Fig. 8.1 shows the results of these experiments.

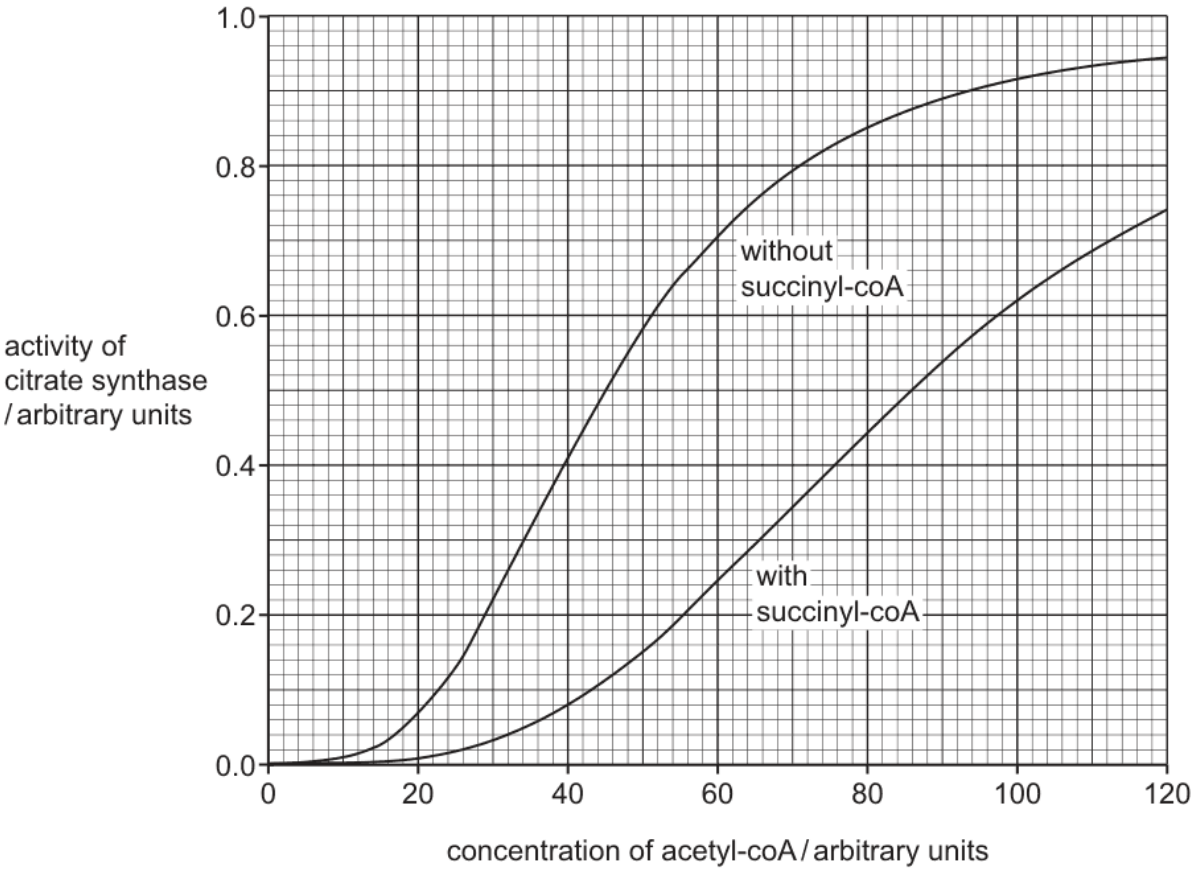


Fig. 8.1

With reference to Fig. 8.1, explain how succinyl-coA could help to regulate the Krebs cycle.

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Question 20 · information from earlier in the question

(e) Some people with lactase non-persistence may be lactose intolerant. They may have symptoms, such as abdominal pain, if their diet contains lactose.

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(ii) The lactose in milk can be hydrolysed using immobilised lactase or using lactase free in solution (free lactase). This results in milk and milk products that do not contain lactose and so are suitable for lactose-intolerant people.

Scientists carried out an investigation to compare the activity of lactase immobilised in very small magnetic beads (magnetic microspheres) with free lactase, at different temperatures and at different pH values.

Fig. 3.2 shows the activity of immobilised lactase and the activity of free lactase at 5 different temperatures.

Fig. 3.3 shows the activity of immobilised lactase and the activity of free lactase at 8 different pH values.

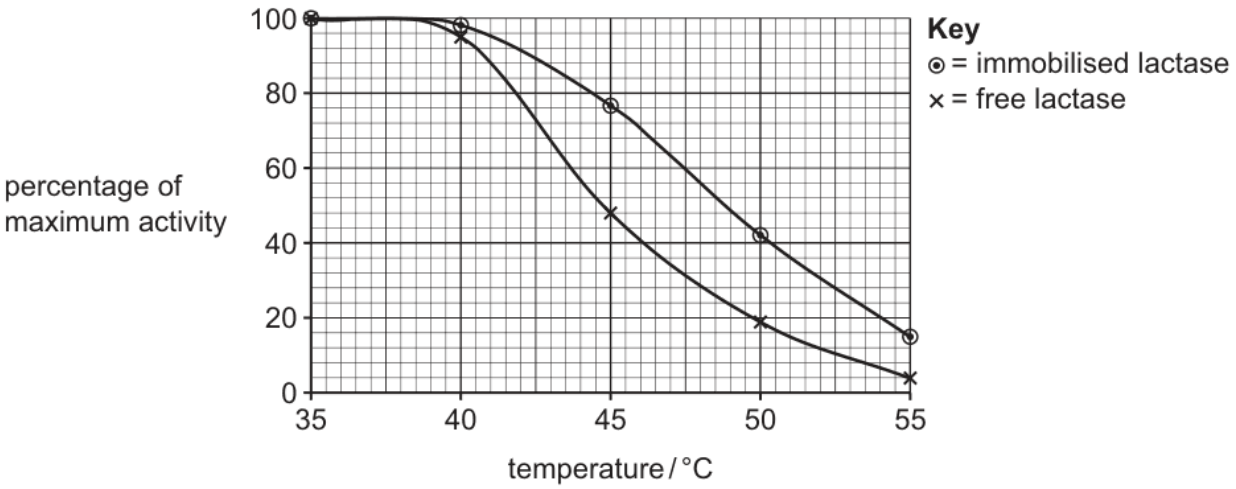


Fig. 3.2

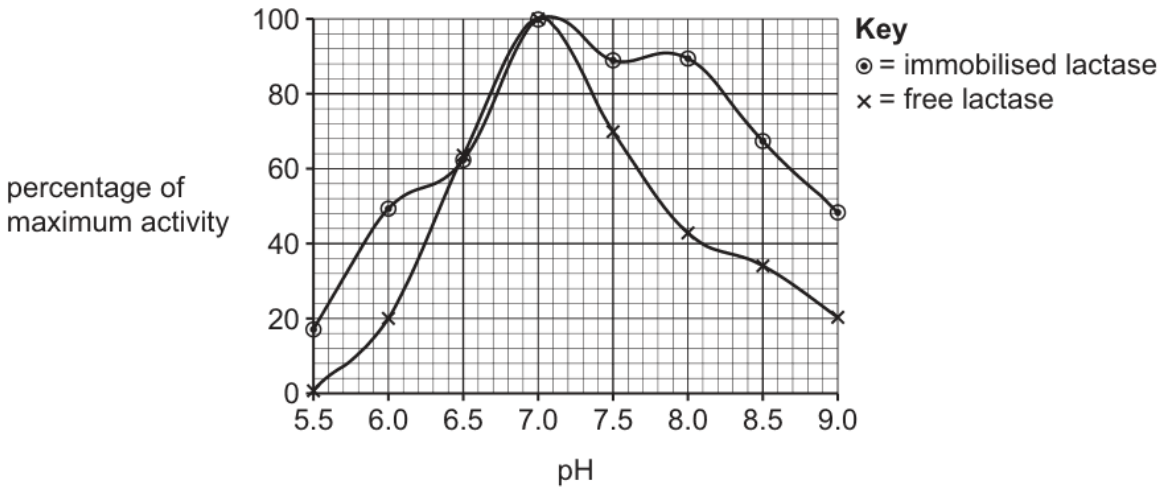


Fig. 3.3

One advantage of using magnetic microspheres with immobilised lactase is that they can be easily recovered using an electric field and reused.

With reference to Fig. 3.2 and Fig. 3.3, explain why the results indicate that there are **other** advantages in using immobilised lactase instead of free lactase to produce

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6 Amylase is an enzyme that catalyses the hydrolysis of starch into reducing sugars.

A student carried out an experiment to investigate the hydrolysis of starch using immobilised amylase. Fig. 6.1 is a diagram of the apparatus that was used in the investigation.

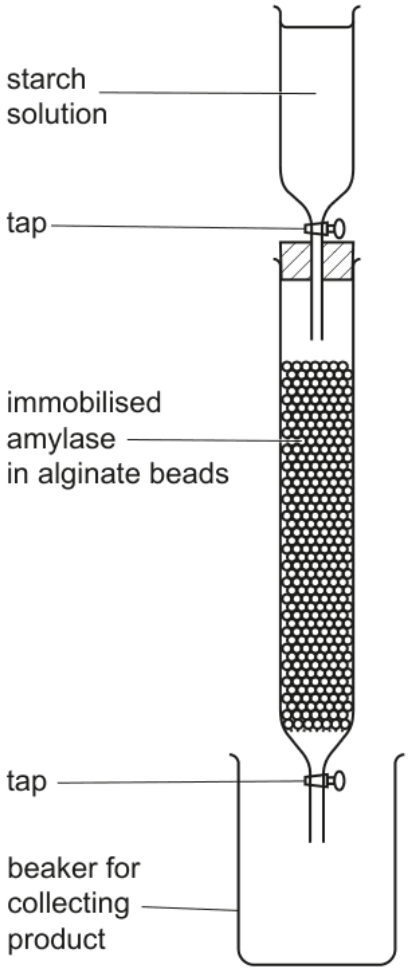


Fig. 6.1

The alginate beads were all the same size.

Both taps were opened to allow the starch solution to flow down the column and for the product to be collected.

The product was tested for the presence of reducing sugar and starch.

(a) The results of the investigation showed that the product collected in the beaker contained reducing sugar **and** starch.

With reference to Fig. 6.1, describe a method that would allow the student to use the immobilised amylase to collect a product that contains **only** reducing sugar.

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(c) An enzyme that catalyses a reaction of commercial interest needs to be investigated to see if it is suitable for use in industry.
For example:

- immobilised enzymes may be used as they have a longer shelf-life than the enzyme free in solution
- many industrial reactions are carried out at higher temperatures to minimise contamination of products by microorganisms.

Fig. 5.2 shows the results of an investigation to compare the activity of sucrose phosphorylase free in solution (free enzyme) with immobilised sucrose phosphorylase (immobilised enzyme) at different pHs.

Fig. 5.3 shows the activity of the free enzyme and immobilised enzyme at different temperatures.

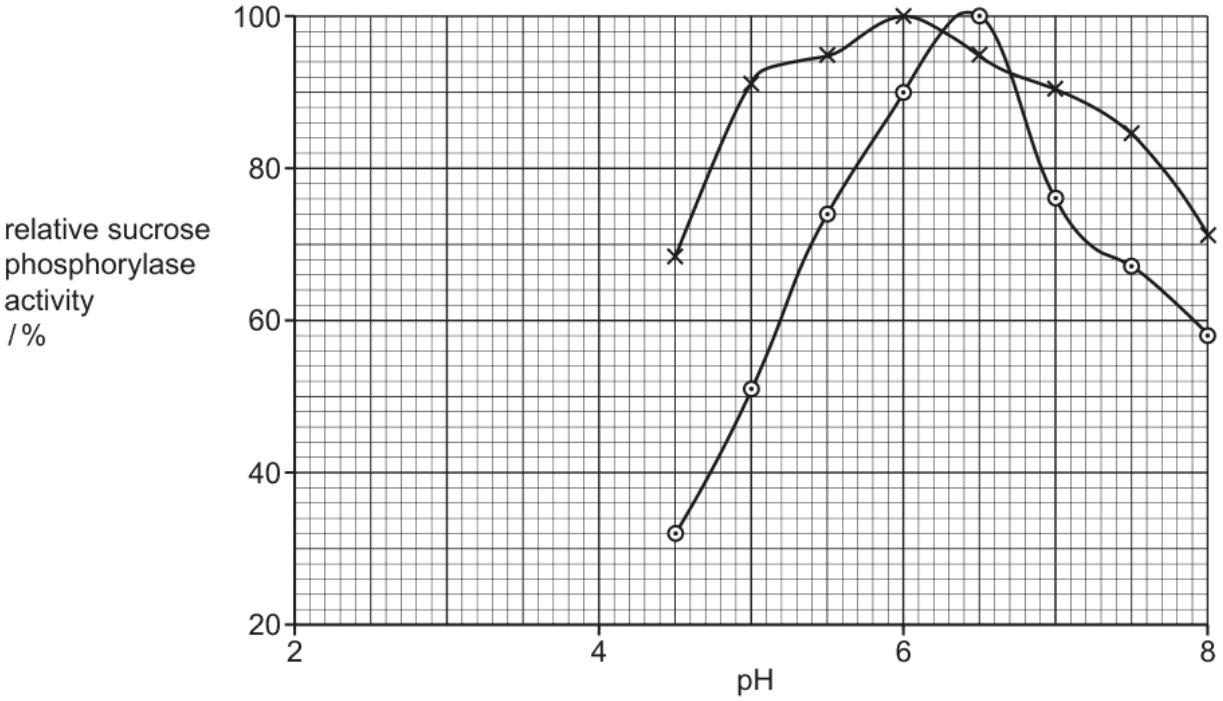
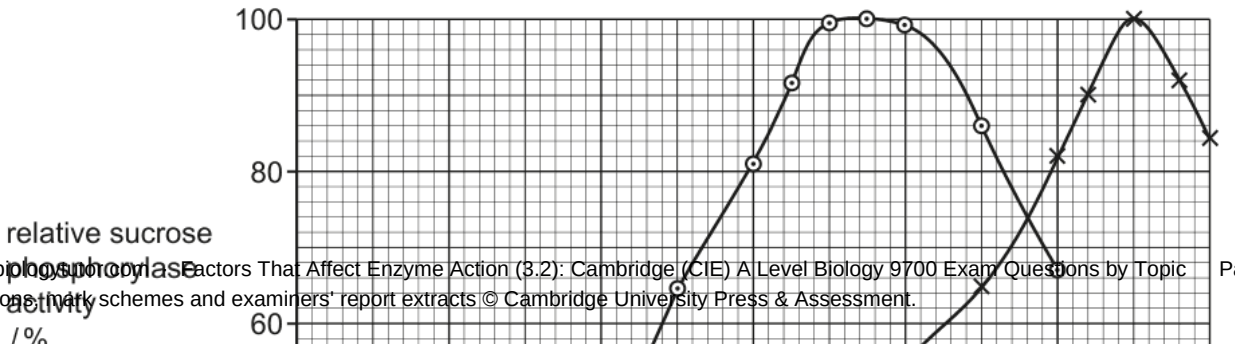


Fig. 5.2



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Mark schemes

Question 1 · Paper 2 · February/March 2023 (22) · Q6(b) · 2 marks

6(b)	*any **two** from:* *at pH 8.0:* ionic / hydrogen bonds, (between R groups) broken / altered ; active site shape altered ; (active site) no longer / less, complementary to substrate / collagen **or** fewer enzyme–substrate complexes formed ; AVP ; e.g. the amino acids in the active site affected by the changing pH ref. to partial denaturation	**2**

Question 2 · Paper 2 · October/November 2023 (22) · Q2(e) · 3 marks

2(e)	*must have pH and °C and %, at least once* *max 2 if only Fig. 2.1 **or** only Fig. 2.2 used for the response.* *any **three** from:* *advantage from **Fig. 2.1** (temperature)* 1 A22 and K12, active / remove stains, at a wide range of temperature **or** stated (active) temperature range of, A22 is 20.5 / 21 °C **or** K12 is 22 °C **or** use of Fig. 2.1 to give % activity and stated temperature of, good / above 60%, activity at, medium / hotter, temperature washes for, A22 / K12 ; **or** K12 active at low temperature (washes) ; **A** data e.g. 37–40% activity at 20 °C / 41–43% activity at 30 °C *disadvantage from **Fig. 2.1** (temperature)* 2 low activity / poor stain removal, at 30 °C / 70 °C / 80 °C, for A22 **or** at 20 °C / 30 °C / 70 °C, for K12 ; **A** data extracted from **Fig. 2.1** *advantage from **Fig. 2.2** (pH) – activity in alkaline conditions* 3 A22, greatest activity / most effective / AW, pH 7.5 – 8.0 **or** (at least 60%) activity between pH 7.5 – 9.0 ; **A** range of pH 6.0 – 9.0 but **R** i*f stated pH 6.0–7.0 is alkaline* **or** K12, active between pH 7.5 – 8.0 / 8.5 **or** optimum activity at pH 8.0 **or** data for (good) activity at stated pH values from Fig. 2.2 for either A22 or K12 ; *disadvantage from Fig. 2.2 (pH)* 4 use of **Fig. 2.3** to show a decrease in activity at higher pH values ;	**3**
2(e)	5 any wash feature in context of mp 1–4 ; e.g. have (some) activity at low temperatures so, more economic /less energy used (to heat water) **A** ora active at high temperatures but, more costly / needs (more) energy can wash at high(er) temperature so, can sanitise clothes / can kill insect pests / can kill pathogens / shorter time to remove stains comparison of K12 and A22 for washes e.g. K12 more, thermostable / stable in (washes at) higher temperatures K12 has higher optimum temperature so more, costly / energy used K12 only useful at lower alkaline pH A22 has wide(r) working range of pH A22 more tolerant to / stable at, higher pH	

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Question 3 · Paper 2 · February/March 2021 (22) · Q2(d)(i) · 3 marks

2(d)(i)	*any **three** from:* **1** increase in temperature increases kinetic energy of, molecules / particles / substrate / enzyme ; **A** moving around faster **2** (increase in temperature) increases rate of, successful collisions between enzyme and substrate / enzyme–substrate complex formation / substrate binding to active site ; **OR** at 48 °C (47 or 49) ref. to, optimum temperature / maximum enzyme activity / active sites binding substrate at maximum capacity / AW ; **3** (at temperatures higher than optimum) ref. to denaturation / loss of shape of active site **(**and decrease in activity**)** ; **4** AVP ; e.g. weaker / hydrogen, bonds break (and ref. to denaturation)	**3**

Question 4 · Paper 2 · February/March 2021 (22) · Q2(d)(ii) · 2 marks

2(d)(ii)	optimum / peak / max activity / at 100% and to right of original ; line beyond optimum must be to right and, reach vertical axis at 50% or below / reach horizontal axis ;	**2**

Question 5 · Paper 4 (A level) · October/November 2021 (42) · Q3(c)(i) · 3 marks

3(c)(i)	*any **three** from:* 1 enzyme, denatured / deactivated / inactivated ; 2 changes, tertiary structure / 3D shape, of amylase ; **A** enzyme 3 changes shape of active site ; 4 active site, no longer complementary / does not bind, to starch / substrate **or** no, ESC / enzyme-substrate complexes, formed ; 5 AVP ; e.g. removes water from embryo for gibberellin synthesis / removes water from endosperm for hydrolytic reactions	**3**

Question 6 · Paper 2 · October/November 2019 (22) · Q6(d) · 2 marks

6(d)	*allow low(er) rate for 0.2 μmol dm^{-3} and high(er) rate for 1.6 μmol dm^{-3}* *any two from:* comparison in terms of active site(s) ; e.g. (low rate) active sites, not all occupied / spare / not all saturated **or** (high rate) active sites all occupied / no spare active sites / active sites saturated bod 'active sites are limited' comparison in terms of enzyme-substrate complexes formed (per unit time) e.g. (low rate) few(er) ES complexes formed **or** (high rate) many / more, ES complexes formed ; **A** (low rate) few(er) collisions between enzyme and substrate ora (low rate) substrate concentration limiting factor **or** (high rate) limiting factor is, enzyme concentration / not substrate concentration;	**2**

Question 7 · Paper 2 · February/March 2022 (22) · Q3(b)(ii) · 1 mark

3(b)(ii)	straight line that continues to rise across all concentrations / line does not level off at higher concentrations ;	**1**

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Question 8 · Paper 2 · February/March 2022 (22) · Q3(c)(i) · 3 marks

3(c)(i)	*any **three** from:* 1 at low, catechol / substrate, concentrations there are many active sites available ; 2 adding more substrate increases the rate of, enzyme–substrate complex formation / more successful collisions / AW ; 3 at high(er) substrate concentrations all the active sites are, occupied / saturated ; 4 at low substrate concentrations the substrate is the limiting factor **or** at high substrate concentrations the enzyme concentration is a limiting factor **or** at high substrate concentrations some factor other than substrate concentration is the limiting factor ; 5 number of active sites does not change / AW ;	**3**

Question 9 · Paper 2 · February/March 2022 (22) · Q3(c)(ii) · 1 mark

3(c)(ii)	0.21 (mol dm^{-3}) ;	**1**

Question 10 · Paper 2 · February/March 2022 (22) · Q3(c)(iii) · 1 mark

3(c)(iii)	catechol oxidase has a higher affinity for methylcatechol than for catechol / ora ;	**1**

Question 11 · Paper 2 · May/June 2025 (21) · Q3(b)(i) · 2 marks

3(b)(i)	V_{max} = 80 (μmol mg^{-1} min^{-1}) and half V_{max} = 40 (μmol mg^{-1} min^{-1}) ; **A** 80 and 40 / horizontal lines shown at 80 and 40 on Fig. 3.4 K_m = 0.014 mmol dm^{-3} / 14 μmol dm^{-3} ; *unit must be given on answer line or in working* **A** in range 0.012 to 0.016 mmol dm^{-3}	**2**

Question 12 · Paper 2 · May/June 2025 (21) · Q3(b)(ii) · 2 marks

3(b)(ii)	*any **two** from:* lower concentration of substrate to reach (½) V_{max} / AW ; **A** faster rate at, the same concentration / lower concentration VpSP37 has a higher affinity for its substrate (than the other enzymes) ; **ora** explained ; e.g. better fit between substrate and active site / AW ; **I** ESCs formed more efficiently / any *ref. to* cost, etc.	**2**

Question 13 · Paper 2 · October/November 2023 (21) · Q6(b)(i) · 2 marks

6(b)(i)	*any **two** from:* (both increase K_m, therefore both) reduce the affinity (of RNA polymerase for its substrate) ; (presence of) F52 reduces the affinity for the substrate more than F47 ; ora for F47 AVP ; e.g. manipulation of data to support described trend correct link stated between K_m values and affinity	**2**

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Question 14 · Paper 2 · October/November 2023 (21) · Q6(b)(ii) · 3 marks

6(b)(ii)	*any **three** from:* *decreased, maximum rate of reactions / rate of transcription because* V_{max} lower, qualified ; binding (of the aptamer) changes, the tertiary structure / shape of, active site (of RNA polymerase) ; **A** binding changes shape of enzyme so active site less complementary to substrate (so) substrate / (RNA) nucleotides, bind less easily to active site ; **A** fewer enzyme-substrate complexes form binding of aptamer prevents, substrate / (RNA) nucleotide, binding to active site ; binding prevents RNA polymerase from binding to template strand ; binding prevents RNA polymerase from forming bond between RNA nucleotides ; AVP ;	**3**

Question 15 · Paper 2 · February/March 2025 (22) · Q5(c) · 3 marks

5(c)	*any **three** from* 1 ref. to non-competitive inhibition ; 2 binding of alpha-amanitin changes shape of active site ; 3 active site, no longer / less, complementary to, substrate / DNA **or** fewer enzyme–substrate complexes formed ; 4 prevents RNA polymerase from, binding to DNA / unwinding DNA / rewinding DNA ; 5 blocks movement of the polypeptides of the enzyme / prevents the enzyme changing shape / hinders induced fit ; 6 RNA polymerase movement along DNA, prevented / slowed down ; **accept** prevents addition of nucleotides to the chain	**3**

Question 16 · Paper 2 · October/November 2022 (22) · Q5(c)(ii) · 2 marks

5(c)(ii)	(V_{max}) decreases ; (K_m) stays the same ;	**2**

Question 17 · Paper 2 · May/June 2025 (22) · Q6(a) · 2 marks

6(a)	curve drawn to the right of original, same shape and lower maximum rate of reaction, passing through plot of 0.055 to 0.06 mmol dm^{-3} on x-axis ; *must start branching away before third plot point at 2au* **R** S-shaped / sigmoid curve K_m = 0.055 to 0.06 mmol dm^{-3} ; *allow ecf if no curve drawn and printed curve used* *incorrect K_m but standard curve for non-competitive inhibition drawn, allow 2 marks if their K_m is correct* *if competitive curve drawn and K_m is correct from their curve allow* ecf *1 mark*	**2**

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Question 18 · Paper 2 · May/June 2024 (21) · Q3(b) · 2 marks

3(b)	*any **two** from:* tenofovir competes with, (activated / phosphorylated) adenine nucleotide / deoxyribose adenosine triphosphate / dATP, for active site / to prevent nucleotides being added to elongating chain ; **A** (tenofovir acts as a) competitive inhibitor (of reverse transcriptase) tenofovir forms a phosphodiester bond to elongating DNA strand, but stops further reactions / AW ; tenofovir has, no 3´ -OH so next nucleotide cannot form a phosphodiester bond / no (deoxy)ribose so cannot form phosphodiester bond ; **A** pentose **R** sugar unqualified	**2**

Question 19 · Paper 4 (A level) · February/March 2025 (42) · Q8(b) · 4 marks

8(b)	*any **four** from:* 1 reduces activity of citrate synthase **or** activity of citrate synthase is higher without succinyl-coA than with succinyl-coA ; 2 comparative data quote ; 3 succinyl-coA binds to citrate synthase active site ; 4 succinyl-coA acts as a competitive inhibitor / described ; 5 slows down Krebs cycle ; 6 prevents build up / reduces production, of, citrate / intermediates ; 7 AVP ;	**4**

Question 20 · Paper 2 · October/November 2025 (22) · Q3(e)(ii) · 4 marks

3(e)(ii)	*any **four** from:* *accept activity for percentage of maximum activity* **1** immobilised (overall) greater, productivity / yield / hydrolysis (of lactose in milk) ; **I** activity **2** (process / immobilised) more tolerant to, temperature / pH, fluctuations / changes ; AW **A** *idea that* can be used in processes covering a wide range of, temperature / pH **3** detail immobilised vs free lactase from Fig. 3.2 ; e.g. (at temperatures) above optimum / higher than 38 / 39 °C, (immobilised) has a higher activity AW above optimum / higher than 38 / 39 °C, (partial / extent of) denaturation (for immobilised) is less at each temperature AW **A** higher than 40 °C *if accompanying context is correct* **R** if optimum stated as 35 °C **4** data from Fig. 3.2 to support ; *any one comparison (units only for temperature required)* **5** detail immobilised vs free lactase from Fig. 3.3 ; e.g. higher activity at all pHs except pH 6.5 and pH 7.0 higher activity, below pH 6.5 / above pH 7.0 free not active at pH 5.5 but immobilised active, at all pHs / for a wider pH range **6** data From Fig. 3.3 to support ; **7** AVP ;; e.g. can use a lower temperature / cheaper, for same quantity of product **8** more, heat stable / thermostable / stable at high temperatures **I** longer shelf-life **R** thermostatic better in alkaline pHs	**4**

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Question 21 · Paper 2 · October/November 2021 (22) · Q6(a) · 3 marks

6(a)	**1** (open top tap and) keep bottom tap closed / close bottom tap for a longer time (and then open bottom tap to collect product) **A** close bottom tap for complete hydrolysis to occur **or** run starch solution through column, collect product, repeat (a number of times) / AW **or** only allow a small quantity of starch solution through at a time / AW **;** *if a different idea suggested to set-up in Fig. 6.1, method must be related to an increase in contact time between beads and starch to award mp1* *any **two** from:* **2** suggestion of modification to experimental set-up in Fig. 6.1 **;** e.g. use smaller beads use more, beads / enzyme in beads **I** use more immobilised enzyme use of visking tubing attached to end of column (so only reducing sugar passes out) use longer, narrower column **I** *ref. to* filtering with filter paper / adding buffers to control pH **3** test (sample of) product with iodine solution, qualified **;** **A** iodine in potassium iodide solution / I in KI solution e.g. to confirm no starch present stays orange (colour) does not change to blue-black (colour) to confirm no starch present **4** ref. to using optimum temperature (for amylase) **; A** e.g. 35 °C temperature	**3**

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Question 22 · Paper 2 · October/November 2020 (22) · Q5(c) · 4 marks

5(c)	**I** *ref. to* denaturation, either mp2 **or** mp10 can be awarded *any **four** from:* *immobilised* **1** higher optimum temperature **;** AW e.g. peak / max activity / 100% activity **;** **2** 74 / 75 / 76°C, v, 56 / 57 / 58 / 59°C **;** **A** one correct value and calculation e.g. 17°C lower **3** *idea that* greater range of pH with, higher activity / greater stability **;** AW *allow use of comparative data at one pH value to support if answer unclear* **4** *idea that* if pH changes, less effect on enzyme activity **;** **A** resists changes in pH **5** *ref. to* more active at, higher temperatures / temperatures above 68°C **;** **A** more thermostable / less effect on enzyme at higher temperatures **A** free enzyme not active above 70°C *allow use of comparative data at one temperature to support if answer unclear*
5(c)	**6** one advantage of immobilised **; e.g.** reusable o less / no, contamination of product (by enzyme) o easier, downstream processing / separation of enzyme from product o longer shelf-life useful if enzyme, difficult to obtain **A** *ref. to* cost effective o can use in continuous processes so more productive o suggestion that (can carry out process at) lower pH (more acid conditions and) may be more effective against microorganisms *free enzyme* **7** higher activity, at lower temperatures / between 35°C to 68°C **;** *in context of a range, quoting one temperature not enough* **8** two temperatures, comparative values for both enzymes to support mp7 **;** **9** optimum temperature lower so cheaper maintaining that temperature **;** AW **A** lower temperatures cost efficient to maintain and enzyme works well / AW **10** (values of optimum) 74 / 75 / 76°C, v, 56 / 57 / 58 / 59°C **;** **A** calculation, e.g. 17°C lower **11** lower optimum temperature still high enough to kill microorganisms **;**

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