EUNOIA JUNIOR COLLEGE
JC2 Preliminary Examinations 2023
General Certificate of Education Advanced Level
Higher 2

| CANDIDATE NAME | **ANSWER KEY** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| CIVICS GROUP | **2** | **2** | **-** | | | REGISTRATION NUMBER | | |

# BIOLOGY

**9744/04**

Paper 4 Practical

**24 August 2023**

**2 hours 30 minutes**

Candidates answer on the Question Paper.

**READ THESE INSTRUCTIONS FIRST**

Write your name, civics group and registration number on all the work you hand in.
Give details of the practical shift and laboratory, where appropriate, in the boxes provided.
Write in dark blue or black pen.
You may use an HB pencil for any diagrams or graphs.
Do not use stapler, paper clips, highlighters, glue or correction fluid/tape.

| **Shift** |
|---|
| |
| **Laboratory** |
| |

Answer **all** questions in the spaces provided in the Question Paper.

The use of an approved scientific calculator is expected, where appropriate.
You may lose marks if you do not show your working or if you do not use appropriate units.

The number of marks is given in brackets [ ] at the end of each question or part question.

| **For Examiner's Use** | |
|---|---|
| **1** | |
| **2** | |
| **3** | |
| **Total** | **55** |

This document consists of **22** printed pages and **2** blank pages.

Answer **all** questions.

**1** Yeast cells contain enzymes that catalyse metabolic reactions. Some of these reactions release carbon dioxide.

You will investigate the release of carbon dioxide from a mixture of yeast and carbohydrate. The mixture is put into dialysis (Visking) tubing.

The dialysis tubing acts as a partially permeable membrane, allowing the carbon dioxide to diffuse out of the dialysis tubing.

You are provided with the materials shown in Table 1.1.

**Table 1.1**

| labelled | contents | hazard | volume / $cm^3$ |
|---|---|---|---|
| **Y** | 1g dried yeast in boiling tube | none | - |
| **G** | 10.0% warm glucose solution | none | 20 |
| **W** | distilled water | none | 50 |
| **B** | bromothymol blue indicator solution | harmful | 10 |
| **V** | 20 cm length dialysis tubing in a beaker of distilled water | none | - |

If any solution comes into contact with your skin, wash off immediately under cold water.

It is recommended that you wear suitable eye protection.

To test for the release of carbon dioxide, a sample of the water surrounding the dialysis tubing is added to drops of an indicator, **B**.

Fig. 1.1 shows the effect of increasing concentration of carbon dioxide on the colour of **B**. Yellow is the end-point.


**Fig. 1.1**

Carry out step 1 to step 21.

step 1 Using the beakers labelled **hot water** and **cold water**, adjust the water in the beaker labelled **water-bath** to 45 °C. You will **not** need to maintain this temperature.

step 2 Put 15 $cm^3$ of **G** into the boiling tube labelled **Y**. Mix well.

Between step 3 and step 4, you will be leaving the apparatus for 15 minutes. Use this time to continue with other parts of Question 1.

step 3 Put boiling tube **Y** into the water-bath for 15 minutes.

step 4 After 15 minutes, remove boiling tube **Y** from the water-bath.

step 5 Stir the mixture in boiling tube **Y** and pour it into a beaker.

step 6 Label the spotting tile with the sample times in minutes, as shown in Fig. 1.2.

step 7 Put 3 drops of **B** onto the spotting tile at each sample time, as shown in Fig. 1.2.

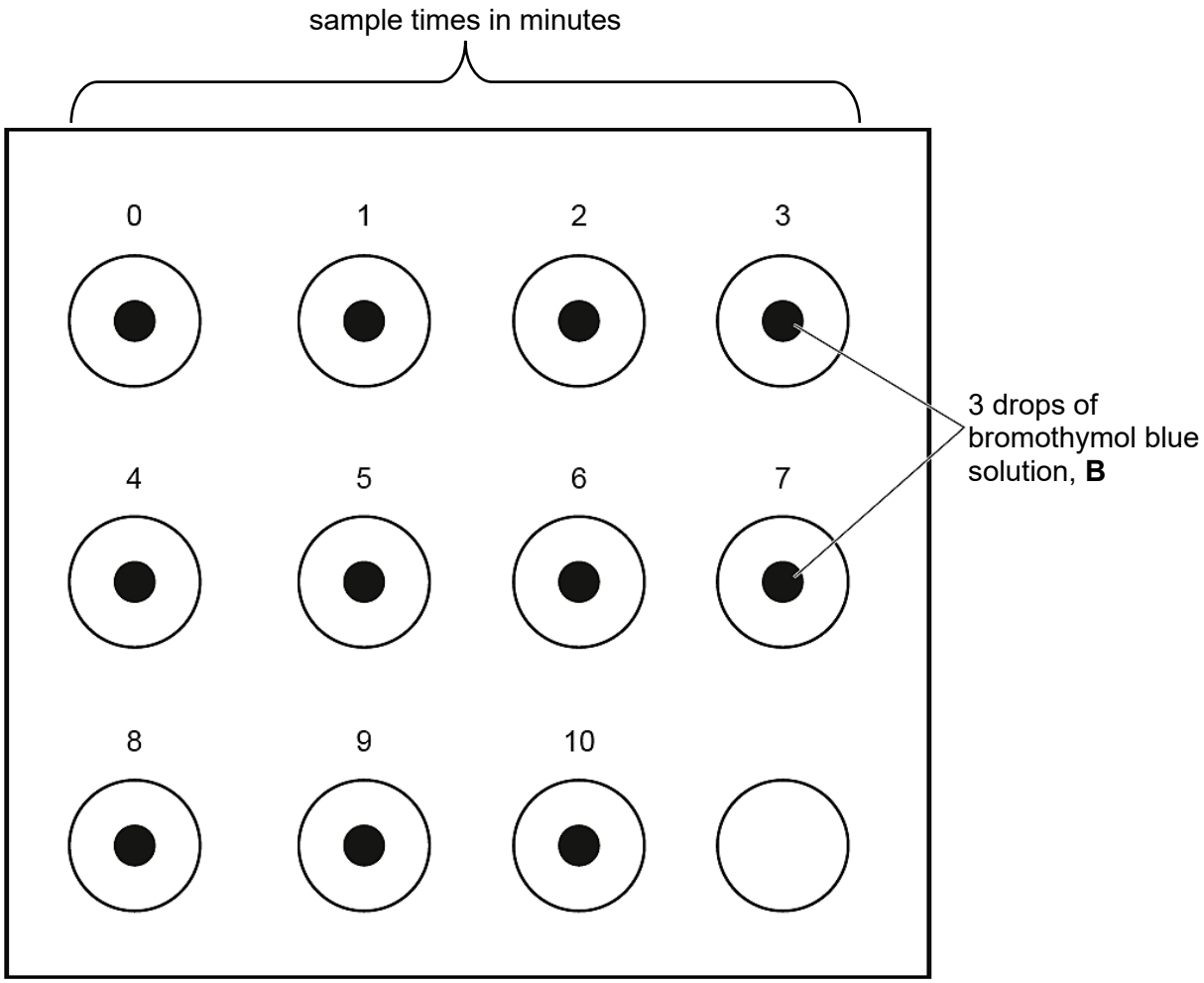



**Fig. 1.2**

Fig. 1.3 shows the apparatus you will set up for this investigation.

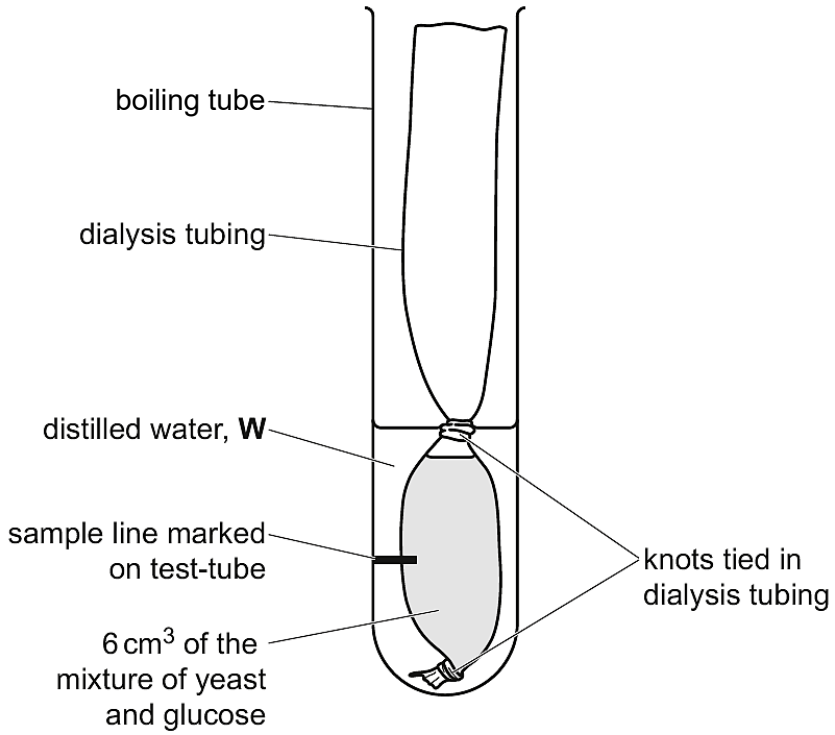



**Fig. 1.3**

step 8 Tie a knot in the dialysis tubing as close as possible to one end, so that the end is sealed.

step 9 To open the other end, rub the tubing gently between your fingers and thumb.

step 10 Stir the mixture in the beaker from step 5 and put 6.0 cm3 of this mixture into a syringe.

step 11 Wipe the outside of the syringe and put the mixture from the syringe into the dialysis tubing.

step 12 Rinse the outside of the dialysis tubing by dipping it into the water in the container labelled **V**.

Look carefully at Fig. 1.3 to help you with step 13 to step 15.

step 13 Tie a knot just above the level of the mixture in the dialysis tubing, as shown in Fig. 1.3.

step 14 Put the dialysis tubing into a clean boiling tube so that it is resting on the bottom of the boiling tube, as shown in Fig. 1.3.

step 15 Draw a line on the boiling tube so that it is half-way between the two knots, as shown in Fig. 1.3. This is where you will take your samples from.

step 16 In this step, you will use a syringe to measure the volume of distilled water, **W**, needed to cover the section of dialysis tubing containing the mixture.

Use a syringe to put **W** into the boiling tube to cover the section of dialysis tubing containing the mixture.

**(a) (i)** State the volume of **W** that you added to the boiling tube in step 16.

volume of **W** = ..... $cm^3$ [1]

1. **8 $cm^3$ to 20 $cm^3$**
   Accept: whole number / 1 d.p
   R: 2 d.p
   **CIE: volume recorded between 5 and 20 $cm^3$**

step 17 Take a sample of **W** from the boiling tube at the point you marked in step 15, using a pipette.

step 18 Put 3 drops of **W** onto **B** at time 0 on the white tile. Put the remaining **W** in the pipette back into the boiling tube.

step 19 Start timing and put the boiling tube containing the dialysis tubing into the beaker labelled **water-bath**.

step 20 Mix the sample of **W** and **B** on the white tile and immediately record the colour in **(a)(ii)**, using the colours stated in Fig. 1.1.

step 21 Repeat step 17, step 18 and step 20 for each of the sampling times until the end-point (yellow) is reached for **two** consecutive samples. If the end-point is not reached at 10 minutes, stop timing.

**(ii)** Record your results in an appropriate table. [3]

| Time / min | Colour |
|---|---|
| **0** | blue |
| **1** | |
| **2** | |
| **3** | |
| **4** | |
| **5** | |
| **6** | |
| **7** | yellow |
| **8** | yellow |
| **9** | |
| **10** | |

1. **H**: Headings
   (independent variable: time and minutes + no units in body of table) note to marker:
   Accept minute / mins
   (dependent variable: colour) A: observation
2. **R**: results (R: colour change under R or H)
   must have at time 0. colour at time 0 must be blue/blue-green
   must have transition of 3 colours minimally
   A: yellow appearance earliest at 5min

(colour for each minute for 10 minutes or until end-point/yellow is reached for two consecutive times)

3. **C**: only used colours from Fig. 1.1

**(iii)** This investigation used colour to indicate the concentration of carbon dioxide in the sample. Suggest **three** improvements to this investigation that would increase the accuracy of the results. [3]

1. Use **colour chart / colorimeter** for a more objective way of determining colour**;**
2. Use **smaller intervals of time (e.g. 30s)** instead of 1 minute**;**
3. **Conduct replicates** to **check for anomalous results / conduct replicates and find the mean;** (R: repeat experiment to check reproducibility of results)
4. Use a **syringe** to measure fixed volume of **B / W** instead of using dropper**;**
5. Use a **thermostatically controlled water bath** to **maintain constant temperature / at 45°C**;
6. **AVP:** Use a **pH meter / carbon dioxide probe;**

**(iv)** A student wishes to repeat the investigation using the same procedure but with different concentrations of glucose. Using a table, show how the different concentrations of glucose can be made. You need to ensure that a sufficient volume is made for each glucose concentration. [4]

| concentration of glucose / % | Volume of 10.0% glucose / $cm^3$ | Volume of distilled water / $cm^3$ |
|---|---|---|
| 10.0 | 20.0 | 0.0 |
| 8.0 | 16.0 | 4.0 |
| 6.0 | 12.0 | 8.0 |
| 4.0 | 8.0 | 12.0 |
| 2.0 | 4.0 | 16.0 |

| concentration of glucose / % | Volume of 10.0% glucose / $cm^3$ | Volume of distilled water / $cm^3$ |
|---|---|---|
| 10.0 | 15.0 | 0.0 |
| 8.0 | 12.0 | 3.0 |
| 6.0 | 9.0 | 6.0 |
| 4.0 | 6.0 | 9.0 |
| 2.0 | 3.0 | 12.0 |

1. Column heading + units + proper lines used for table construction (R: units in table)
   R: if never specify clearly what concentration of glucose is used for dilution
2. Concentration of glucose: 5 different concentrations + equal interval (simple dilution) + highest concentration does not exceed 10%
3. Total volume for each glucose concentration is at least 15 $cm^3$ + accurate volumes to make each glucose concentration
4. Precision: to 1 d.p for both volumes of glucose and distilled water

A: serial dilution

1. Column heading + units + proper lines used for table construction (R: units in table)
   (final) concentration of glucose/%, concentration of glucose **used**/%, volume of glucose **used**/$cm^3$, volume of distilled water/$cm^3$
   R: short forms (e.g. conc., vol)
2. Concentration of glucose: 5 different concentrations + same dilution factor throughout

+ highest concentration does not exceed 10% (R: rounding up of concentration)

3. Total volume for each glucose concentration is at least 15 $cm^3$ + accurate volumes to make each glucose concentration
4. Precision: to 1 d.p for both volumes of glucose and distilled water

**(v)** A student repeated the investigation using the same procedure but with starch as the substrate instead of glucose.

Suggest why it took much longer to reach the end-point when starch was used as the substrate. [4]

1. **Glucose** is the **preferred respiratory substrate**;
2. On the other hand, **starch needs to be broken down/hydrolyse** into **monosaccharides / disaccharides / glucose / simple sugars** first; R: starch needs to be broken down by *amylase* to glucose
3. As such, the concentration of substrate / glucose is not as high as compared to when glucose is used;
4. The **frequency of effective collisions between enzyme and substrate** will be **lower**, and the **rate of formation of enzyme-substrate complex formation** also **drops**;
5. This results in **lower rate of formation carbon dioxide** and hence a longer time to reach end-point; (R: lesser $CO_2$ produced)
   A: takes a loner time to produce the same amount of $CO_2$ to reach end-point

*any three from:*
1 starch takes longer to break down ;
2 less enzymes to break down starch to produce carbon dioxide ;
3 fewer active sites ;
4 so less successful collisions ;
5 fewer enzyme-substrate complexes / ESCs ;

[Total: 15]

**2** **(a)** Many people are intolerant to the disaccharide lactose, which is found in milk. Commercially, the enzyme lactase can be immobilised and used to catalyse the breakdown of lactose to the monosaccharides glucose and galactose. These sugars taste sweeter and are easier to digest than lactose.

One way of immobilising lactase is using alginate beads, where lactase is contained within the beads. A student investigated the effect of alginate bead diameter on the hydrolysis of lactose.

The student:

- put beads with a diameter of 2mm into a syringe, up to the 5 $cm^3$ line
- put of 5 $cm^3$ lactose into this syringe
- left the syringe for 5 minutes
- measured the concentration of lactose after 5 minutes

The student used this method with the bead diameters shown in Fig. 2.1.

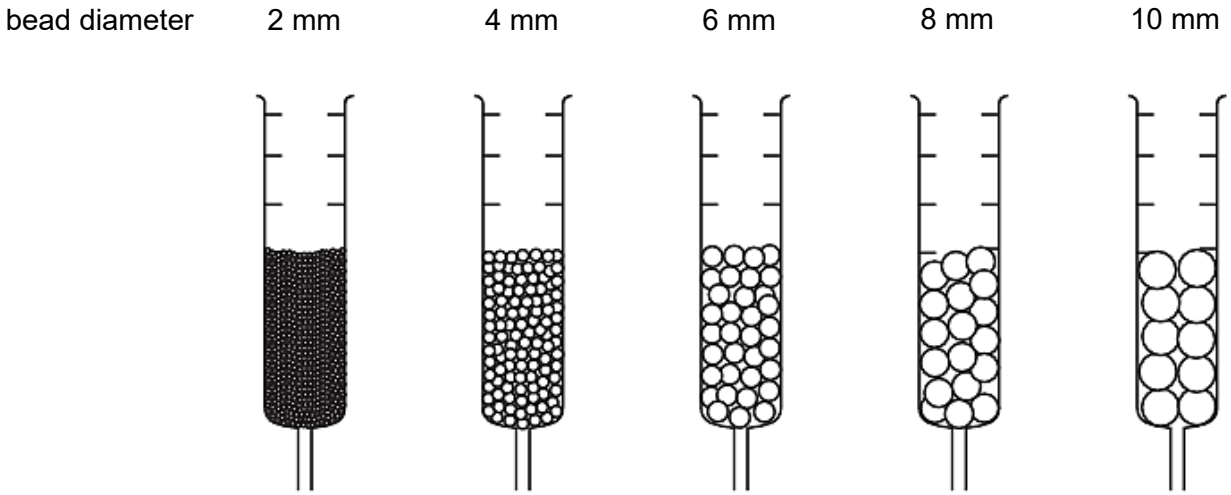



**Fig. 2.1**

Table 2.1 shows the results of this investigation.

**Table 2.1**

| bead diameter / mm | percentage concentration of lactose after 5 minutes / % |
|---|---|
| 2 | 20.5 |
| 4 | 21.0 |
| 6 | 29.5 |
| 8 | 40.5 |
| 10 | 69.0 |

**(i)** Plot a graph of the data shown in Table 2.1 on the grid in Fig. 2.2.

Use a sharp pencil.

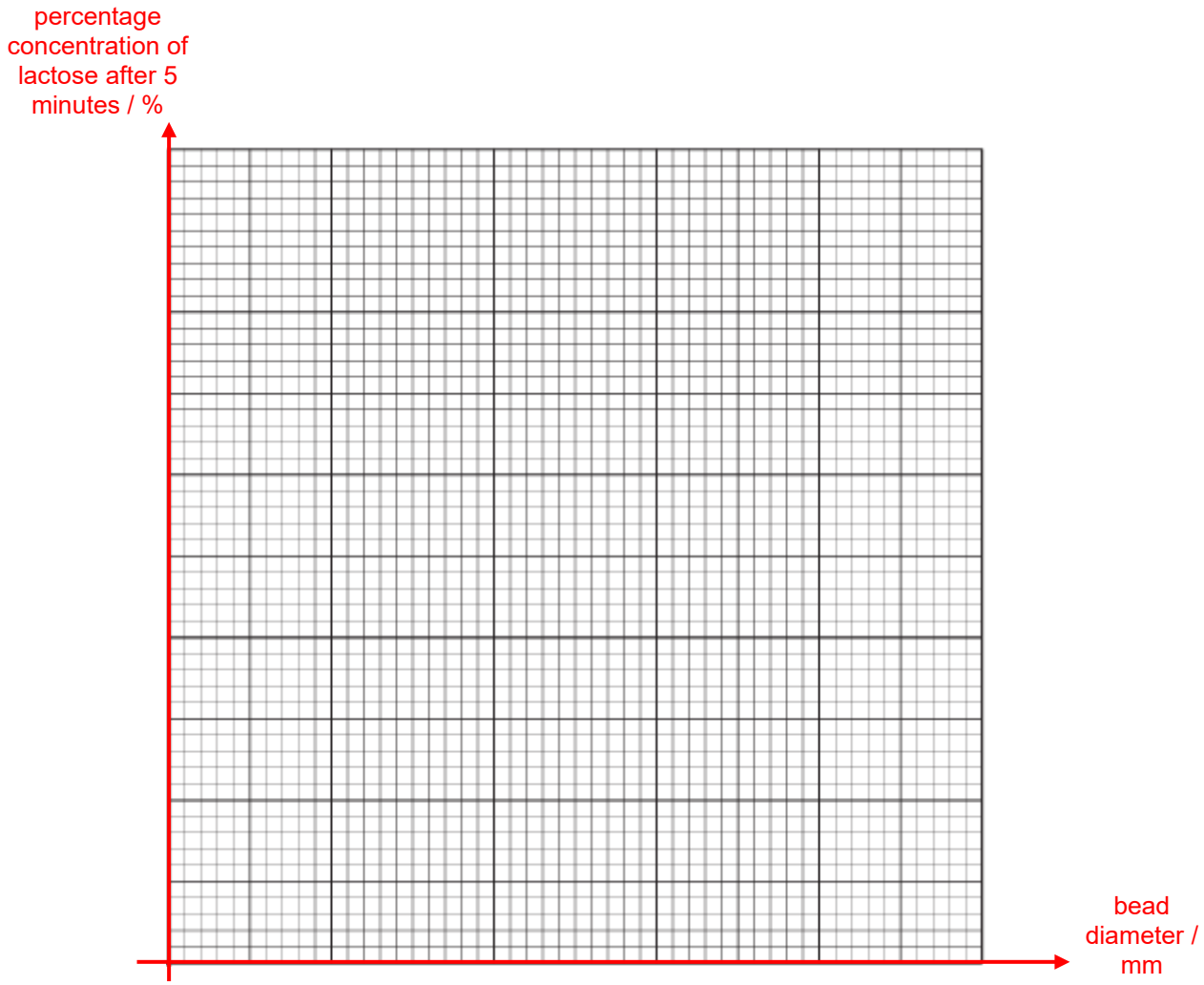



**Fig. 2.2**

[4]

1. **S** – Scale: >1/2 of graph paper used + no odd scale + origin + **T** – Title (good to have) (x-axis) 10 squares to 2mm, labelled each 10 squares + (y-axis) begins with 20, 10 squares to 10%, labelled each 10 squares; R: if lacking interval marking / if origin not labelled for both axes
2. **A** – Axes: correct axes with labels and units
(x-axis) bead diameter / mm + (y-axis) percentage concentration of lactose after 5 minutes / % ;
3. **P** – Plotted points: all points correctly plotted;
4. **L** – Line: best-fit curve/line, or point-to-point + **E** – No extrapolation

**(ii)** Use your graph to find the concentration of lactose in the milk after 5 minutes, when the bead diameter was 5 mm. Show clearly on your graph how the concentration is obtained.

1. Mark based on student's graph + need to show clearly how value is obtained from graph (dotted lines);

concentration of lactose = ……% [1]

Enzymes can be immobilised in a number of different ways, using different materials.

Fig. 2.3 shows three ways of immobilisation of enzymes.

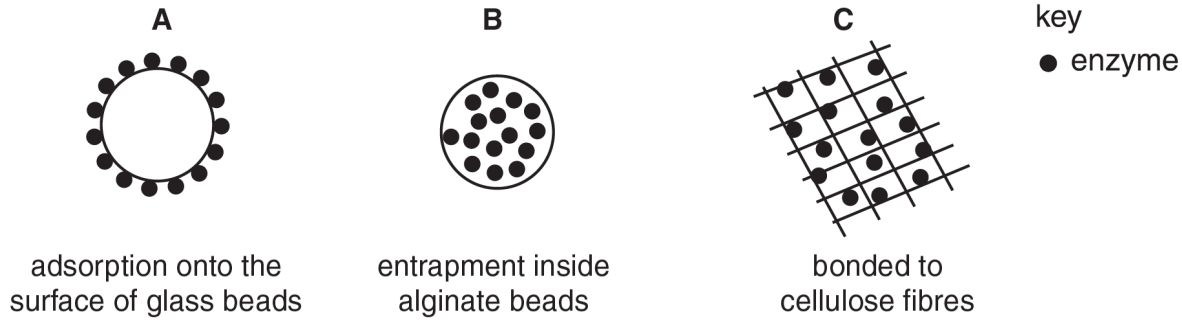



**Fig. 2.3**

A student carried out an investigation to compare the activity of the enzyme lactase that had been immobilised in the three different ways shown in Fig. 2.3.

- A solution containing 20 mg $cm^{-3}$ of lactose was poured through a column containing the immobilised enzyme.
- The solution containing the products was collected and the concentration of glucose measured using a biosensor.

**(b)** State a null hypothesis that the student could make for this investigation. [3]

1. There is **no significant difference** in the activity of the enzyme / lactase (A: rate of reaction / production or amount or concentration of glucose), whatever type of immobilisation is used;

| | |
|---|---|
| there is no significant difference in the activity of, the enzyme / lactase, whatever type of immobilisation is used ; | **A** in terms of, rate of reaction / production or amount or concentration, of glucose<br>**A** if choose 2 of the types of immobilisation<br>**I** 'any difference in the activity of enzymes is due to chance' |

Table 2.2 shows the student's results.

**Table 2.2**

| | way of immobilisation | | |
|---|---|---|---|
| | **A**: adsorption onto the surface of glass beads | **B**: entrapment inside alginate beads | **C**: bonded to cellulose fibres |
| mean volume of solution containing product / $cm^3$ | 21 | 25 | 20 |
| mean glucose concentration / $mg\,cm^{-3}$ | 15 | 10 | 12 |
| mean total glucose collected / mg | | 250 | |

**(c)** Complete Table 2.1 by calculating the mean total glucose collected for **A** and **C**. [1]

**A: 315**
**C: 240**

**(d)** Without a biosensor, it will not be possible to determine the exact concentration of glucose produced by the three ways of immobilising lactase.

However, it is still possible to determine if there is any difference in the concentration of glucose produced using the following apparatus and reagents.

- lactase immobilised via the three methods shown in Fig. 2.3
- 20 mg $cm^{-3}$ lactose solution
- Benedict's solution
- Bunsen burner
- wire gauze
- tripod stand
- beaker
- test tubes
- 5 $cm^3$ syringes
- stopwatch

To determine how the method of enzyme immobilisation affects the rate of lactase activity, you are to plan an investigation using the above apparatus and reagents.

**(i)** State the independent variable. [1]

1. way / method of immobilising lactase;

**(ii)** State the dependent variable and how it can be determined using the above apparatus and reagents. [2]

1. **Rate of lactase activity;**
2. as determined by **1 / (**the **time taken for first colour change for Benedict's test);**

**(iii)** Describe and explain a control that should be included in the investigation. [2]

1. **Keeping all other variables constant, replace lactase** with **boiled and cooled lactase** (R: remove lactase) (A: water)

2. This is to show that any glucose produced is due to the presence of lactase;

   Note to marker: Accept glucose replaced with distilled water.

**(iv)** Other than temperature and pH, identify **two** other variables that should be kept the same (standardised) in the investigation. [2]

1. **concentration of lactase used for each way of immobilisation** (R: volume of lactase)**,**

2. **volume of 20 mg $cm^{-3}$ lactose** (R: concentration of lactose?)

3. **time taken for lactose to pass through the column / fixed time for collection of solution that has passed through the column / lactose solution left in (a closed) column for a fixed time;** (R: time for hydrolysis / experiment)

   R:
   - mention about factors in Benedict's test being controlled
   - collecting fixed volume

**(v)** State a risk and precaution. [1]

1. **Benedict's solution** may be a **skin irritant**. **Wear gloves while handling it**;

R:
- Risk of getting scalded by boiling water bath

**(vi)** You are provided with vials G1, G2 and G3.

Each of these vials contain the glucose collected from one of the three ways of immobilising the lactase. Using Table 2.2 and your answer in **(d)(ii)**, deduce which vial contains glucose collected from **A**. Explain your answer. [2]

1. **Vial G2** contain glucose collected from **A**;
2. This is because the **mean total glucose collected is the highest** amongst the three methods, and hence the **time taken for first colour change for Benedict's test** is the **fastest** amongst the 3 vials;

**(e)** Table 2.3 shows the results of a number of statistical tests to find out if the difference in the rates of reaction were significant.

**Table 2.3**

| statistical tests carried out between different ways of immobilisation | | |
|---|---|---|
| **A** and **B** | **A** and **C** | **B** and **C** |
| $p < 0.05$ | $p < 0.05$ | $p > 0.05$ |

**(i)** State the statistical test that was carried out. [1]

**t-test**;

**(ii)** Explain the results shown in Table 2.3. [4]

1. **With $p < 0.05$**, **method A's results** are **significantly different from methods B and C**;
2. The difference in results is **due to the way of immobilisation** / adsorption onto surface of glass beads, and not due to chance alone;
3. **With $p > 0.05$**, **results of methods B and C** are **not significantly different**;
4. The difference in results **is due to chance** alone;

[Total: 22]

**3** **K1** is a slide of a stained transverse section through a plant stem.

You are not expected to have seen **K1**.

**(a)** **(i)** Draw a large plan diagram of the region of the stem on **K1** indicated by the shaded region in Fig. 3.1. Your plan drawing should include at least 1 large vascular bundle and 2 smaller vascular bundles.

Use a sharp pencil.

Use one ruled label line and label to identify the epidermis.

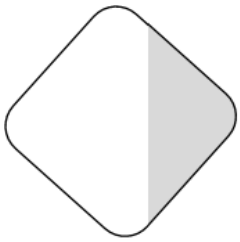
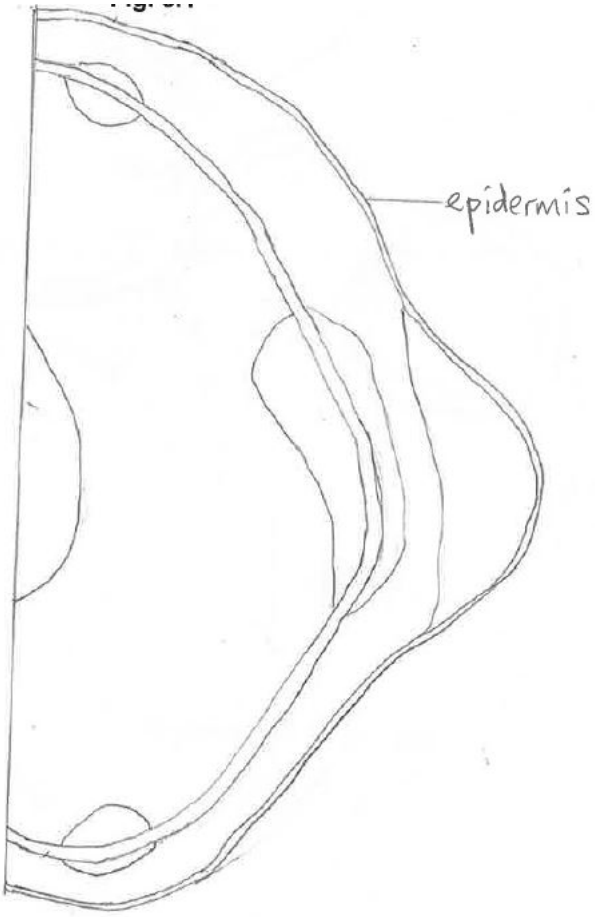
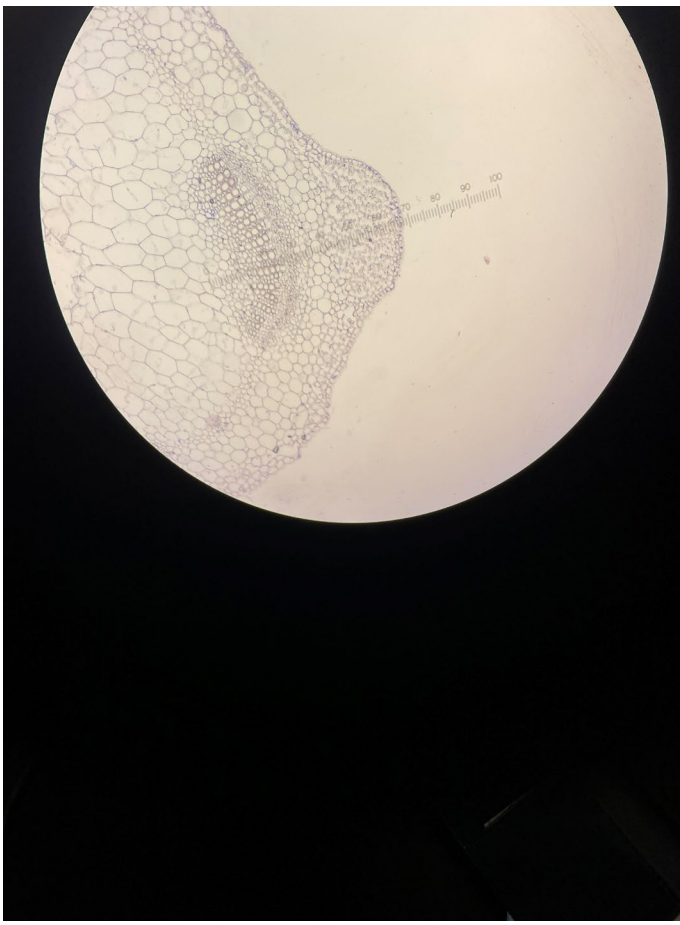


**Fig. 3.1**



[5]

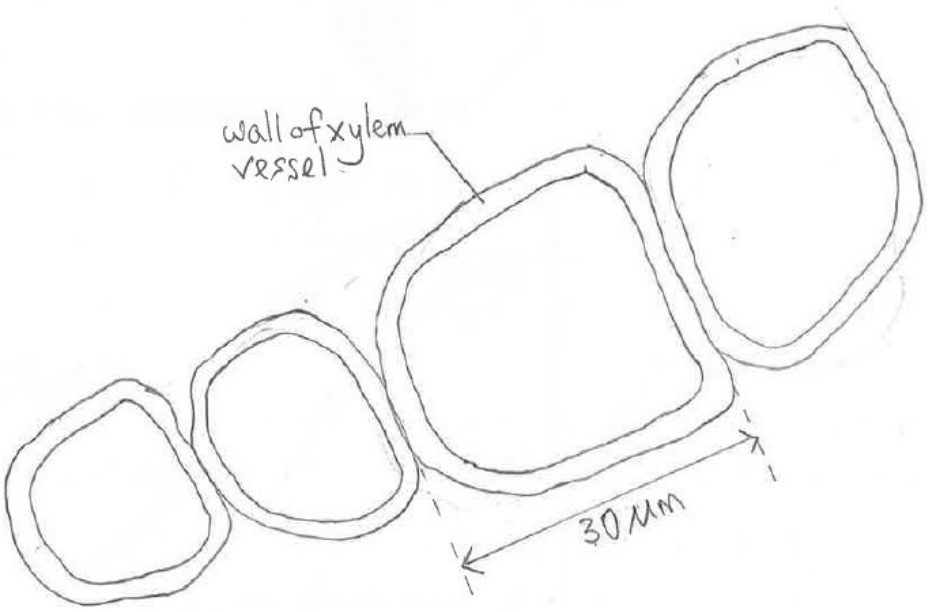
| Plan drawing | |
|---|---|
| **S** | **Smooth**, thin and continuous pencil drawing<br>**Size** of drawing – **suitable size (e.g. at least half of the space given)** |
| **T** | **Title**<br>**Plan drawing of region of stem on K1 (under 10x objective lens)**<br><br>**R:**<br>- 40x objective lens / 400x magnification<br>- 10x optical lens<br>- 10x magnification lens<br>- 10x magnification |
| **D** | **Drawing**<br>**NO cells**<br>**NO shading**<br>**NO ruled lines**<br><br>**Epidermis drawn as two lines**<br>**Correct section of picture drawn**<br>**Correct relative position of vascular bundles**<br>**Must have line to demarcate region of empty pith**<br><br>R:<br>- |
| **A** | **Annotation / label**<br>**Label line (ruled** horizontal **line) + accurate label to epidermis**<br><br>Note to marker: do not mark for “horizontal” |
| **P** | **Proportionate** drawing<br>**Correct proportion of layers drawn** (R: epidermis too thick / thin)<br><br>**Vascular bundle:?**<br>▪ **Shows subdivision of 3 and 2 regions of large and small vascular bundle respectively**<br>▪ **Correct relative proportion of each region in vascular bundle**<br>▪ **Correct shape of vascular bundle (large VB is elongated, and small VB is acorn shaped)**<br><br>**Vascular bundle:?**<br>▪ **divided at least into 3 sections**<br>▪ **shows correct relative proportions of each section** |
| | |

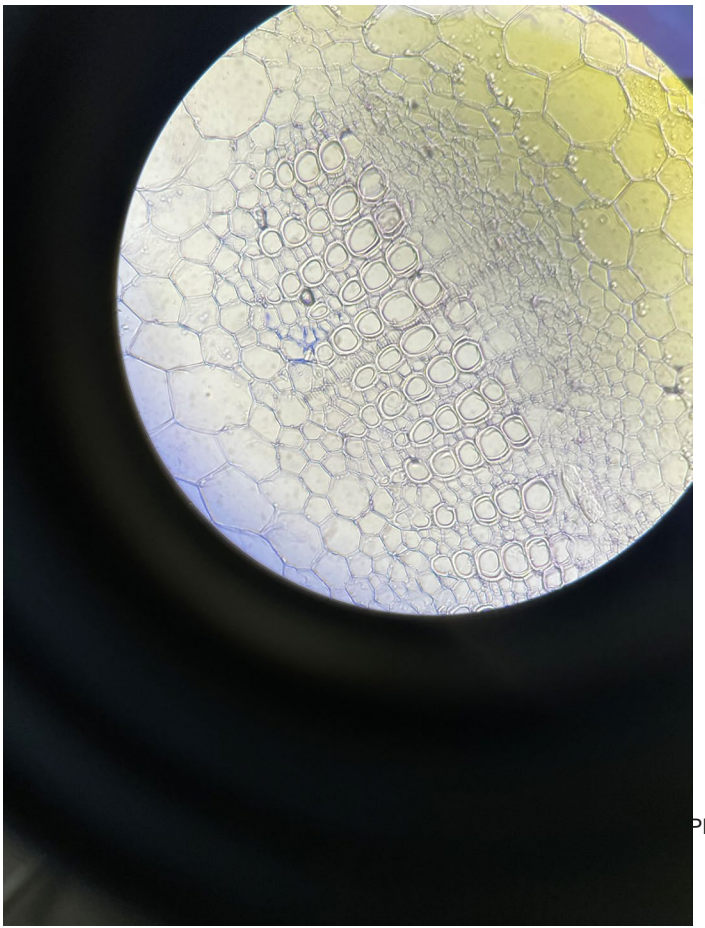
**(ii)** Observe one of the larger vascular bundles on **K1**. The cell walls of a xylem vessel element in the vascular bundle are thickened with lignin.

Select a group of **four** large adjacent xylem vessel elements.

Each xylem vessel must touch at least **one** other xylem vessel element.

- Make a large drawing of this group of four xylem vessel elements.
- Use **one** ruled label line and label to identify the wall of **one** xylem vessel element.
- Include the actual dimension of one xylem vessel element.
- Include the magnification of your drawing and show how this magnification was obtained.



Magnification
$= \frac{5.8\text{ cm}}{30\ \mu\text{m}}$
$= \times 1,933$

[5]

| **Biological drawing** | |
|---|---|
| **S** | **Smooth**, thin and continuous pencil drawing<br>**Scale** of drawing - **drawing should occupy at least half of the given space**<br><br>**Similarity** of drawing to specimen |
| **T** | **Title** (be informative)<br>**Drawing of 4 adjacent xylem vessels viewed under 400X magnification (or 40X objective lens)** A: high power<br><br>R: *just* x40<br>R: x40 magnification (VS objective lens!) |
| **A** | **Annotation / label**<br>- **Label lines are drawn with a ruler and are horizontal**<br>- **Xylem vessel wall is labelled**<br>- **Actual length / diameter of one xylem vessel element should be denoted in micrometer (µm) as part of the drawing + a multiple of 2.5 µm (acceptable range: 20 µm to 60 µm)** |
| **M** | **Magnification** of drawing (with working clearly shown)<br>= **drawing length / actual length** (Both in **µm**)<br><br>**To nearest whole number (R: 3 s.f.)** |
| **P** | **Proportionate** drawing<br>- Xylem vessel element wall drawn using double lines<br>- Thickness of xylem vessel element wall to the entire xylem vessel<br>- Similar size of at least 3 adjacent xylem vessel element relative to each other<br>- Shape of xylem – round / circular |

**(b)** Fig. 3.2 shows a diagram of a stage micrometer scale that is being used to calibrate an eyepiece graticule.

The length of one division on this stage micrometer is 1 mm.

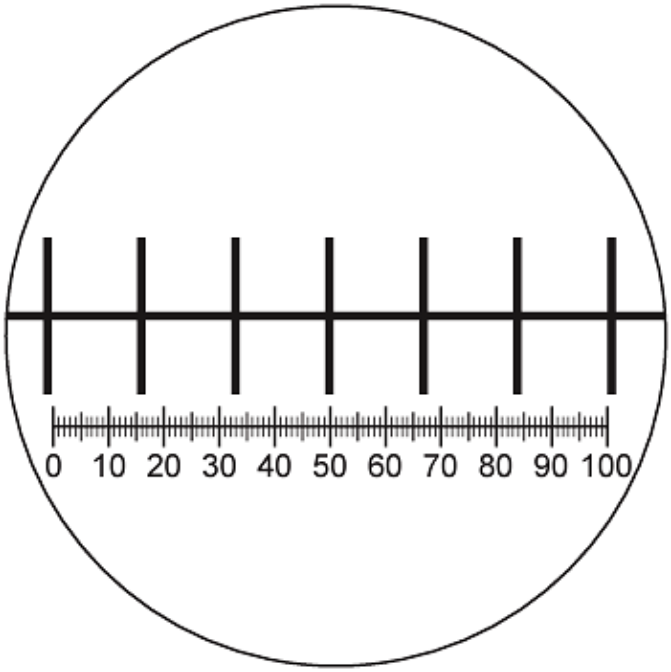



**Fig. 3.2**

**(i)** Use Fig. 3.2 to calculate the actual length of one eyepiece graticule unit.

Show your working.

Length of one eyepiece graticule unit
= 1 mm ÷ 17
= 0.0588 mm
= 58.8 μm

Actual length = ..... [3]

1. Shows division of 1mm by 17;
2. Answer to 3 s.f
3. Appropriate units: μm

Fig. 3.3 shows a photomicrograph of a transverse section of the stem of a different plant to **K1**.
The same microscope and lenses in Fig. 3.2 were used to view this transverse section.
The eyepiece graticule has been placed across the diameter of the stem section.

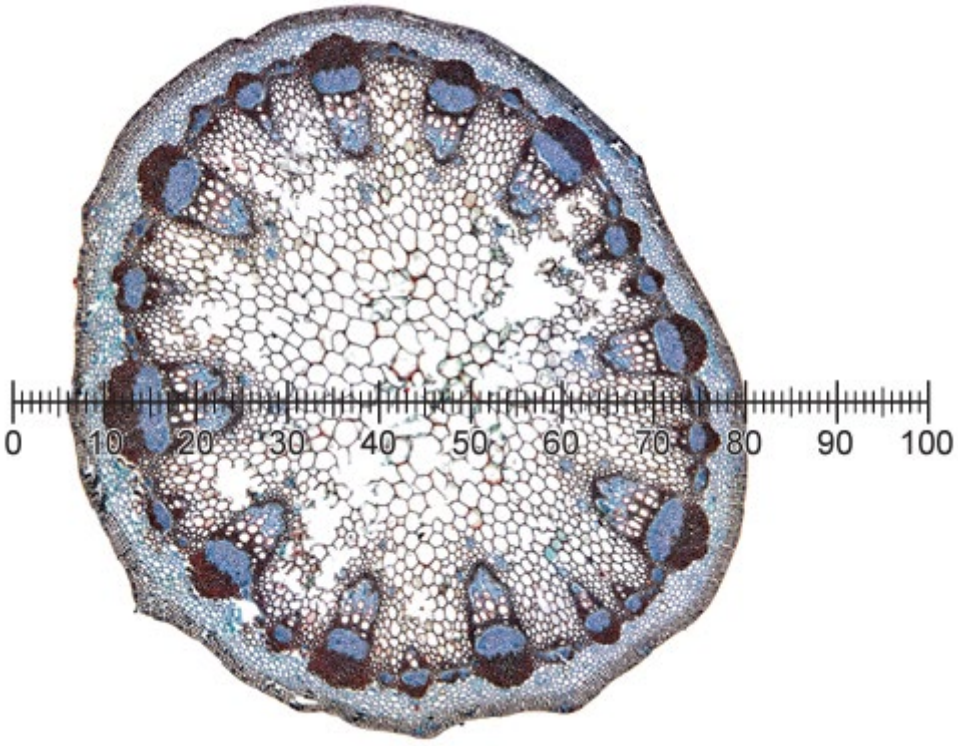



**Fig. 3.3**

**(ii)** Use the calibration of the eyepiece graticule from **(b)(i)** to calculate the actual diameter of the stem section in Fig. 3.3.

Show your working.

Actual diameter
= 74 x 58.8 µm
= 4351.2 µm

Actual diameter = ..... [2]

1. 74 eyepiece graticule units;
2. Shows multiplication of answer from 3(b)(i) by eyepiece graticule units;

**(iii)** Identify **three** observable differences, other than size and colour, between the stem section on **K1** and the stem section in Fig. 3.3.

Record **three** observable differences in Table 3.1.

**Table 3.1**

| feature | K1 | Fig. 3.3 |
|---|---|---|
| Number of vascular bundles | Has lesser vascular bundles | Has more vascular bundles |
| Size of vascular bundle | Smaller | Larger |
| Air space<br>(R: intercellular air space) | Large air space present in the middle | Air space not present |
| Epidermis layer | made up of one layer of cells / lesser layer of cells<br>OR<br>thinner | made up of more layers of cells<br>OR<br>thicker |
| Shape of stem | more squarish (A: square)<br>R: diamond / rectangle | circular / round (A: oval) |

R: have cuticle or not
R: have protrusion from epidermis or not
R: bundle cap thin vs thick
A: bundle cap absent VS present

[3]

[Total: 18]

| feature | K1 | Fig. 2.3. |
|---|---|---|
| shape | triangular | round ; |
| vascular bundle distribution | scattered and at edge | at edge ; |
| vascular bundle shape | circular | oval ; |
| vascular bundle number | more | fewer ; |

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