Mark scheme - Enzymes

20	a	i	*three from* competes (with substrate) / competitive (1) enters / fits in / binds to / blocks, active site (1) prevents substrate from entering active site (1) (binds to active site) temporarily (1)	3	
		ii	(at high substrate concentration) rate approaches rate in absence of inhibitor (1)	1	**IGNORE** idea that increased substrate concentration overcomes the inhibition as answer must refer to evidence from the graph.
	b	i	32 (1) mmol dm^{-3} min^{-1} (1)	2	**ALLOW** mmol dm^{-3} / min' or 'mmol dm^{-3} per, min / minute **ALLOW** 0.53 mmol dm^{-3} / s
		ii	(initial rate likely to be) greater (1) *because…*	3	
		ii	higher concentration of, substrate / amylose, molecules (at start) (1) more chance of, substrate / AW, entering active site (1)		**ALLOW** 'starch'
			Total	**9**	

21		i	*Amanita* ✓	1	First letter must be a capital, the rest must be lower case. **Examiner's Comments** Most candidates selected the correct name and wrote it with a capital letter.
		ii	*one from* 1 (starch) digestion in the regions where the, fungus / hyphae, not present ✓ 2 *enzymes / they, are* released / diffuse away, from the fungus **or** extracellular / secreted ✓	1	1 **ACCEPT** breaks down (starch) in the, region / area / agar, around the fungus **Examiner's Comments** It was surprisingly rare for candidates to apply their knowledge of kingdom Fungi to realise that digestion here is extracellular, involving enzymes being secreted by the hyphae and acting outside of them. Some referred to 'it' and their subsequent answer did not make it clear whether the answer referred to the enzymes or the fungus.
			Total	**2**	
22			prosthetic group ✓ induced fit ✓	3 (AO2.1)	

			non-competitive inhibition ✓		
			Total	**3**	
23			A	1(AO1.1)	
			Total	**1**	
24		i	1. cluster / iron / molybdenum / sulfur , are, cofactors / prosthetic groups ✓ 2. H_2 is a, competitive inhibitor / end product inhibitor ✓ 3. CO is a <u>non-competitive</u> inhibitor ✓ 4. (CO binds to allosteric site and) causes change in shape of active site ✓ 5. energy required (from ATP) ✓ 6. acidic conditions, are tolerated / increase reaction rate ✓	**4 max**	**DO NOT ACCEPT** coenzyme 2. **ACCEPT** H_2, competes / AW, with N_2 for the active site **OR** 'increase in H_2 will reduce the activity of the enzyme' 3. **ACCEPT** CO acts as a cofactor (as candidates may be unfamiliar with CO) 5. **ACCEPT** ATP required as process is active **Examiner's Comments** It was pleasing to see that the majority of candidates were awarded two or three marks for this question accessing marking points 2, 3 and 4, for identifying H_2 as a competitive inhibitor and CO as a non-competitive inhibitor (and then going on to add how this affects the shape of the enzyme's active site). The other two marking points for this question were rarely mentioned, but sometimes the marks for these were missed when candidates did not expressly say that as ATP is needed, the process is active/energy requiring or for saying that acidic conditions are tolerated or increase reaction rate. In addition, some marks were lost for marking points 3 and 4 as students mistake CO for CO_2.
		ii	transport of oxygen, for respiration / to generate ATP (in *Rhizobium*)✓ removes(excess) oxygen so less inhibition (of enzyme / reaction)✓ removes CO to prevent inhibition (of nitrogenase) ✓	**2**	**ACCEPT** removes oxygen / creates anaerobic conditions, for nitrogen fixation **IGNORE** removes H_2 so more N_2 can bind

					(to active site) **Examiner's Comments** Few candidates obtained full marks on this question. Those that did talked about the removal of oxygen and CO and therefore removal of inhibition of the enzyme. Some common errors/omissions on this question included: • Candidates mentioned the removal/ binding of CO/oxygen by leghaemoglobin but did not then mention how this affects the enzyme. • Candidates talked about how leghaemoglobin provides the Iron (from the haem group) for the enzyme's prosthetic group or protons/electrons for the reaction.
			Total	**6**	
25		i	*I*: another named control variable (not mentioned in text) ✓ *E*: *idea of* prevent other factors (other than temperature) affecting results ✓ *I*: *idea of* standardised method ✓ *E*: minimises experimental error ✓ *I*: temperature intervals closer together ✓ *E*: (gives a more) accurate estimate of optimum temperature	4 max (AO3.4)	*Read as prose as improvement mark could be found in explanation e.g. 'I; substrate concentration E; should be kept constant' gets I mp* *Marks for explanation can be awarded if the linked improvement mark is attempted but not given* e.g. area of film / volume of pH buffer / source of trypsin thickness / volume / concentration, of, gelatine / substrate **IGNORE** amount e.g. thickness may affect rate of breakdown of gelatine e.g. film is placed in the solution in the same way each time / measure time for set volume of gelatine to be broken down / use a thermostatically controlled water bath **ALLOW** improves, accuracy / reproducibility/ repeatability / precision **IGNORE** improves reliability **ALLOW** extend temperature range below 10°C

			✓ *I*: control group / tube with no trypsin / tube with boiled trypsin ✓ *E*: to see if gelatine breaks down without trypsin (at different temperatures) / to allow comparison (with experimental data) ✓		**ALLOW** shows the optimum / best temperature (for trypsin) **ALLOW** improves precision **DO NOT ALLOW** improves, reproducibility/reliability **ALLOW** to show trypsin is needed to break down gelatine **ALLOW** to see if heat breaks down gelatine **Examiner's Comments** Candidates did not gain marks for describing improvement aspects of the experiment that were already in place on the exam paper (e.g. controlling pH using a buffer) or variants of this (e.g. saying that the set time period should be stated exactly). The most common correct answers concerned controlling another variable such as the thickness, volume or concentration of the gelatine substrate. Not all could match this improvement with the explanation that variation in this variable would affect the rate being measured. Candidates also sometimes attempted to describe a way of standardising the method, such as using a thermostatically-controlled water bath, although again correct explanations relating to improved precision and reproducibility or repeatability were not always forthcoming. Few candidates considered running a control experiment. Candidates who realised that accuracy could be improved by testing at more temperatures often did not state 'within the range' or to make clear that the more temperature intervals they suggested would be smaller intervals between 10°C and 50°C. Some students did not understand that this question was about practical measurement and talked about improvements relating to calculations and statistical analysis. Correct use of terms such as accuracy, precision, reproducibility and repeatability were important in answering this question. Many candidates justified their suggested

					improvements by simply repeating the term 'validity' from the question.  **AfL** The word 'amount' is not specific enough and should be avoided by candidates.  **OCR support** Appendix 4 of the Practical Skills Handbook, provides information on terms used in measurement and conventions for recording and processing experimental measurements. This is in line with the 'The Language of measurement' booklet: https:/www.ocr.org.uk/Images/294468-biology-practical-skills-handbook.pdf
		ii	mm^2 / cm^2 **and** s^{-1} / min^{-1} ✓	1 (AO2.4)	**ALLOW** /s /min **DO NOT ALLOW** 'per' or 'sec' or 'minute' **Examiner's Comments** A few answers provided correct units for area per unit time such as mm^2 s^{-1} or cm^2 / min. Errors included giving measures of volume (mm^3 and cm^3), combining two conventions such as using a slash and '$^{-1}$' after the time term, and writing in the format of area unit 'per' the time unit. Correct abbreviations of units were needed as opposed to words like 'minutes' or 'sec'.
		iii	*I agree / yes, because…* two mode values exist (for icefish trypsin) ✓ *I disagree / no, because…* outlier / anomaly, included in the mean (for human trypsin) ✓ median / mode, not / less, affected by outliers ✓	2 max (AO3.2)	**IGNORE** references to decimal places **Examiner's Comments** Many candidates provided descriptions of the terms mean, mode and median, but these gained no marks, as they were not related to the question. Some candidates

					showed awareness that the mean calculation included an outlier though not all reasoned that, as a result the student's statement was incorrect. Similarly not all considered that a strength of the median or mode is that they are unaffected by outliers. Very few noticed that the existence of two values for the mode for icefish trypsin was a problem. Some candidates are demonstrating their understanding of the command term 'evaluate' by trying to provide a balanced answer, in this year's exams.
		iv	(Student's)(unpaired) t-test ✓ (they are) comparing **means** (of two data sets) / AW ✓	2 (AO3.1)	**IGNORE** standard deviation **DO NOT ALLOW** paired / dependent / related, t-test e.g. 'finding the difference between 2 means' **ALLOW** 'compare averages of 2 data sets' **Examiner's Comments** Many candidates referred to the correct answer which was t-test. . However, most candidates scored only one mark as they did not explain that this allows comparison of two means (they often just stated two data sets, which is too vague). Some candidates showed extended knowledge of the application of statistics to experimental design with the use of terms like unpaired, unrelated and independent. Incorrect answers included the χ^2 test, standard deviation and Spearman's rank correlation. **OCR support** 'Mathematical skills statistics booklet' can help to develop the correct use of statistical tests: https:/www.ocr.org.uk/Images/338621-mathematical-skills-statistics-booklet.doc
			Total	**9**	
26			enzyme–substrate complex (1) active site (1) tertiary (1)	5	

			induced fit (1) activation energy (1)		
			Total	**5**	
27		i	*At higher temperature / 60°C* more kinetic energy therefore more, successful collisions / ESC formed ✓ initial rate (of reaction) faster ✓ enzyme (eventually) denatured and, less product formed / reaction stopped earlier / not all substrate reacted ✓	max 2	**ORA** for 37°C **ALLOW** description of denatured **Examiner's Comments** This question required careful interpretation of the graphs as well as an understanding of enzyme function. A small number of candidates were able to give clear, logical, explanations to account for the shapes of the curves. In general, it seems that students did not apply a systematic approach to graph analysis. Many *described* the shape of the curves rather than attempting to *explain*. In part (c)(i) the majority of students gave GCSE responses about 37°C being the optimum temperature and this being the temperature that enzymes "work best at". They ignored the evidence in the graph showing a faster rate of reaction at 60°C for the first part of the time period. There were not a lot of direct references to the graph. **OCR support** The Mathematical skills handbook is provided on the OCR website: https:/www.ocr.org.uk/Images/294471-biology-mathematical-skills-handbook.pdf **Exemplar 5**

					At 37°C the enzymes are working at their optimum temperature and so produce the highest yield with the enzyme-substrate complexes being formed. However at 60°C the temperature is higher than 37°C so at first there is a steeper rate of product formation due to the increased number of enzyme-substrate complexes. However at 60° the rate is lower than 37° as the temperature is too high so enzymes' tertiary structure is imposed and so enzymes denature. [2] This response to part(c)(i) is typical of many candidates. The candidate has referenced formation of enzyme substrate complexes but has not related this to increased kinetic energy. The candidate has also referenced the denaturing of enzymes at the higher temperature but has not clearly related the effect that this has had.
		ii	*At lower temperature / 25°C* less kinetic energy therefore less, successful collisions / ESC formed ✓ rate (of reaction) slower / taking more time for product to be formed ✓ not all substrate reacted (after 60 min) ✓	max 2	**ORA** for 37°C **ALLOW** reaction not complete (in 60 min) **ALLOW** substrate (concentration) does not become limiting (in 60 min) **IGNORE** Ref to amount of product formed **Examiner's Comments** As in part (c) (i) candidates concentrated almost exclusively on the 37°C line. Here, the most common reason given for the plateau was that the enzymes had become denatured - despite the fact that this had been described as the optimum temperature in the previous response. Often the 25°C curve was almost completely ignored. It seems that most candidates have not had the opportunity to carry out a range of practicals to investigate other factors that affect enzyme activity. **Exemplar 6** Rate of reaction at 25°C was much lower than that at 37°C, having not completed the reaction within the hour is evidence of this. Reasons can include lower kinetic energy of substrate and enzyme at 25°C meant there were fewer successful collisions per second, thus a slow rate of reaction. [2] This response to part(c)(ii) gained full credit for a clear and concise explanation of the

					difference in the shapes of the curves at 25°C and 37°C. The candidate has clearly stated that 25°C has the lower rate of reaction explaining this by relating it to collision theory. The candidate has also stated that the reaction had not been completed within the one-hour time frame. This candidate has clearly understood the graph well.
			Total	**4**	
28	a	i	1 / time **or 1 ÷** time ✓	**1**	**ACCEPT** 1 / seconds **or 1 ÷** seconds **Examiner's Comments** This question was poorly answered. Very few candidates realised that they could work out the answer by looking at the units in the column heading. Common incorrect answers included enzyme concentration divided by time, or time divided by enzyme concentration.
		ii	**1** (SD) shows spread (of data) around the <u>mean</u> ✓ **2** <u>all</u>, data / concentrations, have small SD ✓ **3** (so) little variation in repeats / high repeatability ✓ **4** as concentration increases the SD increases (in first 4 concentrations) ✓ **5** (so) as concentration increases repeatability decreases ✓	**2 max**	**IGNORE** reliability / accuracy **IGNORE** ref to 'results' **4** **ACCEPT** 0.01% deviated the least **and** 0.075% deviated the most **5** **ACCEPT** greater variability of repeats at higher concentrations **Examiner's Comments** Few candidates managed to gain both marks here. Many candidates recognised that standard deviation shows the spread of the data but failed to mention 'around the mean'

					(some used the term 'average'). Some described the changes in SD in terms of the shape of the graph but not in terms of the repeatability of the results obtained, with a common incorrect response being 'the smaller the SD the more reliable or accurate the results'.
	b		**1** as enzyme concentration increases the rate (of digestion) increases because, more ESCs formed / more active sites available ✓ **2** as the enzyme concentration increases the, concentration / availability, of substrate remains the same ✓ **3** rate, plateaus / levels off, because, many active sites are empty / lack of substrate ✓ **4** substrate concentration is limiting ✓ **5** at high(er) concentrations the, error bars overlap / SD increases, so any difference in the data may be uncertain ✓	**3 max**	**IGNORE** reliability / accuracy **ACCEPT** 'bromelain' or 'protease' for 'enzyme' throughout **1 IGNORE** ref to successful collisions **3 DO NOT ACCEPT** ref V_{max} reached **5 ACCEPT** 'SD bars' for 'error bars' **DO NOT ACCEPT** 'range bars' *Uncertainty may be expressed as:* Greater (potential) error in measuring shorter times The rate of digestion may not plateau at high(er) concentrations There may be no difference between the rate at high(er) concentrations We can't tell if there is any difference in the rates at high(er) concentrations **Examiner's Comments** Few candidates appreciated the difference between 'describe' and 'explain'. The majority of students simply described the

					shape of the graph without explaining the reason for this shape. The increase in rate with enzyme concentration was often explained in terms of more collisions, but not in terms of more enzyme-substrate complexes formed, or more available active sites. Many candidates recognised that the rate plateaus but did not fully understand that it was the substrate concentration that had become limiting and so wrongly suggested that all the active sites were full. Many candidates recognised that the standard deviation increased as the concentration of enzyme or rate of reaction increased, and some concluded that this meant the data was 'less reliable'. Very few correctly used the term 'uncertainty' or explained what this meant for this experiment. The overlap of SD bars was rarely commented on, and candidates found it difficult to link the larger SD or overlapping SD bars with the uncertainty of the data. It appears that whilst candidates are becoming more familiar with the concept of standard deviation and SD bars, they are not yet confident in applying these concepts biologically.
			Total	**6**	
29	a	i	*one from* pH / it, is, the dependent variable / being measured ✓ (pH changes as) fatty acids are produced ✓	1	**ACCEPT** pH (change) indicates the rate of the reaction if pH were controlled there would be no, colour change / end point indicated because the pH (change) shows that the, reaction is happening / lipid is being broken down **IGNORE** we are investigating pH / pH is being investigated **Examiner's Comments** A significant proportion of candidates gave 'stock' answers and did not interpret the information given to realise that pH change is a component of the dependent variable due to the production of fatty acids when

					lipase digests lipid and therefore indicates when the reaction has taken place.
		ii	volume of, alkaline / (alkaline) lipid / substrate, solution **or** concentration of, lipase / enzyme, solution **or** volume of, lipase / enzyme, solution **or** temperature **or** time / intervals, between testing of samples ✓	1	**Mark 1st answer** **IGNORE** amount **IGNORE** 5 cm^3 - this is how the variable was controlled 'volume of 5 cm^3 of alkaline solution' = 1 mark '5 cm^3 of alkaline solution' = 0 marks **IGNORE** 0.5 % - this is how the variable was controlled 'concentration of 0.5% enzyme solution' = 1 mark '0.5% enzyme solution' = 0 marks **IGNORE** 1 cm^3 - this is how the variable was controlled 'volume of 1 cm^3 of lipase solution' = 1 mark '1 cm^3 of lipase solution' = 0 marks **IGNORE** 20°C - this is how the variable was controlled 'a temperature of 20°C' = 1 mark 'keep it at 20°C' = 0 marks **IGNORE** 30 seconds - this is how the variable was controlled 'the times the samples were taken were at intervals of 30 seconds' = 1 mark 'samples taken every 30 seconds' = 0 marks **Examiner's Comments** There were five controlled variables for candidates to select from,but answers commonly lacked an important detail, such as the word 'solution' or a clear description of how the variable was quantified such as volume. Students should be encouraged to replace the imprecise term 'amount' with a more precise descriptor of measurement when talking or writing about experimental variables.
		iii	concentration of, alkaline /	1	**Mark 1st answer** **IGNORE** amount **IGNORE** size / volume, of drops **Examiner's Comments** A surprisingly large number of answers

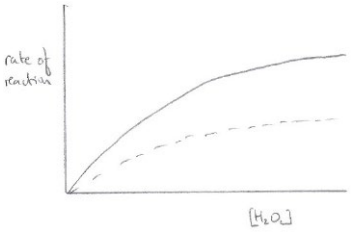
			(alkaline) lipid / substrate, solution **or** volume of indicator (added) **or** number of drops of indicator (added) **or** volume of, sample / mixture / solution (removed) **or** number of drops of, sample / mixture / solution (removed) ✓		stated that temperature was uncontrolled, although the question states that the first run of repeats occurred at 20°C and subsequently at six other temperatures (all of which are listed in the independent variable column in Table 4.1). Correct answers focused on the volume or number of drops of indicator added or sample of reaction mixture solution removed.
		iv	*one from* (looking at, a small volume / against a white background) makes it easier to see the colour change ✓ the indicator (if added to test tube) might affect the progress of the enzyme reaction ✓ better temperature control as test tube not taken in and out of water bath ✓ AVP ✓	1	**ACCEPT** provides a contrasting background to see the colour **ACCEPT ora** e.g. harder to see colour change in the test tube **Examiner's Comments** Many candidates realised the value of a white tile in perceiving a colour change more easily as it provided a contrast.
		v	(the optimum temperature) is between 30°C and 35°C ✓	1	**Must give a range** °C must be stated once **IGNORE** 35°C alone / 'around 35°C' **Examiner's Comments** Most candidates picked a single temperature (35°C) and did not realise that with intervals of 5°C between tests there is a possibility that the true optimum lies to one side of this figure. The correct range was 30°C-35°C based on comparing the data for 30°C and 40°C. Marks were not given on this and the next question if units were omitted.
		vi		4	**Mark the first 2 suggestions seen.** **B mark must relate to the appropriate A mark point** 1A e.g. test, every 2°C / at 1°C intervals

			1A use more intermediate temperature values ✓ 1B in the 30°C - 35°C range ✓ ..... 2A take samples at more frequent intervals (than 30 seconds) ✓ 2B e.g. every 15 seconds ✓ ..... 3A use of colorimeter ✓ 3B colour change would be less, subjective / biased ✓ ..... 4A use of pH, meter / probe / sensor ✓ 4B obtain a numerical value ✓		use temperatures less than 5°C apart 1B **CREDIT** a range of 25°C - 40°C Units must be given once **Note:** 'test a range of temperatures between 30°C and 35°C' 'carry out more experiments between 30°C and 35°C' = 2 marks (mps 1 & 2) 2A **ACCEPT** sample more regularly 2B time interval must be experimentally workable, so should be from 10 and less than 30 seconds. **Note:** 'take samples every 15 seconds' = 2 marks (mps 3&4) 'take samples every 5 seconds' = 1 mark (mp 3 only) 3B obtain a numerical value **Examiner's Comments** Very few candidates scored full or many marks on this task. Candidates needed to focus on the word 'accurate' and consider ways of measurement that would allow the true optimum temperature to be pin-pointed more truly. Refining the temperature range to include smaller temperature intervals in the suspected optimum range, or sampling more often to identify the end point time more closely were the most frequent good suggestions. A few candidates mentioned the use of more sophisticated equipment such as a colorimeter to detect the end point time, or a pH probe to measure the dependent variable without the need for a subjective colour judgement.
	b		**Level 3 (5–6 marks)**	6	***In summary:****Read through the whole answer. (****Be prepared to recognise and***

			• Provides a description of the 2 mechanisms of enzyme action • Provides a description of the ways in which high and low temperature affects the reactants and active site. *There is a well-developed line of reasoning which is clear and logically structured and uses scientific terminology at an appropriate level. All the information presented is relevant and forms a continuous narrative.* Awarding at this Level = L3 & 5 ticks ✓ ✓ ✓ ✓ ✓ Communication = ✓ or ✗ **Level 2 (3–4 marks)** • Describes 1 or both of the mechanisms of enzyme action • Describes some ways in which temperature affects the reactants and / or active site. *There is a line of reasoning presented with some structure and use of appropriate scientific language. The information presented is mostly relevant.* Awarding at this Level = L2 & 3 ticks ✓ ✓ ✓ Communication = ✓ or ✗ ..... **Level 1 (1–2 marks)** • **either** Describes some aspects of the mechanism of		***credit unexpected approaches where they show relevance.**) Using a 'best-fit' approach based on the science content of the answer, first decide which of the level descriptors, **Level 1**, **Level 2** or **Level 3**, **best describes the overall quality of the answer**.* *Then, award the higher or lower mark within the level, according to the **Communication Statement** (shown in italics):* • *award the higher mark where the Communication Statement has been met.* • *award the lower mark where aspects of the Communication Statement have been missed.* • ***The science content determines the level.*** • ***The Communication Statement determines the mark within a level.*** **Use the green dot ● in the margin to indicate places where good scientific points are made about the 2 models of enzyme action.** **Use a highlight square ▪ in the margin to indicate places where good scientific points are made about the effect of temperature.** **Indicative scientific points may include but are not limited to:** *enzyme action* ● **1** enzyme-substrate complex formed **2** enzyme-product complex formed **3** product(s) leave the active site **4** lock and key = shape of substrate and enzyme's active site are complementary and so enzyme is specific **5** induced fit = enzyme active site changes shape to accommodate substrate once substrate binds

			enzyme action **or** Describes an effect of temperature *The information is communicated with some structure but may include a small amount of irrelevant material and some inappropriate use of scientific language.* Awarding at this Level = L1 & 1 tick ✓ Communication = ✓ or ✗ ..... **0 marks** No response or no response worthy of credit.		*effect of temperature* *reactants* **6** increase in temperature increases kinetic energy of molecules **7** results in more successful collisions **8** more enzyme-substrate complexes form **9** decrease in temperature reduces kinetic energy of molecules **10** results in fewer successful collisions**11** fewer enzyme-substrate complexes form *active site* **12** enzymes have an optimum temperature **13** (small) increase in temperature affects the bonds involved in tertiary structure **14** change in shape of active site **15** prevents substrate binding to active site **16** high temperature results in denaturing **17** effects of high temperature are irreversible **18** effects of low temperature are reversible **Examiner's Comments** Most candidates achieved a level two response. Descriptions of what is meant by the lock and key model and the induced fit model were mostly good, as was description of the events that lead to denaturing of enzyme structure at high temperatures. Level three responses also described reaction kinetics at low temperatures. Errors included the belief that enzymes denature at low temperatures. The question referred to temperature change and this was frequently repeated in the answer without stating whether the information was linked to a raising or lowering of temperature. Given the difference in the effect of low and high temperatures on enzyme structure and action, this needed to be clear.
			Total	**15**	
30	a	i	*three from* specify volume of starch and amylase to be added to the tubes (1) specify volume (in ml) of the solution that should be removed for	3	

			testing (1) stir before taking the sample (1) test with iodine (1) all carried out at same temperature (1)		
		ii	*four from* ionic / hydrogen, bonds, disrupted / broken (1) (by) high concentration of, hydrogen ions / H^+ (1) tertiary structure / shape of active site, changed (1) substrate no longer fits into active site (1) (enzyme) denatured (1)	4	 **IGNORE** active site denatured.
		iii	*Evaluation, two from* idea that optimum could be anywhere between pH 6 and pH 8 (1) only one value between pH 6 and pH 8 tested (1) idea that shape of data implies optimum less than pH 7 (1) *Improvement* repeat at more pH values between 6 and 8 (1)	3	
	b		cofactor	1	**IGNORE** coenzyme.
			Total	**11**	
31			D	1	**Examiner's Comments** This question was also straightforward as the material is a clear learning outcome. While many had the ions the wrong way round, the correct choice for the charge of the ions defeated a significant number of candidates.
			Total	**1**	
32		i	cofactor / prosthetic group (1)	1	
		ii	haemoglobin / myoglobin / cytochrome (1)	1	**ACCEPT** other correct named protein
			Total	**2**	

33			*any three from:* non-competitive ✓ PBO / inhibitor, binds to allosteric site ✓ substrate / permethrin, cannot bind / cannot fit into / is not complementary ✓ to, altered / changed, active site ✓	3 max (AO1.1)	**ALLOW** description of allosteric site
			Total	**3**	
34			 ✓	1 (AO2.2)	**ALLOW** any curve that starts at origin and stays below the curve given in Fig. 18.2. **DO NOT ALLOW** negative gradients
			Total	**1**	
35			C	1 (AO2.1)	
			Total	**1**	
36		i	axis labelled 'concentration of malate (mmol dm^{-3})' **AND** *y* axis labelled 'rate of reaction of malate dehydrogenase (mmol dm^{-3} s^{-1})' (1) plotted points use ≥50% of area provided **AND** equidistant scales on *x* and *y* axes (1) points plotted correctly ± 1 mm (1) **smooth** line of best fit (1)	4	**ALLOW** landscape **OR** portrait graph **DO NOT ALLOW** any other units, e.g. mM dm^{-3} / mM/dm^3 / mmol/dm^3 (since units are provided on table) **ALLOW** 'conc.' **DO NOT ALLOW** inversion of axes **ALLOW** solidus instead of brackets **NOTE** *x* axis data are non-linear **DO NOT ALLOW** points joined by straight lines (since candidates should recognise shape of curve)
		ii	6.1 (1)(1) mmol dm^{-3} s^{-1} (1)	3	1 mark for evidence of: (92.3 − 37.7) ÷ 9 2 max if answer is not to 2 SF **ALLOW** mmol dm^{-3}/s
		iii	not an enzyme inhibitor / does not inhibit malate dehydrogenase (1) *idea that* similar curve would be expected in absence of inhibitor /	3	

			in normal conditions (1) allows enzyme / malate dehydrogenase to work at optimal rate / V_{max} (1) *idea that* may inhibit a different enzyme (1)		
			Total	**10**	
37			non-competitive (inhibition) (1) the rate of reaction does not continue to rise as substrate concentration rises / in competitive inhibition the rate of reaction would continue to rise as substrate concentration rises (1)	2	
			Total	**2**	
38			D ✓	1 (AO2.1)	
			Total	**0**	
39		i	**1** inhibitor binds to, allosteric site / enzyme away from active site ✓ changes, tertiary / 3D, structure of, enzyme / active site / protein **OR** **2** <u>active site</u> no longer <u>complementary</u> to substrate **OR** substrate and, enzyme / active site, cannot, bind / fit (together) **OR** E-S compex cannot form ✓	2	**ALLOW** catalase for 'enzyme' throughout **ALLOW** hydrogen peroxide / H_20_2, for 'substrate' throughout **ALLOW** joins / fits into, for 'binds' **ALLOW** shown on diagram **ALLOW** conformation / shape for 'structure' **IGNORE** denatures **<u>Examiner's Comments</u>** This question was well answered with most candidates naming or describing an allosteric site, and giving an appropriate level of detail about the effect of inhibitor binding on the enzyme's tertiary structure or on enzyme-substrate bonding.
		ii	**1** downward-sweeping curve showing negative correlation drawn ✓ **2** *x* axis label = conc(entration) of copper sulfate in moles dm^{-3} **AND**	2	**DO NOT ALLOW** straight line or plotted points that are not joined. Curve may level off at end. Allow 'dot-to-dot' curve. **ALLOW** $CuSO_4$ / copper sulphate, for 'copper sulfate' **ALLOW** slash before unit / slash or 'per' in the unit / brackets round unit **ALLOW** variant symbols: M **OR** moles L^{-1}

			y axis label = vol(ume) of oxygen (gas produced) in cm^3 ✓		**OR** moles / L **OR** mol dm^{-3} **ALLOW** O_2 for 'oxygen' **Examiner's Comments** Most candidates gained one or two marks. See the AfL box for advice on training candidates in this skill. **AfL** 1. Identify the independent variable in the table and label the x axis with the full column heading description plus the full units. 2. Identify the dependent variable in the table and label the y axis with the full column heading description plus the full units. 3. Plot the points roughly by eye. 4. Join them with a clear, single line of best fit, in this case a curve.
		iii	*(trend described)* **1** as (concentration of) copper, sulphate / ions, **increases**, (volume of) oxygen / H_2O_2 breakdown, **decreases** ✓ *(conclusion / inference, about activity of enzyme)*	2 max	**ALLOW** AW for 'decrease' e.g.reduce / decline / drop / fall **ALLOW** AW for 'increase' e.g. go up / rise / climb **ALLOW** AW so long as inverse trend is still made clear by use of comparative terms such as: increases / decreases, higher / lower, more / less E.g. *'when there is more $CuSO_4$, less oxygen is produced'* **ALLOW** ORA, e.g. *'the lower the concentration of Cu^{2+} the higher the volume of oxygen produced'*

			2 copper, sulphate / ions, inhibit(s) / decrease(s), <u>catalase</u> activity ✓ *(detail)* **3** at high concentrations / 0.15 / 0.20 **EITHER** most enzymes, (irreversibly / already) damaged / inhibited **OR** adding more copper (sulphate / ions) has little effect ✓		**IGNORE** *'disturbs the action of catalase'* **<u>Examiner's Comments</u>** Most candidates showed they are able to describe a relationship between two variables using data from a table or graph. Higher ability candidates went on from this to explain the relationship in terms of copper ions inhibiting the activity of catalase.
		iv	**1** compare / measure / test, catalase activity / oxygen produced ✓ **2** experimental detail ✓ **3** further experimental detail ✓ **4** less, oxygen / catalase (activity), means more, copper / pollution ✓ **5** use, Table 4 / graph, to estimate copper (ion) concentration ✓	3 max	**IGNORE** how much oxygen is in each fish **IGNORE** how much catalase is in each fish *experimental detail points:* **ALLOW** AW throughout **IGNORE** amount throughout **i** prepare a , catalase / fish / tissue, extract / sample (e.g. ref. pestle and mortar / chopping / liquidiser) **ii** equal / known / controlled, volume / sized samples (of fish / tissue / extract) **iii** equal / known / controlled, concentration / volume, of hydrogen peroxide **iv** measure, volume of, oxygen / gas, in a given time **v** use gas syringe / collect gas under water **ALLOW** correct statement of relationship between copper or pollution **and** oxygen **or** amount of catalase present **or** catalase activity **even if wrong experiment is done** (e.g. adding catalase or copper sulphate to fish) or measuring 'how much oxygen is in fish' **<u>Examiner's Comments</u>** While a generous mark scheme enabled candidates to score one or two marks, very few candidates were able to fully integrate the information gained from (a) parts (i) to

					(iii) to understand how to tackle this question. The correct line of thinking underpinning an experiment to use the three fish to compare the copper ion pollution in the rivers they came from is given in the AfL box. Errors in thinking included adding catalase to the fish (instead of realising the fish are a source of catalase and adding hydrogen peroxide) or thinking that the relative oxygen content of each fish could be measured. The main marking point that was credited was re-stating the relationship that more copper ions means less oxygen produced or catalase activity. Higher scoring answers mentioned a way of controlling a variable in their experimental design, such as cutting equal-sized samples of each fish, or referring to the table or graph to read off a concentration of copper ions. The higher order skills being tested here were firstly, to interpret and evaluate the information given, and secondly, to creatively develop a practical procedure based on these facts.  **AfL** Steps in thinking required to design an experiment to use the three fish to compare the copper ion pollution in the rivers they came from: 1. Copper ions bind irreversibly to catalase enzyme (information from the first line of (b)). 2. Catalase is found in all living things (information from (b) (ii)), including fish. 3. A fish from a more polluted river will have less working catalase. 4. Catalase activity can be measured by adding hydrogen peroxide and measuring oxygen production (information from (b) (ii)). 5. Refinement of procedure with consideration to controlling variables and interpreting results.

					Candidates could be asked to consider each step 1-5 and whether the skill needed in each case is interpretation or evaluation of information given, or creative design with the ability to visualise an experimental procedure.
			Total	**9**	