3. Enzymes

3.2 Factors that Affect Enzyme Action

Paper 2

Marking Scheme

Q1.

(e)	*any **five** from:* caspofungin / drug, binds to site on, enzyme / glucan synthase, other than active site ; **A** binds to allosteric site changes shape of active site ; **A** enzyme changes, shape / tertiary structure, so active site is changed substrates cannot bind to active site / active site no longer complementary to substrates / enzyme-substrate complexes do not form ; **A** fewer enzyme-substrate complexes form less / no, (1,3- β-D) glucans / products, synthesised / produced ; cell wall, weakened / synthesis hindered / AW ; **A** cell wall not formed (leads to osmotic) lysis of, *P. jirovecii* / cell / fungus ; **A** bursting / cytolysis reduces / prevents, population growth ; AW e.g. decrease number of *P. jirovecii* **A** decreases ability of *P. jirovecii* to colonise / AW AVP ; e.g. *in context of lysis ref.* water entry into cell, by osmosis / down water potential gradient / AW (reduced number) increases chance of immune system eliminating the fungus	**5**

Q2.

(a)(i)	*correct answer as a whole number* 34(%) ;	**1**
(a)(ii)	*allow enzyme for trypsin* *allow substrate for casein* *any **five** from:* **1** at lower concentrations there are not enough, active sites / enzymes ; **2** idea of substrate molecules cannot enter active site until product released ; **3** as concentration increases, increased number of active sites ; **4** idea that as concentration increases there are more successful collisions between enzyme and, substrate / casein ; **5** (so) allows increase in (number of) enzyme-substrate complexes (formed per unit time) ; **6** increased rate of casein breakdown as enzyme concentration increases ; **7** *ref. to* casein reaction e.g. milk becomes transparent as solution contains amino acids ; **8** AVP ; e.g. depends on how many active sites available at any one time *ref. to* enzyme-substrate complex formation per unit time at high concentration substrate concentration is becoming the limiting factor when clear, total hydrolysis of casein has occurred	**5**

(b)(i)	*any **one** from:* immobilising the enzyme may have altered the, tertiary structure of the enzyme / shape of the active site ; *suggestion that* material may not be inert and may have an inhibitory effect at lower temperatures ; immobilising may have covered, part of the active site / active site of some enzymes ; *idea that* trypsin free in solution has increased chance of collision with substrate ; immobilising changes the optimum temperature of the enzyme ;	**1**
(b)(ii)	*any **two** from:* immobilisation, protects / stabilises / AW, the, enzyme / trypsin ; from (thermal) denaturation ; **ora** enzyme free in solution denatured maintains shape of active site ; immobilisation may result in lower, kinetic energy / vibration, of the enzyme molecules compared to free (so protects from denaturation) ; **ora** prevents the breaking of bonds holding the tertiary structure (of immobilised enzyme molecule) ; **ora** *ref. to*, hydrogen / ionic bonds ;	**2**

Q3.

(d) **4**

keratinase	temperature range at 60% relative activity / °C	pH range at 60% relative activity
K12	41 – 63	6.8 / 6.9 – 8.4 ;
A22	36 / 36.5 – 57 ; **A** 57.1 / 57.2	6.0 – 9.0
P3	29 – 56	5.3 – 7.5

P3 ;

A22 ;

(e) **3**

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** *if 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 ;

Q4.

(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**

(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**

Q5.

(f)	*any **three** from:* **1** acetazolamide / inhibitor, binds / attaches / AW, to, allosteric site / site other than active site ; **R** alternative active site **2** (which) changes, shape / tertiary structure / 3-D structure, of active site ; **A** active site distorted **I** protein structure *allow ecf for mp3 and 4 if competitive described (to max 2)* **3** substrate(s) / CO_2 / HCO_3^-, cannot, enter / bind to / fit in / AW, active site ; **A** active site no longer complementary to, substrate / AW **A** enzyme substrate / ES, complexes cannot form **A** ESCs cannot form **I** 'substrate / CO_2 / HCO_3^- / 'cannot bind to enzyme' *without a link to active site* **4** product, not formed / released ; **A** named product **5** *ref. to* acetazolamide / inhibitor, leaves, allosteric site / enzyme ; **I** reversibly binds	**3**

Q6.

(b)	*any **three** from:* **1** at low(er) concentrations / AW, of, substrate / ATP, lower rate of activity with GNF-5 ; **2** as substrate concentration increases, effect of GNF-5 decreases ; **3** maximum rate of reaction / V_{max} / described, is the same ; **4** Michaelis-Menten constant / K_m, is higher with inhibitor ; ora *accept comparative data quotes to support answers* *for ATP concentration, must use μmol dm^{-3} at least once*	**3**
(c)(i)	*without inhibitor, activity of T315L* *any **one** from:* **1** no activity at 0.1 μmol dm^{-3} (ATP) ; **2** maximum rate of reaction / V_{max}, is reached / AW, (for T315L) at a higher concentration (of ATP) ; **3** Michaelis-Menten constant / K_m, is higher (for T315L) ; ora *accept comparative data quotes to support answers* *for ATP concentration, must use μmol dm^{-3} at least once*	**1**
(c)(ii)	*with inhibitor, activity of T315L* *any **two** from:* **1** activity does not reach TKR, maximum rate of reaction / V_{max} ; **2** *idea that* drug / GNF-5, acts as a non-competitive inhibitor of T315L ; **3** drug / GNF-5, has less of an effect at low concentrations on activity (of enzyme / T315L) ; ora *accept comparative data quotes to support answers*	**2**

Q7.

(c)(i)	*any **two** from:* collagen, not broken down / not hydrolysed / breakdown prevented ; **A** ECM *for collagen* **A** less hydrolysis / AW detail ; e.g. suggestion that, collagen does not fit into active site / active site changes shape no / few, ESC / enzyme substrate complexes, form collagen, continues to be, synthesised / released ; **A** increase in collagen / maintenance of collagen	**2**
(c)(ii)	(V_{max}) decreases ; (K_m) stays the same ;	**2**

Q8.

(c)(iii)	*allow two suggestions or a suggestion with further detail, for two marks* active site becomes complementary to substrate ; (because) bonds altered between R groups of amino acids ; **A** hydrogen / ionic, bonds removal of a portion that blocks the active site ; AW substrate can enter active site ; *idea of* cofactor / AW, associates with the enzyme ; enzymes becomes functional ; sets off an enzyme cascade / ref. cell signalling ; AVP ;	**2**

Q9.

(c)	*accept substrate for cellulose and enzyme for cellulase* *allow use of extracted data to support a response to confirm awarding a mark* 1 correct description ; *overall trend or more detailed break down* e.g. as, cellulose / substrate, concentration increases, rate of reaction increases **or** as, cellulose / substrate, concentration increases, steep increase in rate of reaction then, less steep increase / (begins to) plateau **I** 'rate of reaction is fast and then slows down' *needs ref. to rate of reaction, describing 'gradient of curve' is not enough* *plus any two from* 2 increasing cellulose concentration increases number of, collisions between enzyme and substrate / enzyme-substrate complexes / ESCs ; **A** collisions between active site and substrate **A** *idea of* increased chances of cellulose binding to active site 3 at low cellulose concentrations, (many) active sites, available / not all used / not saturated ; 4 at higher cellulose concentrations, active sites becoming saturated / most active sites occupied / most active sites not available / AW ; **A** actives sites are, saturated / occupied 5 named limiting factor(s), qualified ; e.g. at low(er) cellulose concentrations, cellulose concentration limiting / enzyme concentration not limiting **A** idea of, not enough cellulose / enzyme in excess as cellulose concentration increases, change from cellulose concentration limiting to enzyme concentration as limiting factor at higher cellulose concentrations, enzyme concentration limiting / cellulose concentration not limiting **A** idea of, cellulose in excess / not enough enzyme / enzyme working at its fastest rate / active sites not emptying quickly enough for substrate available	**3**

(d)(i)	*mark to max 2 if microorganisms are considered to be enzyme inhibitors* *lower K_m for pectinase, any two from* *ultrasound* increases affinity of enzyme for substrate ; makes shape of active site more complementary ; makes (position of) active site more accessible (to substrate) ; **A** makes it easier for substrate to enter (active site) breaks up pectin, aggregates / AW, to expose more substrate for binding ; *higher Vmax for xylanase, any two from* *ultrasound* increases rate of, collision between enzyme and substrate / more enzyme-substrate complexes per unit time ; **I** more ESC form *needs idea of increased rate* increases rate of catalysis after binding ; may change substrate for easier hydrolysis ; may lower, more than normal, activation energy required for reaction ; **A** described, e.g. increased ability to, break / form, bonds	**3**
(d)(ii)	yes (as all have) increased, catalytic efficiency / productivity / V_{max}/K_m ; **A** manipulated data for increased catalytic efficiency *any <u>two</u> from* pectin 24.5 / 25% cellulase 17.25 / 17% xylanase 16.8 / 17%	**1**

Q10.

(b)(i)	***A** peroxidase for enzyme* *any three from* *rate of reaction increases as substrate concentration increases to 0.33 – 0.35 mmol dm^{-3}* **1** (up to 0.33 to 0.35 mmol dm^{-3}) some active sites are not occupied ; **2** more collisions between, enzyme / active site, and substrate molecules ; **A** more collisions lead to increase in formation of enzyme-substrate complexes *rate of reaction remains constant above 0.33–0.35 mmol dm^{-3}* **3** all active sites, occupied / saturated ; **4** correct reference to limiting factor for slope ; **A** limits / limiting e.g. at low substrate concentrations, substrate concentration limiting / enzyme concentration not limiting **5** correct reference to limiting factor for plateau ; **A** limits / limiting e.g. at high substrate concentrations, enzyme concentration limiting / substrate concentration not limiting	**3**
(b)(ii)	*idea that* determined V_{max} / maximum rate / *ref. to* 5.6 (µmol min^{-1}) ; **A** if V_{max} shown on graph K_m is the substrate concentration at half the V_{max} ;	**2**

Q11.

(b)	*any **two** from:* (highly) alkaline / acidic (conditions) ; **A** (very) low / high, pH disruption of, hydrogen / ionic, bonds, of (polyhedron) proteins / polyhedrins ; (presence of) digestive enzymes / hydrolytic enzymes / hydrolases ; **R** if stated as within lysosomes *but allow detail of enzyme action ecf* detail ; e.g. extracellular enzymes / proteases / peptidases peptide bonds broken **A** amide bond for peptide bond optimum pH ; *must be in context of enzyme presence*	**2**

Q12.

(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**

(b)	*any **one** from:* surface area to volume ratio / SA:V, decreases with increasing size / increases with decreasing size ; SA:V ratio is different, with consequence ; e.g. smaller beads means increased contact of substrate with enzyme (in same conditions) *idea that overall,* larger surface area exposed using smaller beads ; **ora** *(for the same experimental set up)* larger beads, slows rate of diffusion into beads / increases diffusion time to reach enzyme ; **ora** larger beads will increase rate of flow through column ; **ora**	**1**

Q13.

(a)(i)	*one mark for units* *one mark per column* V_{max} µmol min^{-1} ; K_m mmol dm^{-3} ; **J** 60 0.2 **K** 50 ; 0.2 ;	**3**
(a)(ii)	*K compared with J* *max 3 from mp4–mp8* *any four from:* 1. rate of reaction is lower at all substrate concentrations ; 2. *ref. to* greater difference as substrate concentration increases ; 3. rate of reactions for **J** and **K** compared at two substrate concentrations to support mp1 or mp2 *or* manipulated data at two substrate concentrations to support mp2 ; 4. less efficient / acts more slowly / AW ; 5. active site is still binding substrate ; 6. *idea that* active site is less effective at catalysing reaction 7. *or* enzyme has lower affinity for substrate ; 8. (replaced / changed) amino acid is important in, shape / structure, of active site ; 9. AVP ; e.g. how the mutation may have changed interaction between substrate and, active / catalytic, site e.g. *ref. to* hydrogen bonding / transfer of electrons	**4**

Q14.

(b)(ii)	4 (hours) ;	**1**
(b)(iii)	*any **four** from:* **1** triglyceride hydrolysis / action of lipase, produces fatty acids ; **2** decrease in pH / described, because of production of (fatty) acids ; **3** steepest rate of (pH) decrease at start because, substrate / triglyceride, concentration highest ; **4** rate of pH decrease / AW, slows because decrease in rate of formation of fatty acids ; **5** rate of pH decrease /AW, slows because enzymes, not at optimum / begin to denature ; **6** rate of pH decrease slows because, substrate / triglyceride, being used up **or** substrate concentration becomes a limiting factor / AW ; **7** *ref. to* plateau at 18 hours / at pH 6.1, enzymes are denatured ; **8** *ref. to* plateau at 18 hours / at pH 6.1, as all the, triglyceride / substrate, has been, hydrolysed / AW ; **9** optimum pH for lipase activity, is pH 8 / alkaline conditions / at the start ; **10** AVP ; e.g. *ref. to* tertiary structure of lipase	**4**

(b)(iv)	*any **two** from:* (initial) decrease in pH would be more rapid ; plateau would be reached earlier ; AW plateau would be at the same pH (as the previous experiment) ;	**2**

Q15.

(b)(ii)	*any **three** from:* **I** stops transcription **1** alters shape of / blocks, active site ; **R** enters / fits into, active site *as it is <u>not</u> competitive* **R** *ref. to* choice of competitive or non-competitive **A** alters shape of enzyme *only if mp2 gained* **2** substrate / nucleotides, cannot, bind to / enter, active site ; **A** fewer / no, enzyme-substrate complexes / ESCs, form *allow ecf from mp1 if rifampicin described as, competitive / enters active site* **3** complementary (base) pairs / (complementary) base pairs, cannot form / form less easily (between DNA and RNA nucleotides) ; **4, 5, 6** *(in context of rifampicin binding to RNA polymerase)* prevents / AW, unwinding of DNA (double helix) ; **A** uncoiling **I** unzipping prevents / AW, attachment to DNA (strand) **A** prevents / AW, attachment to promoter prevents / AW, movement along DNA (strand) ; prevents / AW, elongation (of polynucleotide) / formation of polynucleotide /nucleotides being joined ; prevents / AW, phosphodiester bond formation ; prevents / AW, proof reading ; **7** AVP ; e.g. prevents / AW, tRNA / rRNA, formation	**3**

Q16.

(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*	

(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 ;	

Q17.

(b)(i)	*three from* changes the tertiary structure / shape of active site, of , enzyme / RNA polymerase ; active site no longer complementary to, RNA nucleotides / substrate ; AW RNA nucleotides not joined / mRNA not synthesised ; **A** transcription does not occur translation prevented ; **A** described, **I** polypeptide / protein synthesis prevented AVP ; *ref. to* other roles of RNA polymerase not occurring, e.g. does not bind to DNA, double helix does not unwind	**3**

Q18.

(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**

Q19.

(a)(ii)	*accept **ora** where appropriate* **1** copper sulfate, decreases / AW, the activity of Neutrase (*ref. to* **A** or **B**) ; **2** 0.01 (mol dm^{-3}) / low concentration, $CuSO_4$ has less of an effect than, 0.05 (mol dm^{-3}) / high concentration (*ref. to* **A** and **B**) ; **3** potassium sulfate has, little / no, effect on activity (*ref. to* **C**) ; **4** data quote to show absorbance for two different lines at the same time ; *one time and two absorbance readings from different lines on the graph with the unit for time used once anywhere in answer, allow 'at the end' for 100 s* **5** copper sulfate is an inhibitor of Neutrase ; **6** potassium sulfate is not an inhibitor ; **A** 'less of…' **7** copper sulfate binds to Neutrase ; **A** anywhere **8** substrate cannot enter active site / ESCs do not form (so protein not hydrolysed) ; **A** fewer ESCs	

(b)	*higher productivity / higher yield and fewer costs because* enzyme can be re-used ; enzyme can be easily recovered ; downstream processing is easier ; product, not / less, contaminated ; **A** less purification needed longer shelf-life of enzyme ; reduces product inhibition ; enzyme is, more stable / less likely to denature *or* described ; **A** thermostable / can work at high temperatures **A** in context of change in pH **I** 'can withstand changes in temperature'	**2**

Q20.

(a)(ii)	*any two from:* greater surface area (over which catalase released) ; greater number of cells, damaged / cut open, to release catalase ; higher concentration of, catalase / enzyme ; **A** more, catalase / enzyme **A** more active sites available *must be linked to idea of more enzyme (i.e. must get mp 2 or 3)* so, higher rate of reaction / more enzyme-substrate complexes / more oxygen released ; AW	**2**
(b)	increase to optimum and steeper decrease ; **A** if curve does not touch one or both axes	**1**

Q21.

(a)	*three from:* same / similar, shape as, HMG CoA / substrate ; complementary (shape) to active site (shape) ; binds / attaches / fits into / active site (of, enzyme / HMG CoA reductase) ; HMG CoA / substrate, cannot bind (to active site) ; **A** no / few, enzyme/substrate complexes form mevalonic acid production, decreases / stops ; **I** product not made AVP ; e.g. increase in mevinolin concentration increases inhibition / ora	**3**

Q22.

(a)	*four from:* activity increases and decreases / activity peaks, for both ; **A** both have maximum activity / optimum temperature, at 60 °C activity is 76% at 40 °C for both ; (overall) activity decreases more steeply for free enzyme after, optimum / maximum / 60 °C ; **A** ora at 80 °C free enzyme is inactive / (fully) denatured / 0%, but immobilised enzyme, is still active / is partially denatured / 27% ; for immobilised enzyme, activity is higher (than free enzyme) at all temperatures above 60 °C ;	**4**
(b)	*two from:* (alginate / immobilisation), is protective / has stabilising effect ; **A** enzyme, less / not, exposed (to solution) hydroxide ions do not penetrate the alginate beads ; shape of active site (of immobilised enzyme) is, less / not, disrupted / AW ; **A** active site is (more) complementary to substrate **A** few(er) active sites are, altered / changed few(er) bond(s) within (immobilised) enzyme break ; **A** hydrogen / ionic **R** peptide / disulfide at pH 8 immobilised enzyme is not fully denatured ; **A** pH 7 / 7.5	**2**

(c)	*explanation must match variable stated* *standardised variable* concentration / volume, of, enzyme / substrate, solution ; *explanation for standardised variable* *idea that:* concentration / volume, of, enzyme / substrate, influences the probability of collisions between enzyme and substrate molecules / formation of enzyme/substrate complexes ; **A** number of / frequency of, collisions between substrate and enzyme **or** *standardised variable* use the same method for determining enzyme activity ; e.g. time when samples are taken *explanation for standardised variable 3* ref. to how enzyme activity is measured by conversion of substrate to product ;	**2**

(d)	*two from:* 1 (may be able to) obtain more product (per unit time) ; 2 can use higher temperatures (to obtain more product) / still active at higher temperatures ; **A** thermostable 3 does not denature (as easily as free) if temperatures increase / AW ; 4 immobilised enzyme can be reused / immobilised enzyme can be recovered ; 5 enzyme does not contaminate product ; **A** no effect on quality of product, e.g. taste 6 less, purification (of product) / downstream processing, needed ; **A** idea that downstream processing less, complex / difficult 7 longer shelf-life of enzyme / AW ; **A** durability 8 AVP ; e.g. allows continuous production (rather than batch) some enzymes are difficult to extract **I** ref. to cost / cost effective unqualified **R** reduces effect of end product inhibition	**2**

Q23.

(c)(iii)	*any four from* **1** (reversibly) binds / attaches / AW, to, allosteric site / site other than active site ; **2** (which) changes, shape / tertiary structure / 3-D structure, of active site ; **A** active site distorted **I** protein structure **3** substrate / ATP, cannot, enter / bind / fit / AW, to active site ; **A** active site no longer complementary to substrate **A** enzyme substrate / ES, complexes cannot form **A** ESCs cannot form **I** ATP / substrate, cannot bind to enzyme *without a link to active site* **4** no / less, hydrolysis of ATP **A** breakdown **or** no / less, energy released ; **I** no energy, synthesised / created / produced **5** Na^+ not moved, out and K^+ not moved in ; **I** active transport, stops / decreases	**4**

Q24.

(c)(i)	*any two from* pack more beads into the column ; slows the passage of milk through the column ; more time for enzyme to be exposed to substrate ; (small beads have a) larger surface area to volume ratio ; rate of reaction will be faster / AW ; **A** faster reactions will take less time to collect results (at each temperature) ; fast(er), rate of diffusion of, substrate / product ;	**2**
(c)(ii)	*any three from* both have same activity between 0 and 20 °C ; **A** described with values from **Fig. 2.2** between 20 °C and 40 °C, the activity of **F** increases more steeply than **I** ; **F**, has a lower optimum temperature / peaks at a lower temperature / AW, than **I** ; **A** optimum temperature, **F** is 40 °C v **I** is 50 °C at 70 °C, no activity for **F** but there is activity for **I** ; **A** 4% for **I** and 0% for **F** **A** denatured for 0% above the optimum temperature **I** has a steeper decrease of activity ; **I** is active over a greater range of temperatures (than F) ;	**3**