RIVER VALLEY HIGH SCHOOL

JC 2 PRELIMINARY EXAMINATION

CANDIDATE NAME											
CENTRE NUMBER	S					CLASS		INDEX NUMBER			

H2 BIOLOGY **9744/04**

Paper 4 Practical **24 Aug 2023**

2 hours 30 minutes

Candidates answer on the Question Paper.

Additional Materials: As listed in the Confidential Instructions.

READ THESE INSTRUCTIONS FIRST

Write your Centre number, index number, class and name 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 staples, paper clips, glue or correction fluid.
DO **NOT** WRITE ON ANY BARCODES.

Answer **all** questions in the spaces provided on 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.

At the end of the examination, fasten all your work securely together.
The number of marks is given in brackets [ ] at the end of each question or part question.

Shift
Laboratory

For Examiner's Use	
1	
2	
3	
Total	

Answer **all** questions.

1 Cells contain enzymes that catalyse metabolic activities. Some of these reactions release carbon dioxide.

A scientist engineered a new strain of yeast, **BY62**, which he believes has higher rates of metabolic activities.

You will investigate the release of carbon dioxide from mixtures of yeast and carbohydrate, one comprising a common yeast strain, **Y**, and the other comprising **BY62**. Each mixture is put into a 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 required to:

- perform serial dilution to obtain different concentrations of **P**
- collect results and present it in a suitable table
- estimate the concentration of carbon dioxide in **BY62** mixture

You are provided with:

- 1 g dried yeast, in a test-tube labelled **Y**
- 10.0 % glucose solution, in a container labelled **S**
- Mixture containing carbon dioxide produced from **BY62** and carbohydrate, in a container labelled **P**
- bromothymol blue indicator solution, in a container labelled **B**
- dialysis tubing, in a beaker of distilled water labelled **D**
- distilled water, in a beaker labelled **W**

If any solution comes into contact with your skin, wash off immediately under tap water. Suitable eye protection should be worn.

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

*Before starting the investigation, read through steps **1 – 22** and prepare a table in **(b)(i)**.*

Proceed as follows.

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

2 Put 15 cm^3 of **S** into the test-tube labelled **Y**. Mix well.

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

You will leave the apparatus for 15 minutes. Use this time to continue with **(a)**.

(a) You are required to make a serial dilution of mixture in the container labelled **P**.

P is obtained by subjecting yeast strain **BY62** to the same conditions and experimental step 1 to step 3 as yeast strain **Y**.

You are to reduce the concentration of **P** by **five-fold** between each successive dilutions.

After the serial dilution is completed, you will need to have at least 8 cm^3 of each concentration available for use.

Complete Fig. 1.2 to show how you will dilute **P**.

For each plastic vial:

- state, under the plastic vial, the volume and concentration of **P** in the vial that will be available for use in the investigation, after the serial dilution has been completed
- use one arrow, with a label above the plastic vial, to show the volume and concentration of **P** added to prepare the concentration of **P** in the vial
- use another arrow, with a label above the plastic vial, to show the volume of distilled water, **W**, added to prepare the concentration of **P** in the vial.

[2]

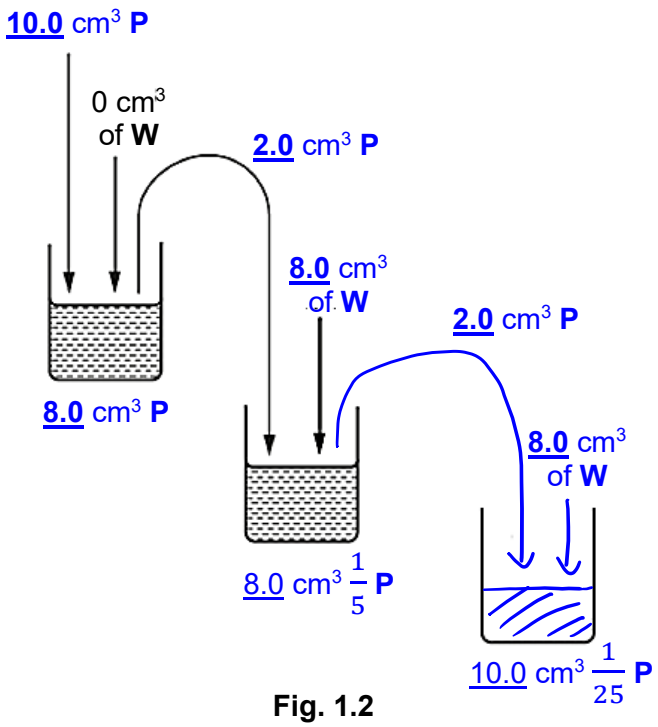


Fig. 1.2

		1. **Ca: Correct calculations for all concentrations of P and W;;** Transfer 2.0 cm^3 of **P** and 8.0 cm^3 of **W** 2. **P: Correct precision for volume transferred;** 1 d.p. 3. **V: Correct volume of P prepared for each concentration;** At least 8.0 cm^3 each of concentration	

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

5 Stir the mixture in test-tube **Y** and pour it into a beaker.

6 Label **two** spotting tiles with the sample times in minutes, as shown in Fig. 1.3.

7 Put 3 drops of **B** onto the spotting tile at each sample time, as shown in Fig. 1.3.

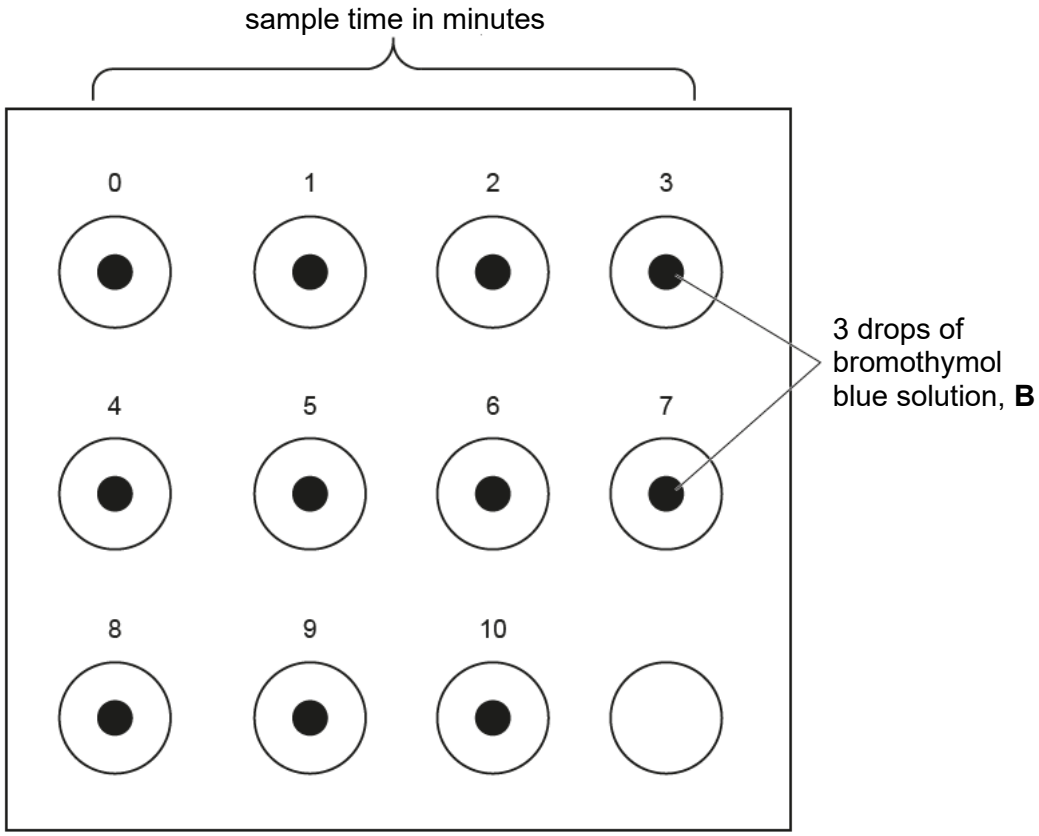


Fig. 1.3

Fig. 1.4 shows the apparatus you will set up for this investigation.

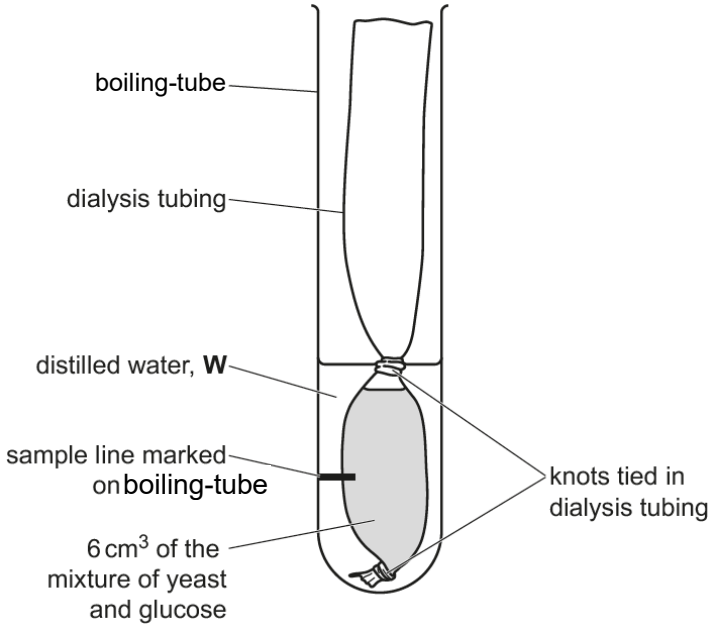


Fig. 1.4

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

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

10 Stir the mixture in the beaker from step 5 and put 6.0 cm^3 of this mixture into a syringe.

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

12 Rinse the outside of the dialysis tubing with distilled water.

13 Repeat steps 8 to 12 using the **final diluted concentration** of mixture **P** obtained in **(a)**.

Look carefully at Fig. 1.4 to help you with step 14 to step 17. You will be performing the following steps for **both** mixtures **Y** and **P at the same time**.

14 Tie a knot just above the level of the mixture in the dialysis tubing, as shown in Fig. 1.4.

15 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. 1.4.

16 Using a syringe, add **15 cm^3** of distilled water, **W**, and ensure that each of the section of dialysis tubing containing the mixtures for **Y** and **P** is fully submerged.

17 Draw a line on the boiling-tube so that it is half-way between the two knots, as shown in Fig. 1.4. This is where you will take your samples from.

18 Start timing and put the boiling-tubes containing the dialysis tubing into the beaker labelled **water-bath**.

19 Take a sample of **W** from the boiling-tube at the point you marked in step 16, using a pipette.

20 Put 4 drops of **W** onto **B** at time 0 on the spotting tile. Put the remaining **W** in the pipette back into the boiling-tube.

21 Mix the sample of **W** and **B** on the spotting tile and immediately record the colour in **(b)(i)**, using the colours stated in Fig. 1.1.

22 Repeat steps 19-21 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.

(b) (i) Record your results in a suitable table. [4]

Table showing the colour for Y and P over time

Sampling time, T / minutes	Colour of Y	Colour of P
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

1. **T: Title;;**
   "Table showing colour of **Y** and **P** over time

2. **H&U: Heading with units;;**
   IV: Sampling time, T / minutes
   DV: Colour

3. **D: Data;;;;**
   Colour for each minute for 10 minutes or until end-point is reached for two consecutive times;
   Uses only colours from Fig. 1.1;
   Colour recorded for both **Y** and **P**;;

Table 1.1 shows the concentration of carbon dioxide present in a mixture if the end-point was detected at the sampling time.

Table 1.1

sampling time / minutes	1	2	3	4	5	6	7	8	9	10
concentration of carbon dioxide in mixture / mol dm^{-3}	4.6	2.1	1.4	0.9	0.6	0.2	0.07	0.039	0.005	0.001

(ii) Using your results in **(b)(i)**, estimate the concentration of carbon dioxide produced by **BY62** mixture in the container labelled **P**.

Show your workings clearly. [2]

			End-point of P is X minutes, therefore its carbon dioxide concentration is between _____ mol dm^{-3} and _____ mol dm^{-3} **The upper estimate concentration of CO_2 in BY62 mixture is** **= 5 x 5 x upper estimate** **= _____ mol dm^{-3} (same precision given in table) ;;** **The lower estimate concentration of CO_2 in BY62 mixture is** **= 5 x 5 x lower estimate** **= _____ mol dm^{-3} (same precision given in table) ;;** **The carbon dioxide concentration produced by BY62 is between _____ mol dm^{-3} and _____ mol dm^{-3}**	
		(iii)	Explain the trend observed in Table 1.1 in relation to a named metabolic activity of the yeast cells.	[4]
			1. As sampling time increased from 1 minute to 10 minutes, the concentration of carbon dioxide present in mixture decreased from 4.6 mol dm^{-3} to 0.001 mol dm^{-3} ;; **The more metabolically active the yeast cells,** **2. The higher rate of fermentation of glucose;** **3. More pyruvate converted to ethanol per unit time;** **OR** **4. The higher rate of aerobic respiration of glucose;** **5. Higher rates of oxidative decarboxylation in link reaction & Krebs cycle;** **6. The larger volume of carbon dioxide released per unit time (award once);** **7. The faster; carbon dioxide diffuses from yeast cytoplasm to outside of yeast cell across cell surface membrane / and across dialysis tubing into W;** **8. From region of higher concentration to region of lower concentration / down concentration gradient;** **9. Resulting in shorter sampling time to reach end-point;** **Max 3m**	
	(c)	An accumulation of dissolved carbon dioxide in yeast cells is toxic. Explain how the change in pH affects the rate of carbon dioxide produced.		[3]
			Accumulation of dissolved carbon dioxide **1. Results in higher acidity/decreased pH/higher concentration of H^+ ions;** **2. Disrupts ionic bonds and hydrogen bonds;**	

		3. Results in enzymes losing specific 3-dimensional conformation; **4. Active site no longer complementary to substrate;** **5. Fewer enzyme-substrate complexes formed per unit time;** **6. Less carbon dioxide produced per unit time;** **7. Enzyme is denatured;**	
	(d)	This investigation used colour to indicate the concentration of carbon dioxide in the sample. Complete Table 1.2 to: • identify **one** significant source of error in the procedure • identify **one other** significant source of error in this investigation • suggest how to make improvements to reduce these sources of error.	[4]
		Table 1.2 significant source of error / how to make an improvement 1. **Visual determination of end-point colour (yellow) is subjective;; OR Visual determination of colour (yellow) to determine the end-point is subjective;;** / **Use a colourimeter to more precisely determine when end-point colour has been reached;; OR Use a colour chart to compare and more precisely determine when end-point colour has been reached;;** 2. **End-point colour may be present before sampling time is reached;;** / **Use smaller intervals between sampling time, example 30s;;** 3. **Size of drops of W is not constant;;** / **Use a syringe to add the same volume of W to B, example 1 cm^3;;** 4. **Sampling time for Y & P is not the same at 1 minute interval;;** / **Carry out experiment for Y & P individually so that sampling time occurs at exactly 1 minute interval;;** 5. **AVP;;** Any two.	
	(e)	Describe a suitable control for the investigation conducted.	[1]
		1. A negative control is subjected to the same factors as that for the experiment; **2. except that yeast is replace by an equivolume of distilled water / boiled and cooled yeast;**	

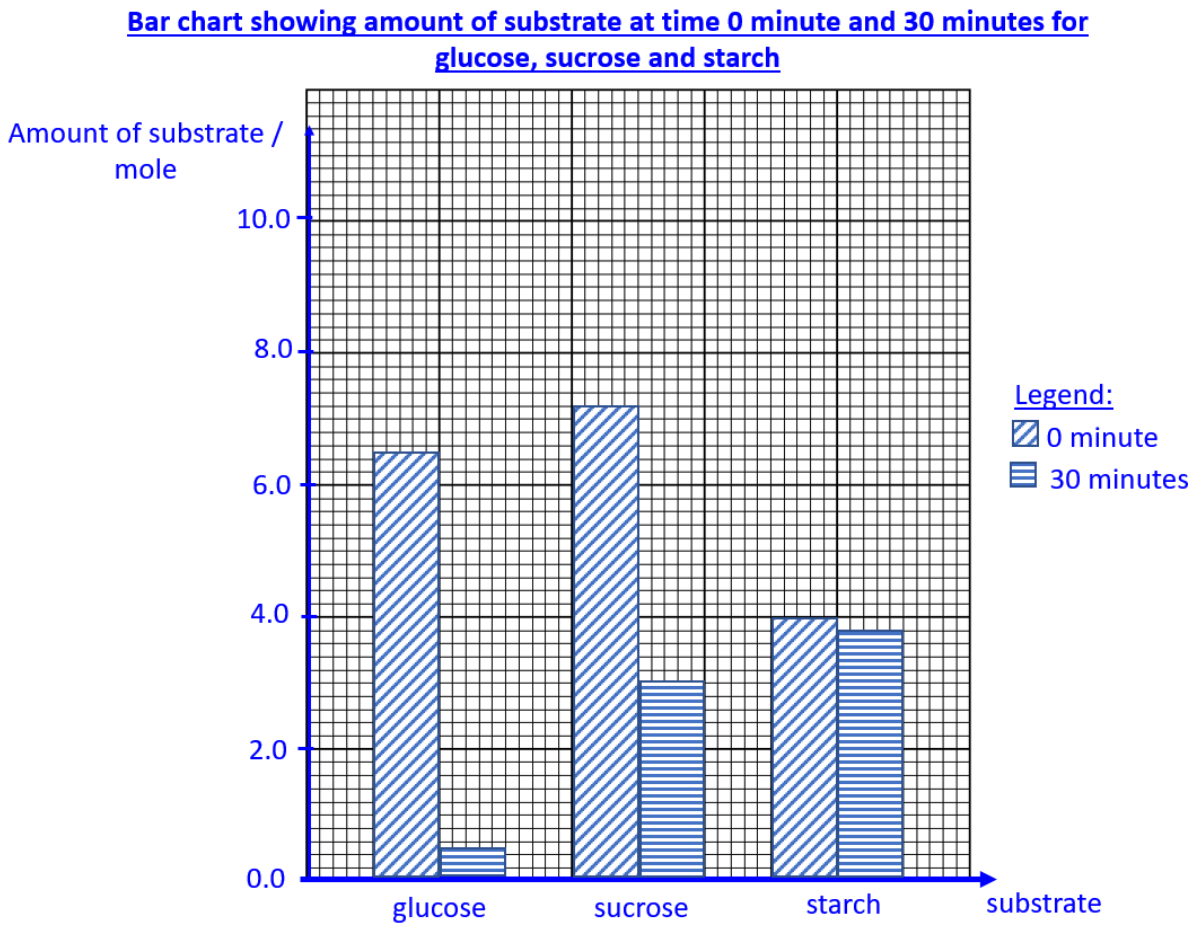
(f) A student repeated the investigation with yeast strain **BY62** using the same procedure but with different types of substrates. To identify which substrate is most effectively used by yeast, the student measured the amount of substrate at the start and at the end of the experiment duration of 30 minutes.

The results are shown in Table 1.3.

Table 1.3

substrate	amount of substrate at time / mole	
	0 minute	30 minutes
glucose	6.5	0.5
sucrose	7.2	3.0
starch	4.0	3.8

(i) Using an appropriate method, present the data shown in Table 1.3 on the grid provided. [4]



			1. Title (T); "Bar chart showing amount of substrate at time 0 and 30 minute for glucose, sucrose and starch" **2. Heading with Units (H&U);;** x-axis: "Substrate" y-axis: "Amount of substrate / mole" **3. Precision (P);** x-axis: nil y-axis: 1 d.p. **4. Scale (S);** Graph takes up at least 50% of grid Divisions are equidistant Y-axis must start from zero **5. Plot points (PP);;** Correct points plotted **6. Type of graph;** Bar chart (gaps between bars, same thickness for all bars) Chart for the same substrate is grouped together	
		(ii)	From the results in Table 1.3, the student concluded that glucose is the substrate that releases the most carbon dioxide in 30 minutes by yeast strain **BY62**. Comment on the validity of the student's conclusion.	[2]
			1. Conclusion is not valid;; **2. Substrate amount present at the beginning is not the same, therefore no basis for comparison / confounding factor;;** **OR** **3. The amount of substrate absent from the mixture may not be equivalent to amount of substrate used and subsequently releases carbon dioxide;;** **OR** **4. Starch and sucrose consist of more than one glucose monomer, therefore amount of carbon dioxide released by 0.2 mole of starch is more than that of 6 moles of glucose;;** **5. AVP;;** **Max. 1m**	
				[Total: 26]

2 The rate of photosynthesis in plants is affected by the concentration of carbon dioxide.

A student proposed two different methods to determine the effect of carbon dioxide concentration on the rate of photosynthesis in Brazilian waterweed, *Egeria densa*. This plant is found in freshwater ponds.

In **Method 1** proposed by the student, the rate of photosynthesis is to be measured by the set-up shown in Fig. 2.1. A stand and clamp were used to hold the apparatus in a vertical position.

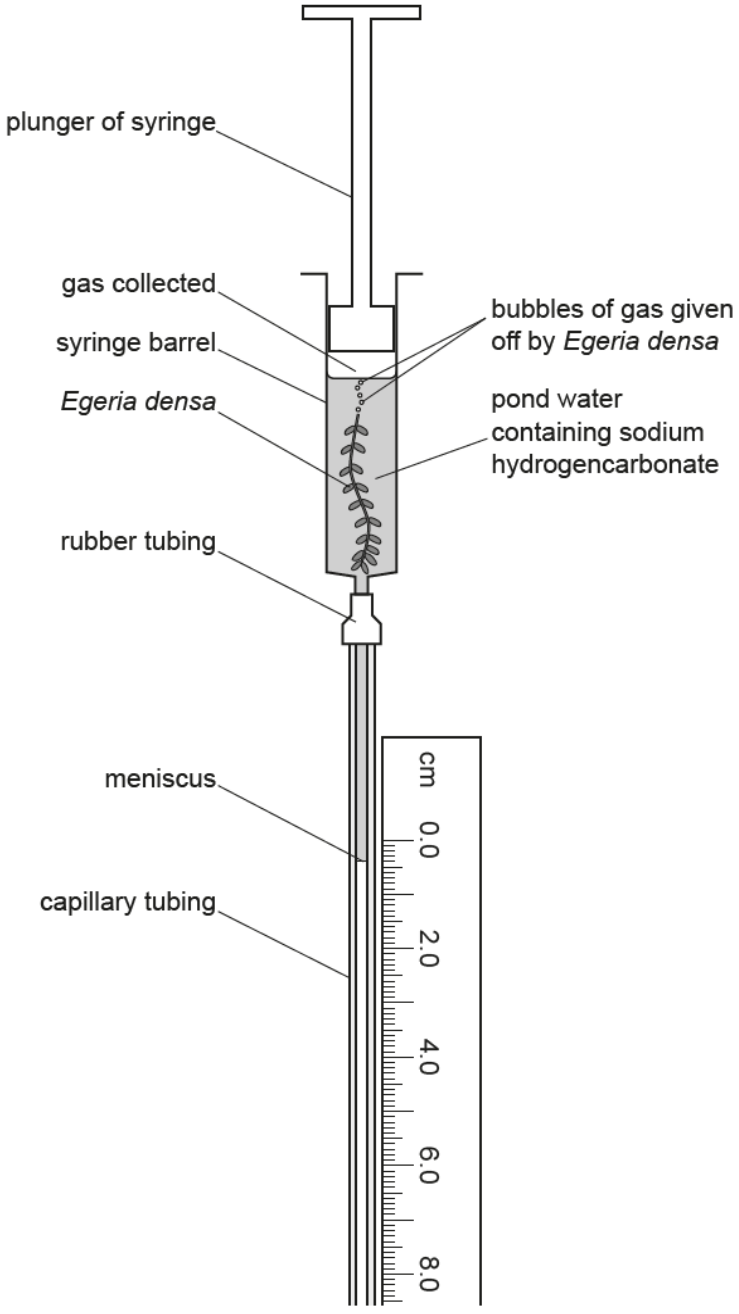


Fig. 2.1

(a) Use the information provided to plan a method by which the student could use in his investigation.

In your plan, you must use:

- *E. densa* plant that has been kept in the dark for at least 24 hours
- 1.0% sodium hydrogen carbonate solution
- distilled water
- apparatus shown in Fig. 2.1

You may also select from the following apparatus and use appropriate additional apparatus:

- any normal laboratory glassware e.g. test tubes, beakers, measuring cylinders, graduated pipettes, glass rods, etc.
- syringes
- pipette fillers
- ruler
- timer, e.g. stopwatch
- 60W bench lamp

Your plan should:

- have a clear and helpful structure such that the method you use is able to be repeated by anyone reading it
- identify the independent variable, dependent variable and the variables that you will need to control
- include details to ensure that results are as accurate and reliable as possible
- use the correct technical and scientific terms
- include reference to safety measures to minimise any risks associated with the proposed experiment

[9]

Independent variable	**Concentration of sodium hydrogen carbonate solution – 1.0%, 0.8%, 0.6%, 0.4%, 0.2%**
Dependent variable	**Distance moved by meniscus over 3 mins**
Controlled variables	**1. Light intensity as determined by distance of lamp from syringe, 10.0 cm**
	2. Volume of sodium hydrogen carbonate solution, 10.0 cm^3
	3. Duration to observe distance moved by meniscus, 3 min

	4. Temperature of experimental setup, 37 °C
	5. Length of waterweed, 7.0 cm

Suggested procedure

1. Place waterweed into syringe
2. Add 10.0 cm^3 of 1.0% sodium hydrogen carbonate
3. Insert plunger
4. Place the lamp 10 cm away from the syringe
5. Switch on the lamp
6. Leave set up for 2 mins to observe movement by meniscus
7. Pull plunger to draw meniscus back to starting position
8. Start the stop watch
9. Stop timing after 3 min and record distance moved by meniscus
10. Repeat the steps using other concentrations of sodium hydrogen carbonate

Accurate & reliable results

11. Perform two replicates and two repeats with new reagents. Carry out a t-test to determine whether there is any significant difference between the means.
12. Decrease the intervals of the concentration of sodium hydrogen carbonate solution to obtain more data for accurate determination of the rate of photosynthesis.

Control

13. Set up a negative control

    A negative control is subjected to the same factors as that for the experiment except that sodium hydrogen carbonate is replaced by an equivolume of distilled water.

    It is expected that the meniscus will not move regardless of the incubation time.

    This proves that it is indeed the sodium hydrogen carbonate that causes the meniscus to move.

Risk assessment and precautions taken

14. Handling lamp with wet hands may cause electrocution. Handle lamp with dry hands.
15. Bulb of lamp is hot and may cause scalds / burns. Do not touch with bare hands.
16. Broken capillary tubes may cause cuts. Handle with care.

Mark scheme

1. **(Independent variable) Concentration of sodium hydrogen carbonate solution – 1.0%, 0.8%, 0.6%, 0.4%, 0.2%;;**
2. **(Dependent variable) Distance moved by meniscus over 3 mins;;**
3. **(Controlled variable 1) Ref to standard volume (e.g. 10 cm^3) of 1% sodium hydrogen carbonate;;**
4. **(Controlled variable 2) Ref to fixed distance of lamp from syringe (e.g. 10 cm);;**
5. **Ref to linear dilution to obtain different concentrations of sodium hydrogen carbonate;**
6. **Ref to allow time for equilibration;**
7. **Switch on the lamp and start the stopwatch;**
8. **Repeat the steps using different concentrations of sodium hydrogen carbonate;**
9. **Conduct replicates and repeats with new reagents;**
10. **Decrease intervals of sodium hydrogen carbonate concentration;**
11. **Set up a negative control where sodium hydrogen carbonate is replaced by an equivolume of distilled water;**
12. **It is expected that the meniscus will not move regardless of the incubation time. This proves that it is indeed the sodium hydrogen carbonate that causes the meniscus to move;**
13. **2 risks and precautions;;**

You will now perform **Method 2**, proposed by the student, to determine the effect of carbon dioxide concentration on the rate of photosynthesis. In this method, you will investigate the sugar content in 2 leaf extract samples from the same plant, **S1** and **S2**. Each sample was obtained from leaves after exposure to different carbon dioxide concentrations.

Before proceeding further, use the plastic container labelled **hot water** to collect approximately 300 cm^3 of hot water from where it is provided in the laboratory. Heat the water to a suitable temperature for use in the Benedict's test.

Suitable eye protection must be worn during heating.

Proceed as follows.

1 Add 2 cm^3 of **S1** and **S2** to two separate test tubes.

2 Add 2 cm^3 of Benedict's reagent to each of the samples and heat them in a boiling water bath for 2 minutes.

(b) (i) Record, in Table 2.1, your observations for **S1** and **S2**. [1]

Table 2.1

sample	observation	carbon dioxide concentration
S1	**Blue solution / suspension with a red tinge observed. Small amount of red ppt formed upon settling ;**	**low;**
S2	**Red suspension observed. Moderate amount of red ppt formed upon settling ;**	**high;**

(ii) Complete Table 2.1 to state the concentrations of carbon dioxide at which **S1** and **S2** were initially exposed to, by using the terms 'high' and 'low'. [1]

(iii) Explain your answer in **(b)(ii)**. [2]

At higher carbon dioxide concentration present / maintained

1. **More carbon dioxide combines with ribulose bisphosphate per unit time;**
2. **More glyceraldehyde-3-phosphate (GALP) produced per unit time;**
3. **More glucose produced;**
4. **Larger amount of precipitate observed in Benedict's test;**

(c) Out of the two methods proposed by the student, state which method is less suitable to determine the effect of carbon dioxide concentration on the rate of photosynthesis.

Give a reason for your answer. [2]

1. **Method 1;;**
2. **measures rate of photolysis / light-dependent /; OWTTE**
3. **independent of carbon dioxide concentration / excess NADP or not limiting (during this period); OWTTE**

[Total: 15]

3 During this question you will require access to a microscope and slide **L1**.

L1 is a slide of a stained transverse section through a plant stem.

You are not expected to be familiar with this specimen.

(a) Select a field of view so that you can observe the different tissues as shown by the shaded area in Fig. 3.1.

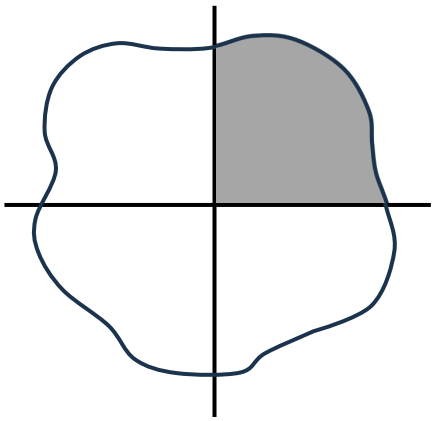


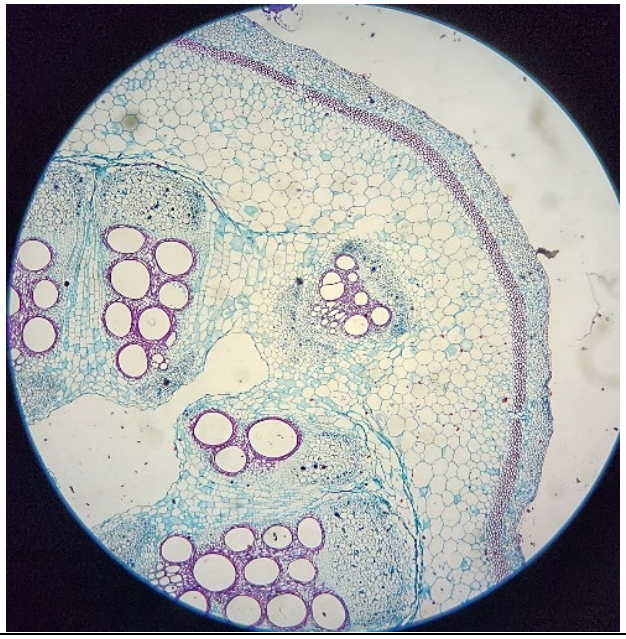
Fig. 3.1

(i) Draw a large plan diagram of the region of the stem on **L1** shown by the shaded area in Fig. 3.1. A plan drawing shows the arrangement of different tissues. Individual cells must **not** be drawn in plan diagrams.

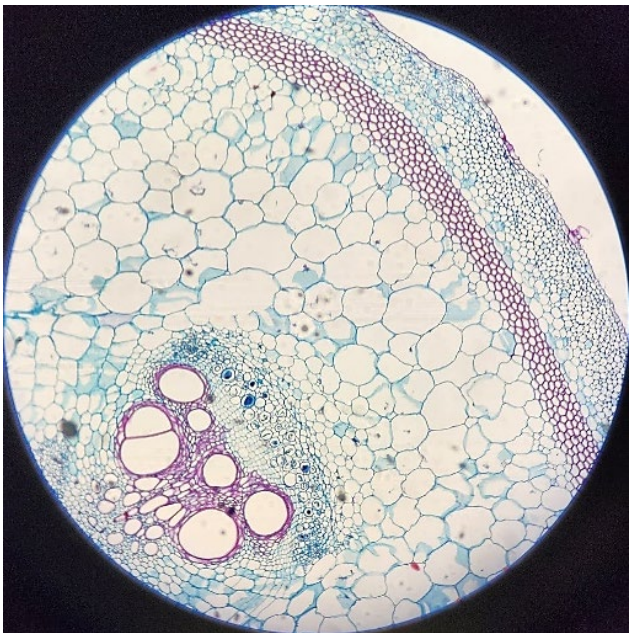
Your drawing should show the correct shape and proportion of the different tissues.

Select **one** vascular bundle to include in your drawing.

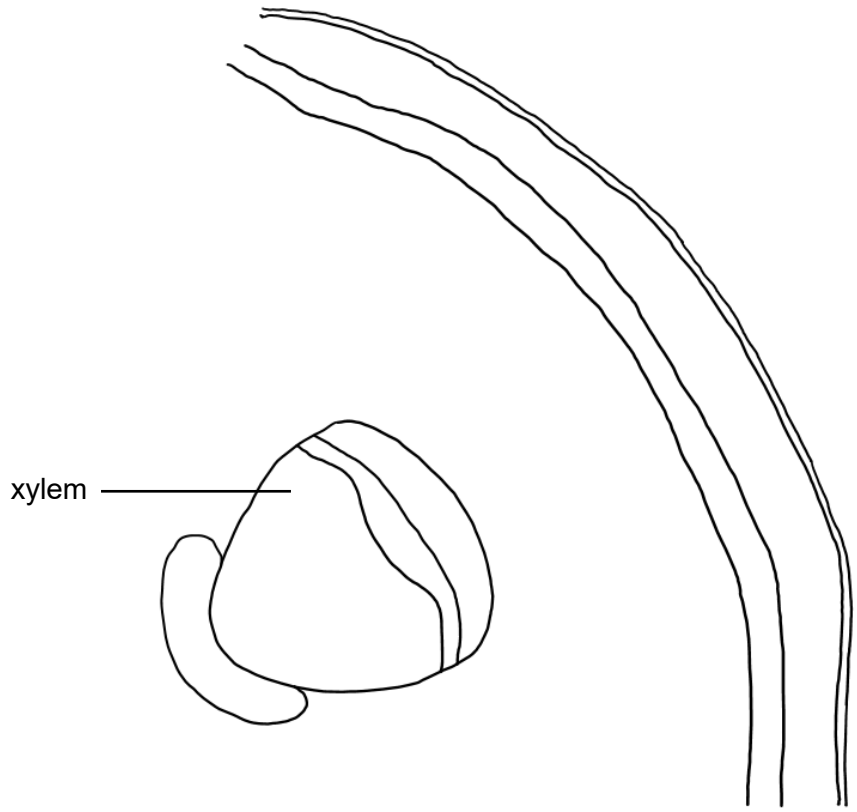
Use **one** ruled label line and label to identify the xylem.



[4]



<table>
<tr>
<td></td><td></td><td></td>
<td>
<u>Plan drawing of plant stem (t.s.) (x100.0)</u>



T: Title;
“Plan drawing of plant stem (t.s.) (x100.0)”

P: Proportion;;
P1: ratio of epidermis to stem radius = 1:10
P2: ratio of vascular bundle to stem radius = 1:4 to 1:3

S: Structures;;
S1: min. 5 different tissues – epidermis, cortex with 3 separate layers (collenchyma, parenchyma, sclerenchyma), vascular bundle
S2: vascular bundle – <u>2 layers</u> of phloem, xylem

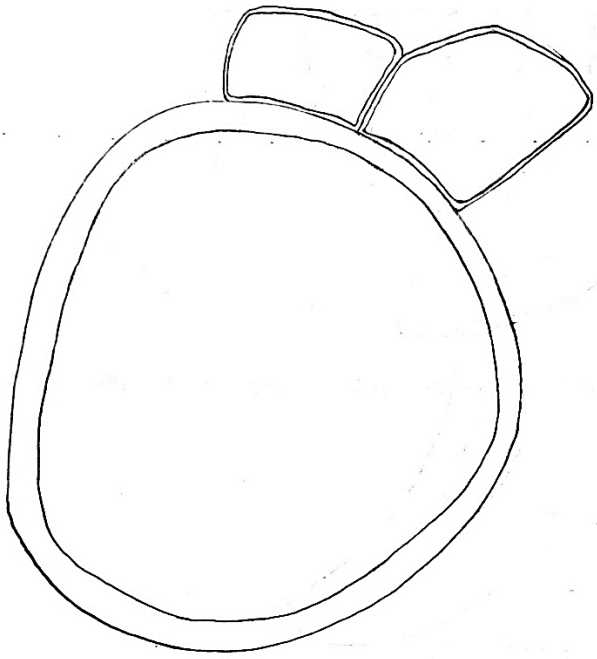
L: Line;
Continuous lines (i.e. without gaps)
Smooth line of consistent thickness

La: Label;;
Correct tissue labelled as xylem
</td>
<td></td>
</tr>
<tr><td></td><td></td><td></td><td></td><td></td></tr>
<tr>
<td></td><td></td><td>(ii)</td>
<td>Observe the xylem vessel elements in the stem on L1.

Select one large xylem vessel element and two adjacent, touching, smaller cells.

Each smaller cell must touch the xylem vessel element and the other smaller cell.

Make a large drawing of this group of three cells.</td>
<td></td>
</tr>
</table>



P: Proportion;;
P1: ratio of xylem wall to cell wall thickness = 3:1
P2: cell wall thickness <3% of cell width

S: Shape;;
S1: correct shape of xylem vessel
S2: correct shape of adjacent cells

CL: Cell layout;;
CL1: only 3 cells drawn as per instruction
CL2: cells are touching each other and the xylem vessel

L: Line;;
Continuous lines (i.e. without gaps)
Smooth line of consistent thickness

If drawing size <70mm penalise ½ mark

Note: not awarding if wrong cells drawn [4]

(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.

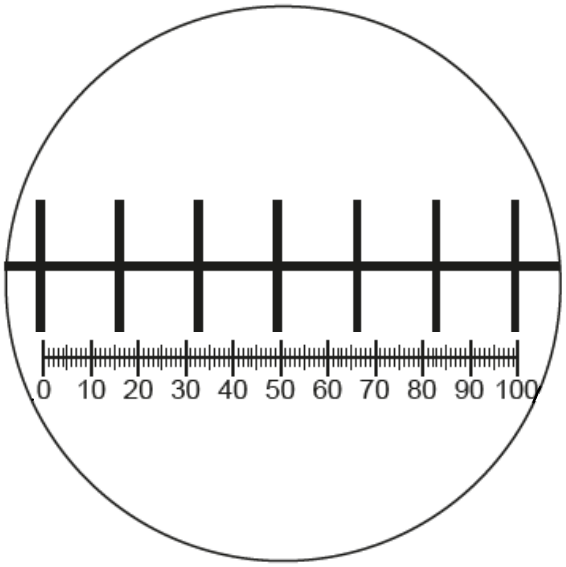


Fig. 3.2

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

100 eyepiece graticule units = 6 stage micrometer units
100 eyepiece graticule units = 6 mm on stage micrometer
1 eyepiece graticule unit = 6 mm / 100;
= 0.06; mm; OR 60; μm;

actual length = _____ [2]

Fig. 3.3 is a photomicrograph of a stained transverse section through a root of a different plant species. This was taken with the same microscope and lenses used to take Fig. 3.2. The eyepiece graticule has been placed across the diameter of the section.

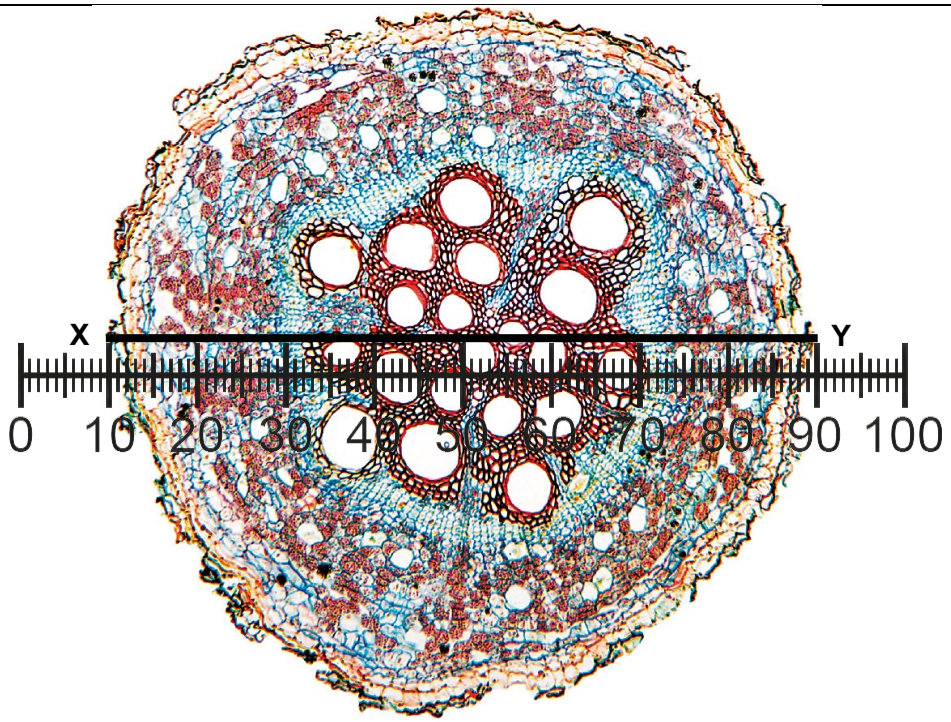


Fig. 3.3

(ii) The line **X–Y** is drawn across the diameter of the root section.

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

Show your working.

Number of eyepiece graticule units of diameter of root = 80;;

Actual diameter = 80 x 60 μm; = 4800 μm;

actual diameter = _____ [2]

(iii) Identify the observable differences between the root section in Fig. 3.3 and the stem section on **L1**.

Record the observable differences in an appropriate table. [2]

Table showing differences between root section in Fig. 3.3 and L1

feature	Fig. 3.3	L1
location of vascular tissue	**center of root**	**scattered around periphery of stem;;**
arrangement of vascular bundle	**clustered in the middle of the root**	**arranged in a ring;;**
number of vascular bundle	**one**	**many;;**

hollow centre	**absent (center of root is packed with cells)**	**present;;**	
overall shape	**circular**	**irregular;;**	

Any two

[Total: 14]