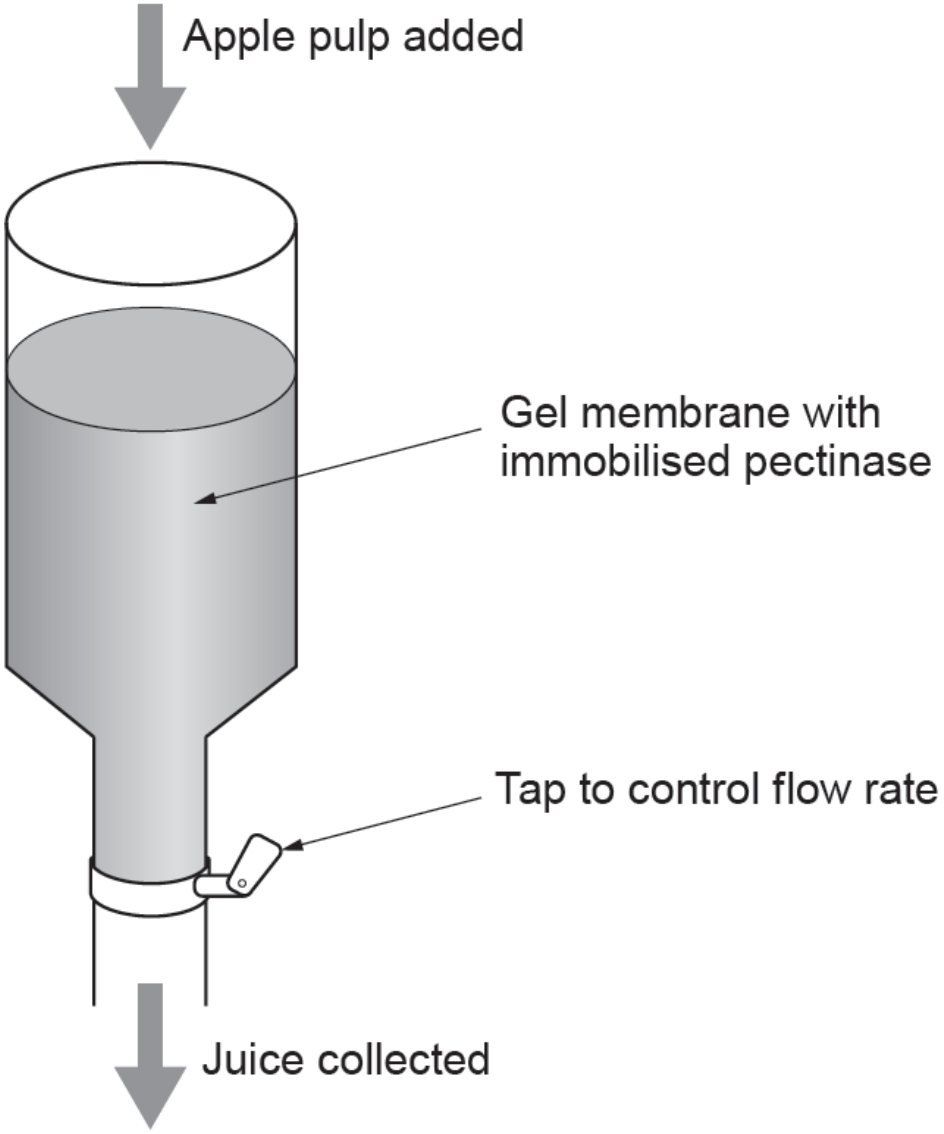
My Question Paper

1. Pectin is a structural polysaccharide found in the cell walls of plant cells and in the middle lamella between cells, where it helps to bind cells together. Pectinases are enzymes that are routinely used in industry to increase the volume and clarity of fruit juice extracted from apples. The enzyme is immobilised onto the surface of a gel membrane which is then placed inside a column. Apple pulp is added at the top, and juice is collected at the bottom. The process is shown in the diagram below.



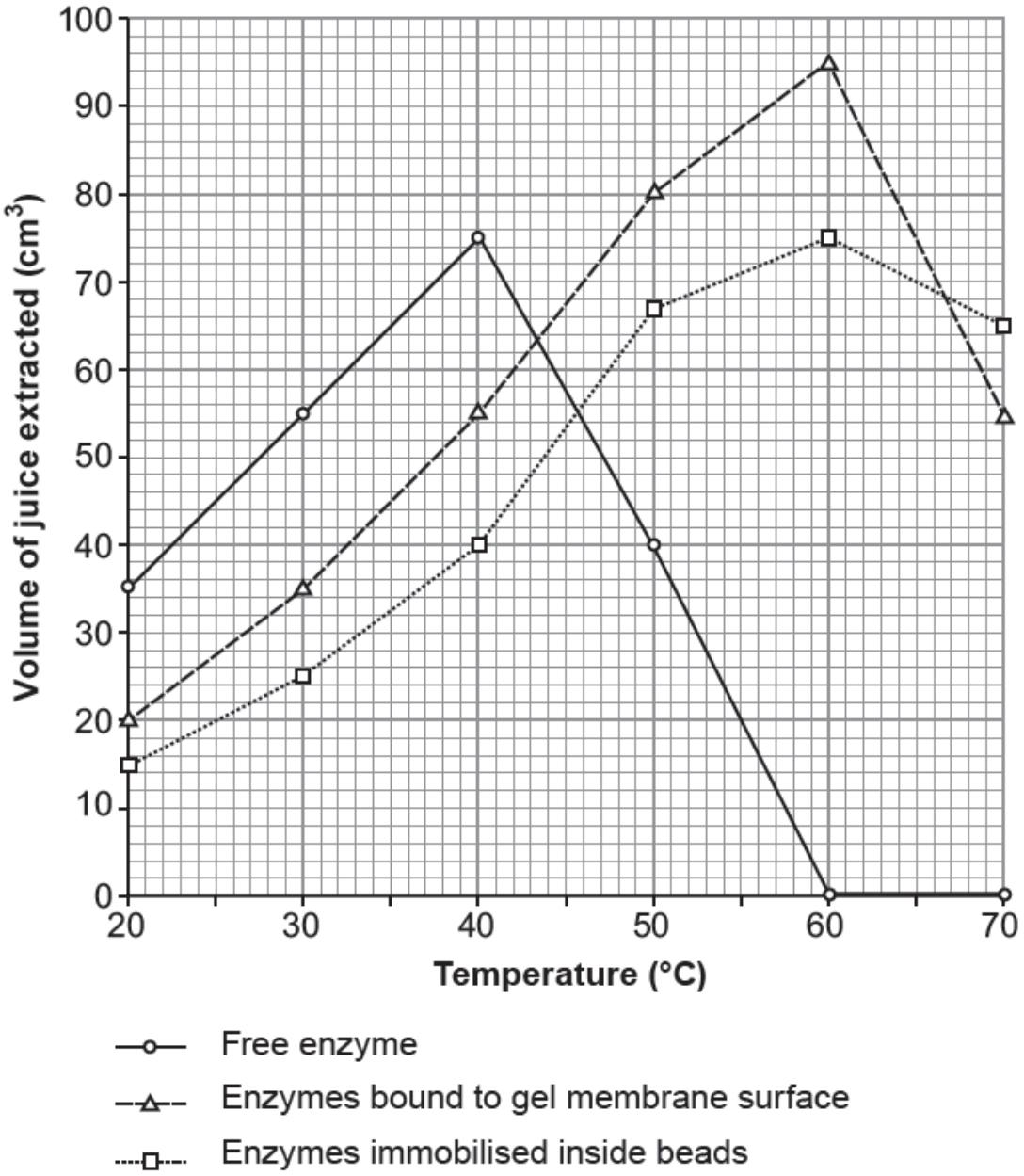
(a) Immobilising enzymes can increase the temperature range over which they can be used.
Describe **two** other advantages of immobilising pectinases

[2]

(b) Suggest why reducing the flow rate of material through the column would result in an increased volume of juice being collected.

[2]

(c) The extraction of juice using pectinase was compared using equal volumes and concentrations of free enzyme, enzymes bound to the surface of a gel membrane and enzymes immobilised inside alginate beads. The results are shown in the graph below.



Using the graph and your own knowledge of enzymes, answer the following questions.

(i) Describe and explain the results for the free enzyme at temperatures above 40°C.

[4]

(ii) Explain why a higher yield of juice was obtained when using free enzyme between temperatures of 20°C and 40°C than when using immobilised enzyme.

[2]

(iii) Suggest a reason for the differences seen in the results for the enzymes bound to the gel membrane surface with those immobilised inside the beads, between temperatures of 20°C and 60°C.

[2]

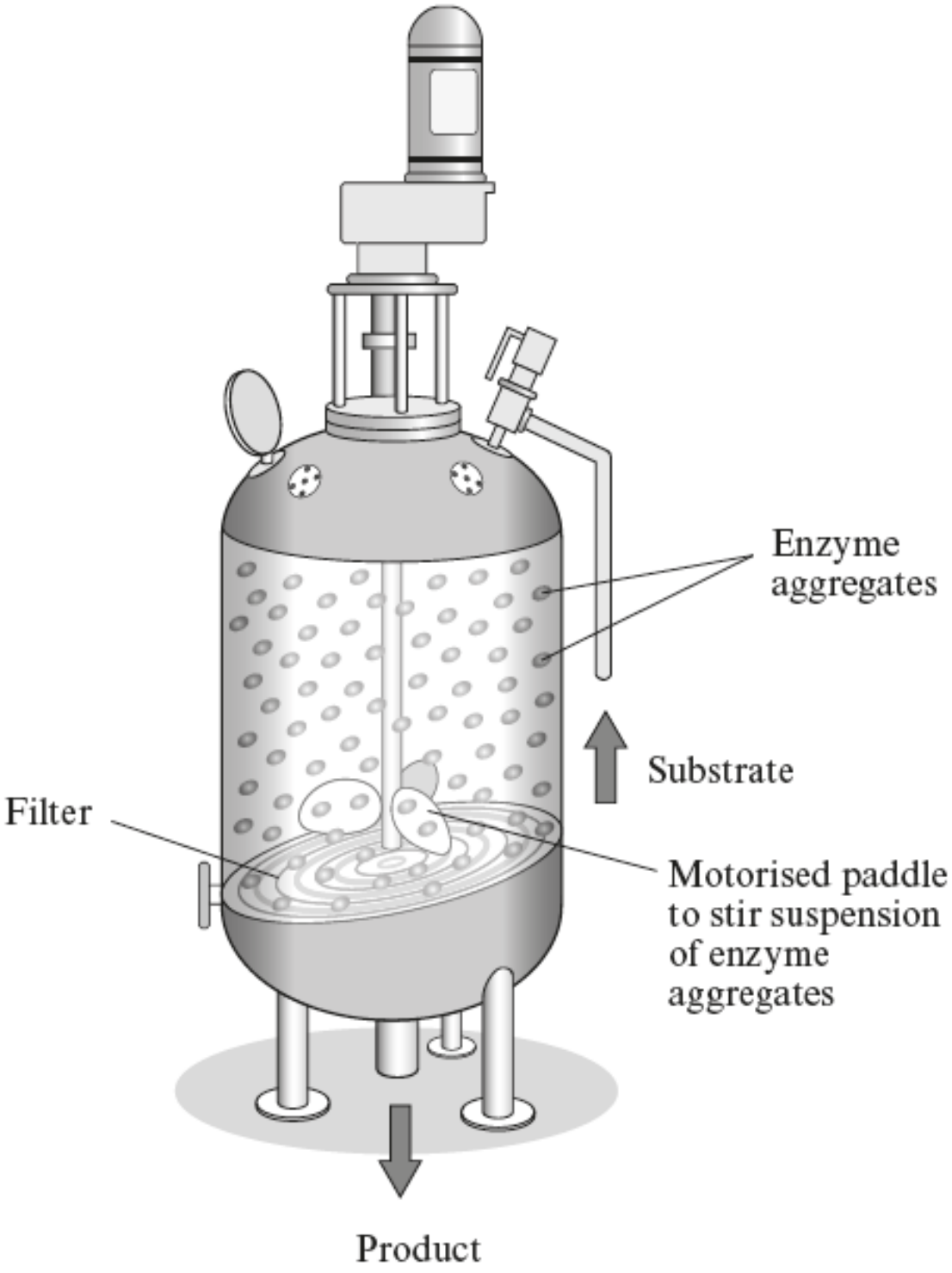
2.

Answer **one** of the following questions.
Any diagrams included in your answer must be fully annotated.

Either, *(a)* Describe the biological principles involved in the use of immobilised enzymes including the detection of blood sugar using biosensors. [10]

Or *(b)* Describe the structure and role of proteins in living organisms. [10]

3. Immobilised enzymes are prepared for industrial use in a number of ways. In the vessel shown below, the enzymes have been formed into clumps called enzyme aggregates. These are held together by cross-linking without altering their tertiary structure. They are permanently insoluble but maintain their catalytic activity.



(a) Why is it important that the tertiary structure of these enzymes is not altered by the cross-linking?

[2]

(b) Using your own knowledge and the diagram opposite, explain why it is necessary for these enzyme aggregates to be insoluble.

[2]

(c) State **three** advantages in using immobilised enzymes in industry.

[3]

(d) Name another method of immobilising enzymes, other than cross-linking.

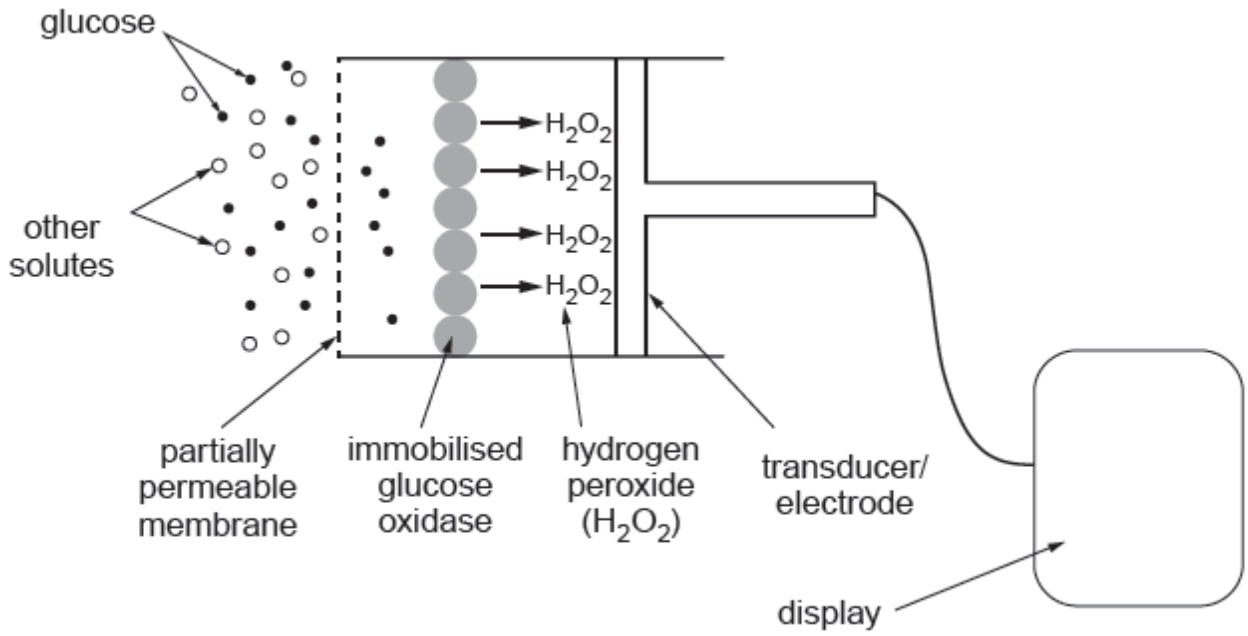
[1]

Total

[8]

4.

Biosensors make use of immobilised enzymes to detect specific molecules in a mixture. The diagram below shows a possible structure of a biosensor used to monitor blood glucose concentration.



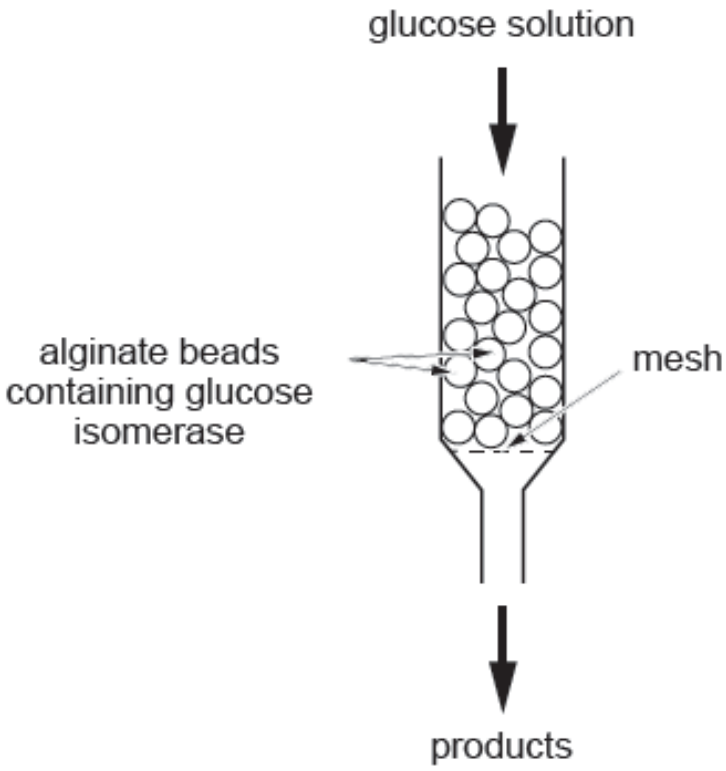
(a) (i) Describe the function of the partially permeable membrane in this biosensor.

[2]

(ii) With reference to the diagram above, describe how the concentration of glucose is transmitted to the display.

[3]

Immobilised enzymes are also used in the food industry to produce many useful substances, for example fructose syrup. The diagram below shows a simplified version of this process. A glucose solution is passed through a column of the immobilised enzyme glucose isomerase and fructose is released as a product.



(b) (i) Suggest why the enzyme involved is called glucose isomerase.

[1]

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(ii) One of the advantages of using immobilised enzymes is that the product does not contain the enzyme and therefore does not need to be purified. Describe a biochemical test that could be used to show that the product has not been contaminated by the **enzyme**.

[2]

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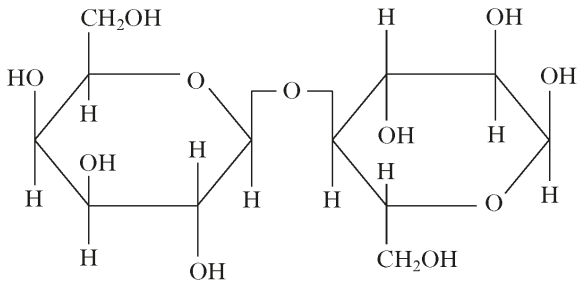
(iii) Describe **two** other advantages of using immobilised enzymes.

[2]

5. Lactose is a disaccharide found in milk. The diagram below shows the structure of lactose.



(a) Lactose can be broken down into its constituent monosaccharides.

(i) Complete the diagram above to show how lactose is broken down. [2]

(ii) State the type of reaction involved in the breakdown of lactose. [1]

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(iii) Name the bond that is broken during this reaction. [1]

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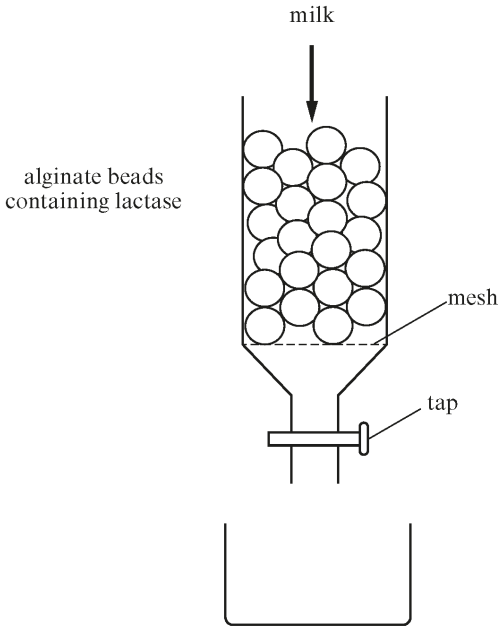
(iv) Name the molecules produced when lactose is broken down. [1]

.....

(b) The enzyme lactase can be used to break down lactose. In an experiment lactase was immobilised inside alginate beads and placed in a column, as shown in the diagram below. Fresh milk was then poured into the column and left for one minute before being allowed to drain into the beaker below. As the milk passes through the column the lactose in the milk is broken down.



(i) What is meant by the term immobilised enzyme? [1]

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(ii) Describe **two** advantages of using immobilised enzymes in this way. [2]

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(c) (i) The products produced from the breakdown of lactose are reducing sugars. Describe how you could test for the presence of a reducing sugar. [2]

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(ii) The products produced could also be detected by a biosensor. What is meant by the term biosensor? [1]

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(iii) What would be the main advantage of using the biosensor to detect the products? [1]

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(d) Some bacteria which are found in milk can convert sugars within the milk to lactic acid. Over time the number of these bacteria increase and this eventually causes milk to go sour. The experiment above was repeated with milk that had been left for seven days. State and explain the effect this would have on the concentration of reducing sugars detected. [4]

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(Total 16 marks)

Marking Scheme

1.

Question			Marking details	Marks Available
7	(a)		Any **two** from Product not contaminated with enzyme; Enzyme can be re-used/ small quantity of enzyme required; Can {withstand/tolerate} a wider range of pH; Can be used in a continuous process;	Max 2
	(b)		Increases (contact) time between enzymes and substrate/ more time for pectinase to digest {apple pulp/pectin}; More successful collisions/more enzyme substrate complexes formed; NOT ESC	2
	(c)	(i)	40°C to 60°C {decrease in/less} (volume of) juice extracted; NOT less juice extracted above 40 °C Above 60 °C no juice extracted; Between 40 °C and 60 °C enzymes are denaturing/ above 60°C they are denatured; Hydrogen bonds break; {Tertiary structure deformed / active site changes shape} {Substrate can no longer fit/ fewer enzyme substrate complexes formed};	Max 4
		(ii)	(Free enzymes) can move; Increased chance of successful collision / more enzyme substrate complexes formed;	2
		(iii)	(Increased juice extracted with membrane bound enzymes) because membrane bound enzymes are {more accessible/OWTTE} to substrate; (Enzymes immobilised inside bead) substrate has to {diffuse/pass} into bead;	2
			Question 7 Total	**[12]**

2. (b)

A primary structure, {sequence/ order} of amino acids in its polypeptide chain

B linked by peptide bonds

C secondary structure consists of – α helix/ pleated sheet

D hydrogen bonds

E tertiary structure described - 3D folding/ irregular/ further folding

F as shown by globular proteins

G disulphide bridges/ ionic/ hydrogen/ hydrophobic (any two)

H Quaternary structure described- combination of two or more polypeptide chains

I Some proteins have non-protein groups/ prosthetic groups

J enzymes – function or description of

K antibodies/hormones/ plasmaproteins with function

L haemoglobin – {carries/ transport} of oxygen

M fibrous proteins + example connective tissue/ keratin/ collagen

N Function of fibrous protein - strength

O carriers in active transport/ facilitated diffusion /fibrinogen in blood clotting /histones/ ribosomal proteins

(Any 10 out of 15 points)

(b)

A primary structure, {sequence/ order} of amino acids in its polypeptide chain

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L haemoglobin – {carries/ transport} of oxygen

M fibrous proteins + example connective tissue/ keratin/ collagen

N Function of fibrous protein - strength

O carriers in active transport/ facilitated diffusion /fibrinogen in blood clotting /histones/ ribosomal proteins

(Any 10 out of 15 points)

3.

Question			Marking details	Marks Available
6.	(a)		Causes change in <u>shape</u> of enzyme/active site; So substrate no longer fits into active site; {No/ fewer} enzyme substrate complexes;	2 max
	(b)		{(Insoluble) enzymes/ (enzyme) aggregates} cannot pass through the filter/ ORA; So the product is uncontaminated with enzymes/ ORA;	2
	(c)		Can tolerate {<u>higher</u> temperatures/greater <u>range</u> of pHs}; NOT range of temperatures Easily <u>recovered</u> for reuse/ enzymes stay in aggregates/ reused qualified/ uncontaminated product/ separated from product; NOT reused unqualified/ enzymes reused Several enzymes can be used together; Easy addition/removal of enzymes;	3 max
	(d)		Any one from : Gel capsule/alginate beads/ gel beads; cellulose fibres; gel membrane; porous glass beads; NOT inert matrix unqualified/ encapsulation unqualified **Question 6 Total**	1 max **[8]**

4.

Question			Marking details	Marks Available
6	*(a)*	(i)	Allows the glucose molecules to pass through (to the enzyme layer); Prevents the passage of other solutes ; so they can't {affect results / affect enzyme / reduce enzyme activity};	2 max
		(ii)	glucose broken down by enzyme; the {hydrogen peroxide/oxygen} is {detected/absorbed} by electrode; an electric signal is generated/ changes chemical to electrical signal; the greater the concentration of {glucose/hydrogen peroxide/oxygen} the greater the signal;	3 max
	(b)	(i)	The enzyme converts glucose into it's isomer fructose / glucose and fructose are isomers;	1
		(ii)	Add Biuret solution / sodium hydroxide solution & copper sulphate; (reject if reference to heat) The solution would remain blue / no colour change would occur;	2
		(iii)	can be re-used; has greater stability/denature at higher temperatures; can catalyse reactions/greater stability over a wider range of pH; More than one enzyme can be used/enzymes added or removed easily/ greater control over process/ can be used in a continuous process; (Reference to cost is neutral)	2 max
			Question 6 Total	**[10]**

5.

(a)	(i)	Molecule of water (drawn with arrow towards the O atom of the glycosidic bond); NOT water going out Monosaccharides drawn with –OH groups in correct position on C1 and C4 (involved in bond);	2
	(ii)	Hydrolysis; NOT hydrolysation (ignore reference to acid)	1
	(iii)	Glycosidic;	1
	(iv)	Glucose and galactose; ignore alpha/ beta	1
(b)	(i)	An enzyme that has been fixed to an inert {matrix/support/ substance};	1
	(ii)	The enzyme can easily be recovered/ reused; The product is free from contamination; Enzyme is {stable at / tolerates/ withstand} higher temperatures/denatures at a higher temperature/ functions over a wide range of pH; NOT wider range of temperature alone Several enzymes with differing optima can be used at the same time; More control over the reaction/enzymes easily added or removed/ can be used in a continuous process;	Max 2

(a)	(i)	Molecule of water (drawn with arrow towards the O atom of the glycosidic bond); NOT water going out Monosaccharides drawn with –OH groups in correct position on C1 and C4 (involved in bond);	2
	(ii)	Hydrolysis; NOT hydrolysation (ignore reference to acid)	1
	(iii)	Glycosidic;	1
	(iv)	Glucose and galactose; ignore alpha/ beta	1
(b)	(i)	An enzyme that has been fixed to an inert {matrix/support/ substance};	1
	(ii)	The enzyme can easily be recovered/ reused; The product is free from contamination; Enzyme is {stable at / tolerates/ withstand} higher temperatures/denatures at a higher temperature/ functions over a wide range of pH; NOT wider range of temperature alone Several enzymes with differing optima can be used at the same time; More control over the reaction/enzymes easily added or removed/ can be used in a continuous process;	Max 2

Examiner's Comments

1. This question proved to be a good discriminator, requiring candidates to relate and apply both enzyme kinetics and immobilised enzyme knowledge.

   Part (a) was well answered, however some candidates quoted general advantages of immobilizing enzymes e.g. that several enzymes could be used together, rather than relating to pectinases.

   For part (b) candidates were able to identify that decreasing flow rate would increase contact time between enzyme and substrate. However, many stated that this would give more time for successful collisions to occur rather than more successful collisions. These candidates failed to gain the second marking point. Some candidates are still using the abbreviations ES-complexes or ESC rather than the full term - this is not acceptable.

   For (c)*(i)* most candidates picked up two marks for hydrogen bonds breaking and the affect this would have on the enzyme. Many candidates failed to describe the results at all, or gave vague descriptions of the volumes of juice produced, often just referring to “above 40oC volume of juice decreased”, but failed to comment that above 60oC no juice is collected. Moreover, candidates often stated that “above 40oC enzymes were denatured”, which was not the case as juice was still being extracted up to 59oC. It is only at or above 60oC that all enzymes are denatured. It is important to remind candidates to read questions carefully - many described free enzymes from 20oC or even referenced immobilised enzymes which were not asked for in the question. For (c)*(ii)* candidates often described that the different enzymes had different optimum temperatures whilst failing to explain why a higher yield was produced. Part*(iii)* proved challenging for candidates and many explanations involved higher surface areas.

   *This comment originally referred to question **7** on paper **1071/01 (21/05/2014)***

2. The essay on proteins proved to be far more popular than that on immobilised enzymes. In (a) those that answered the question on immobilised enzymes scored highly although the descriptions of biosensors lacked detail at times. In (b) well prepared candidates scored highly on the structure and role of proteins. The majority of candidates described in detail the levels of protein structure. Less well prepared candidates were confused and failed to describe successfully the roles of proteins and neglected to describe specific examples and their functions.

   *This comment originally referred to question **8** on paper **1071/01 (11/01/2012)***

3. Another application of knowledge question where it was apparent that many have learned the work by rote, but do not understand the principles, so cannot transfer the knowledge to a novel situation. (a) linked back to how enzymes work, so most answered correctly, but a few got it completely wrong and said that the cross linking would stop the enzymes working - very confused. Better answers, using the diagram, explained that aggregate insolubility meant that the enzymes would not pass through the filter and so the product would not need to be separated from enzymes. Many did not even mention the filter or link it with the aggregates, yet the answer was on the diagram. The remainder of the question was straight recall and was well done.

   *This comment originally referred to question **6** on paper **1071/01 (21/05/2013)***

4. Many candidates picked up the majority of the marks in part (a) although there was some confusion as to the role of the transducer. Quality of written communication was also an issue for some and their descriptions were not logically set out. Part (b) proved more difficult with only a small minority interpreting the information and realising the link between isomerism in monosaccharides. The majority of candidates incorrectly stated that they would use Benedict's reagent to test for the protein. Those that did state that they would use Biuret then explained that there would be a colour change and therefore didn't answer the question being asked. The majority of candidates were able to give advantages of using immobilised enzymes.

   *This comment originally referred to question **6** on paper **1071/01 (08/01/2014)***

5. (a) The majority of candidates recognised that water was required for the reaction, but very few drew an arrow indicating the water would be inserted into the molecule of lactose; and many went on to correctly draw the resulting monosaccharides. Some candidates lost marks by drawing the 'OH groups' at the bottom of the molecules or they unsuccessfully tried to invert one of the molecules.

(b) Questions on immobilised enzymes are common and the majority gained 2 marks, however here were some candidates who thought that because the enzymes were immobilised this would allow them to function in extremes of temperature and pH.

(c) Many candidates stated that the Benedict's test would be required and that if a reducing sugar was present the solution would turn red, however a significant number failed to state that the solution needs to be heated and/or there would be a colour change from blue to red.

Many candidates failed to state what a biosensor is but instead gave detailed descriptions of how digital biosensors work. Vague responses were given regarding the advantages of using them, such as they are 'more accurate', 'precise' give a faster result' and very few related it to the experiment.

(d) Responses to this question were disappointing. Most candidates stated that there would be a decrease in reducing sugars detected but then simply went on to rewrite the stem of the question. Only the better candidates could make the link between the effects of a reduced substrate concentration and/or a decrease in pH on the rate of the enzyme catalysed reaction.

*This comment originally referred to question **6** on paper **1071/01 (09/01/2013)***