

BIOLOGY **Higher 2**

9744/04 17 August 2023

Paper 4 Practical

2 hours 30 minutes

Candidates answer on the Question Paper. Additional Materials: As listed in the Confidential Instructions

READ THESE INSTRUCTIONS FIRST

Write your class, index number 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.

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

> For Examiner's Use 1 2 3 Total

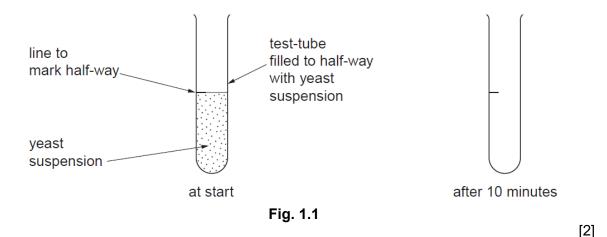
Shift

Laboratory

This document consists of 16 printed pages.

Answer **all** questions.

- 1 There are molecules on the surface of yeast cells which cause the cells to stick together. When a yeast suspension is placed in a test-tube some of the cells sink slowly to the bottom.
 - (a) (i) Show clearly on Fig. 1.1 what you would expect the contents of the test-tube to look like after 10 minutes. You will gain marks for clear annotations.



- 1. Line draw level with half-way mark + more yeast drawn towards bottom of tube or another line to show a separate region ;
- 2. One annotation/label/description e.g. yeast cells sink ;

You are required to investigate the effect of the independent variable, pH, on the sedimentation of a yeast cell suspension over a period of 10 minutes.

(ii) State the time intervals you will use and what you will use the graph paper scale to measure.

......[2]

- 1. Uses 10 minutes + one/two minutes intervals ; R: if does not divide into 10 e.g. 3 minutes
- 2. Measures or describes measuring e.g. (use graph paper) to find height + description of what is measured e.g. from halfway mark to top of sediment or bottom of tube to top of sediment/AW ;

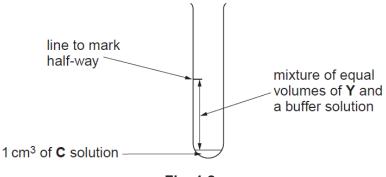
You are provided with:

- a suspension of yeast cells, Y, in a beaker labelled Y
- calcium chloride solution, C, in a container labelled C
- buffer pH 3, pH 4, pH 5 and pH 6 in containers labelled pH 3, pH 4, pH 5 and pH 6.

Proceed as follows.

- **1** Label one test-tube for each pH.
- **2** Use the marker provided to mark a line half-way along the length of each test-tube as shown in Fig. 1.1.

You will need to put 1 cm³ of **C** in each test-tube, and then an equal volume of **Y** and each buffer solution so that the mixture will fill the test-tube to the half-way mark as shown in Fig. 1.2.





(iii) Use the test-tube labelled **T** and the other apparatus provided and decide on the volume of **Y** and the volume of each buffer solution to use.

Describe all the steps you used to work out the volumes.

......[1]

1. Describes all following steps: add 1 cm³ C + measure volume up to the halfway line + divides volume by half ;

State the volume of Y and the volume of each buffer solution to use.

volume of Y4.5 cm³ (4-4.5 cm³).....

volume of each buffer solution

(10cm³ is more than half of test tube)

[1]

1. Volume of Y equal to volume of buffer AND cm³ on both ; Allow up to 1 decimal place i.e. to 0.1 cm³

- **3** Put the volume of buffer solution labelled **pH 3**, stated in (iii), into the test-tube labelled **pH 3**.
- 4 Put 1 cm^3 of **C** to the same test-tube.
- 5 Repeat steps 3 and 4 with each of the other buffer solutions **pH 4**, **pH 5** and **pH 6**.
- 6 Stir the yeast suspension **Y** with a glass rod.
- 7 Put the volume of **Y**, stated in (iii), into each test-tube to make the total volume up to the half-way mark.
- 8 Put a bung or cork into the test-tube and invert the test-tube twice to mix well. Repeat with each test-tube.
- **9** Immediately start timing. At your selected times, record your observations. You may need to lift each test-tube to eye level to take each reading. Take care not to disturb the contents of the test-tube.
 - (iv) Prepare the space below and record your observations.

Table showing the length of halfway mark to top of sediment OR bottom of tube to top of sediment at each time interval

рН	time / minutes	height / mm	OR <mark>height / mm</mark>
	2	3	37
	4	4	36
3	6	6	34
	8	9	31
	10	11	29
	2	2	38
	4	3	37
4	6	4	36
	8	7	33
	10	10	30
	2	1	39
	4	2	38
5	6	2	38
	8	3	37
	10	3	37

	2	1	39
	4	1	39
6	6	1	39
	8	1	39
	10	1	39

OR

time/min	height / mm at each pH			
	рН 3	pH 4	pH 5	рН 6
2				
4				
6				
8				
10				

- 1. Table with all cells drawn with suitable column / row headings with correct units; pH (at top or left), time / min(utes), height/length/depth/AW with mm R: if units anywhere else except headings
- 2. Collects results for all four pH (ECF if alr penalised in (a)(ii) regarding number of data) ;
- 3. Recording height to correct precision (whole number) ;
- 4. Different results/observations for different pH (minimum 2 pH) for final set of data at 10 min ;

[4]

- (v) Use your results to state the effect of pH on the yeast suspension.
 -[1]
- 1. (yeast settles) <u>more / higher rate</u> of sedimentation at some pH OR correct example of pH with results ; R: activity OR
- 2. According to candidates' results e.g. As pH increases from pH 3 to pH 6, rate of sedimentation increases / there is more sedimentation ;
- (vi) Identify two significant sources of error in this experiment. [2]
- 1. Difficulty in measuring/determining/judging the length/boundary/top of layer (due to bubbles on surface)
- 2. Difficulty in lining up the graph paper with the test tube (which might be different for each reading) ;
- 3. Difficulty in taking readings/adding solutions/mixing solutions at the same time for different pH solutions (so timing was different);

- 4. CV: Hold test-tube vertically in retort stand/attach graph paper to test-tubes ;
- 5. Carry out repeats or replicates ;
- (viii) Suggest a suitable control that could have been set up for this investigation. [1]
- 1. Replace pH solutions with equal volume / <u>(according to (a)(iii)</u> cm³ of distilled water ;

[Total: 17]

2 Fig. 2.1 is a photomicrograph of a transverse section through part of a stem.

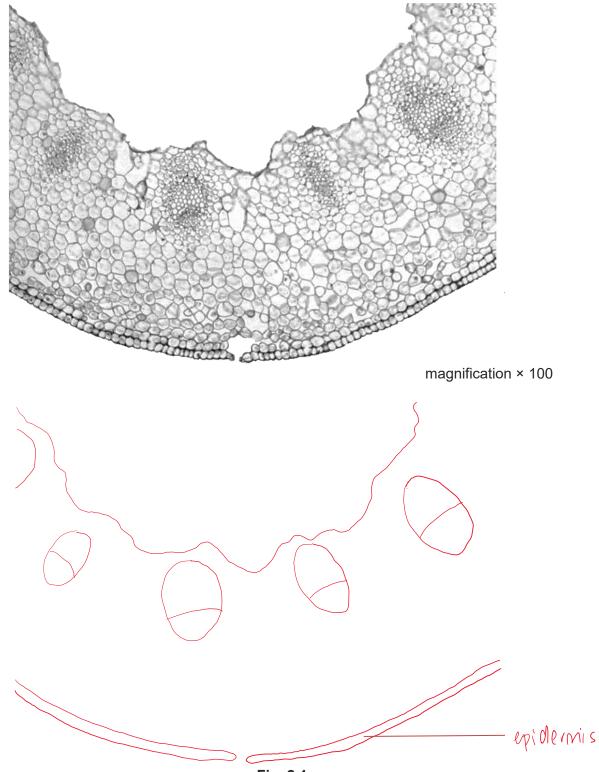


Fig. 2.1

(a) Draw a large plan diagram of the specimen shown in Fig. 2.1.

A plan diagram shows the arrangement of different tissues. Your drawing should show the correct shape and proportions of the different tissues.

No cells should be drawn.

Label the epidermis.

- 1. Clear, sharp, unbroken lines + no shading + suitable size (larger than 50mm across top to bottom of arc);
 - R: draw over print
- 2. Draws only outline of tissues with one inner layer (irregular shape) + no cells ;
- 3. Drawing accuracy
 - at least 3 lines
 - 4.5 complete vascular bundles
 - vascular bundles with xylem & phloem
 - stoma drawn as gap at lowest point of epidermis
- 4. Correct proportion of tissues drawn ;
- 5. correct label with ruled label line to epidermis ;

[5]

Fig. 2.2 is a photomicrograph of a transverse section through part of a different plant organ from a different plant species.

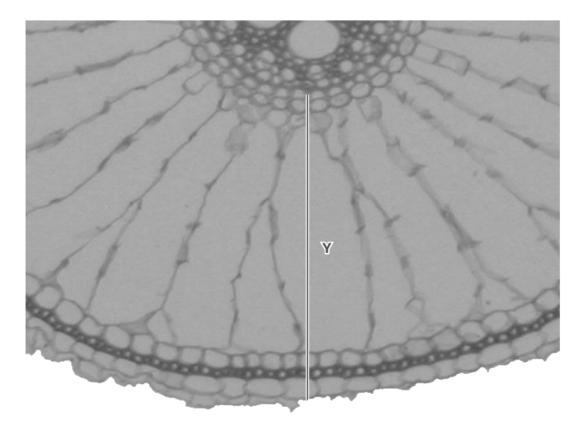


Fig. 2.2

(b) (i) Prepare the space below so that it is suitable for you to record **two** observable differences between the specimens in Fig. 2.1 and in Fig. 2.2.

Record your observations in the space you have prepared.

1. Table drawn with Fig. 2.1 and Fig. 2.2 as headings ;

	Feature	Fig. 2.1	Fig. 2.2
2.	vascular bundle	bundles / more / separate	(no) bundle / one / less ;
	/ xylem / phloem	more than one	only one ;
		near middle / edge	middle / centre ;
3.	hollow centre (pith)	present	absent ;
4.	air spaces /	smaller / less	larger / more ;
	shape of cells	round / circular	long ;
		R: 3D shapes such as spherical	_
5.	epidermis	regular / smooth	irregular / rough ;
		thinner / 2 layers	thicker / 3 layers
6.	gap / stomata / guard cells	present / one	absent / none ;
7.	number of cells	more	less

[3]

(ii) The actual length of line Y is 495 μ m. Use this measurement to calculate the magnification of Fig. 2.2.

You may lose marks if you do not show your working or if you do not use appropriate units.

Measured length of line Y = <u>81 mm</u> ; (A; 80-81)) = 81 <u>x 1000</u> μm ; = 81000 μm

Magnification = 81000 <u>/ 495</u> ; = 163.63 = 164 (whole number) ;

magnification ×[4]

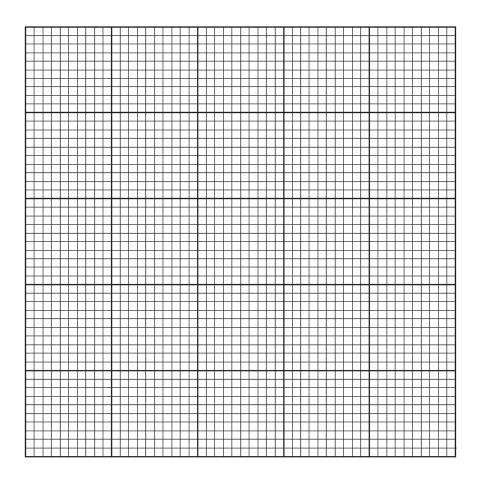
(c) Rice is often grown with its roots submerged in water. Barley is a crop that is quickly killed by such conditions. Respiration rate of root cells can be measured as rate of production of carbon dioxide.

In an investigation into the rate of respiration in rice and barley root cells, the data shown in Table 2.1 were obtained.

conditions	rate of production of carbon dioxide / mmol g ⁻¹
rice root cells with oxygen (RO)	4.5
rice root cells without oxygen (RW)	5.6
barley root cells with oxygen (BO)	9.2
barley root cells without oxygen (BW)	3.0

Table 2.1

(i) Use the grid provided to display the results shown in Table 2.1 in an appropriate form.



- 1. x-axis: conditions <u>and</u> y-axis: rate of production of carbon dioxide / mmol g⁻¹;
- scale on x-axis: bars of equal width and evenly spaced (total squares of all bars more than half of graph) + scale on y-axis is 2.0 to 2 cm + labelled each 2 cm ; (R: no origin / no 10.0 labelled at y axis (to cater to BO reading / double bars chart)
 correct plotting of 4 bars with bars in order of table ;
- 4. bars drawn with thin visible vertical and horizontal lines joining up precisely + labels (RO, RW, BO, BW) ;
 - (ii) Explain the difference in the rates of respiration in the sets of seeds used in this investigation.

......[3]

- 1. rice without oxygen grows better than rice with oxygen but barley with oxygen grows better than barley without oxygen ;
- 2. rice is adapted to grow in anaerobic/water logged conditions, grows better than barley without oxygen ;
- 3. rice can tolerate the ethanol produced by anaerobic respiration/barley seeds killed by ethanol produced by anaerobic respiration ;
- 4. aerobic respiration releases more energy than anaerobic, barley grows faster/more with oxygen ; (Any 3)

[Total: 19]

3 Methylene blue stains dead cells blue. Living cells are not stained blue so they will appear white or clear.

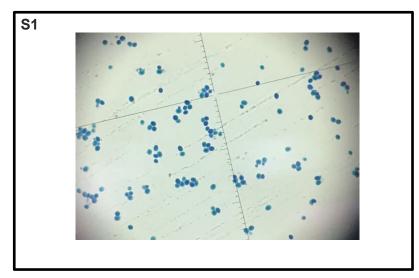
You are provided with:

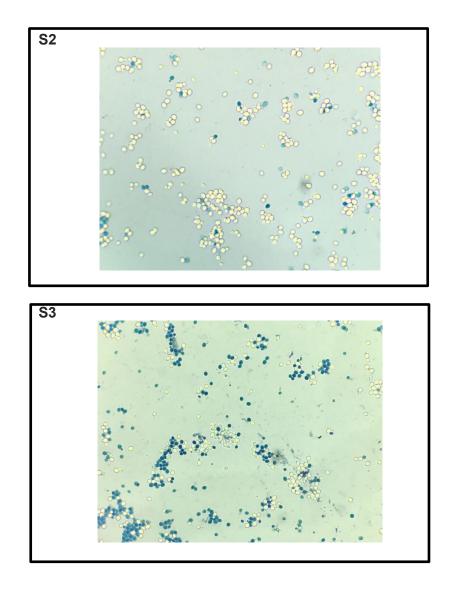
- methylene blue solution, **M**, (handle carefully as it will stain your skin)
- suspensions of yeast cells, labelled **S1**, **S2** and **S3**.

Each suspension, **S1**, **S2** and **S3** has been heated for ten minutes at 45 °C or 80 °C or 100 °C.

You are required to:

- use the microscope to observe the colour of the yeast cells from S1, S2 and S3, after M has been added
- record your observations by using annotated drawings of three yeast cells from each of S1, S2 and S3
- identify the temperature at which each of **S1**, **S2** and **S3** was heated.
- 1 Label three microscope slides **S1**, **S2** and **S3**.
- 2 Place **one drop** of **S1** onto slide **S1** and add **one drop** of **M**. Mix carefully using a glass rod. (If **M** comes into contact with your skin rinse with cold water.)
- **3** Repeat step 2 with **S2** and **S3**.
- **4** Leave for five minutes.
- **5** Add a coverslip to each slide.
- 6 Use the paper towel to dry off any excess liquid around the coverslip.
- 7 Use the microscope to observe the yeast cells on each slide, then select cells which you can draw and annotate to describe the effect of the methylene blue, **M**.
- (a) (i) Prepare the space below and record your observations by:
 - making drawings of **three** cells from **each** of the slides in the boxes provided
 - annotating your drawings to describe the effect of methylene blue, **M** on the cells.





- [4]
- 1. At least 9 separate cells (with cell wall) in total drawn in boxes S1, S2 and S3 + size at least <u>10mm across smallest cell</u>, in any box ;
- 2. <u>Drawing and quality</u>: Do not give mark for any shading, any ruled lines, or any line is too thick, has any feathery or dashed lines or gap in line, has any overlaps ;
- 3. Drawn only <u>3 cells</u> in each of the three boxes ;
- 4. At least one colour stated for each of the cells in the boxes S1, S2 and S3; Each point must be fulfilled across ALL 3 drawings in order for the mark to be awarded.
- (ii) Use your observations to identify the temperature that was used to heat each of the suspensions S1, S2 and S3.
 Complete the table.

suspension	temperature / °C
S1	100
S2	45
S3	80

(iii) Explain how you identified the yeast cells that had been heated at 100 °C.

......[1]

1. Yeast cells blue + therefore inactive or dead ;

(iv) A student was provided with a suspension of yeast cells which had been heated at a temperature between 45 °C and 80 °C.

Describe how you could modify this investigation to provide **quantitative** measurements that can be used to estimate this temperature.

- 1. Heat to five named temperatures between 45 °C and 80 °C, e.g. 50 °C, 55 °C, 60 °C, 65 °C, 70 °C ;
- 2. Count dead / blue yeast cells (from each sample of yeast cells) ;
- 3. Plot graph, draw a straight line from x-axis to the graph and from the intercept, draw a line to cut the y-axis to find unknown temperature (idea marking);

(b) Baker's yeast was dissolved in water and provided with glucose to give active yeast suspension **BA**.

Brewer's yeast was dissolved in water and provided with glucose to give active yeast suspension **BR**.

A student suggested the hypothesis that:

'brewer's yeast has a lower rate of respiration than baker's yeast".

Methylene blue solution acts as an electron acceptor and becomes colourless when reduced in the process of respiration, making it suitable to be used to test out his hypothesis. As the blue solution may float to the top of the yeast suspension, the mixture needs to be stirred constantly to observe the colour change.

The student carried out some preliminary trials to find the volumes of suspension and methylene blue solution to use. The student found that the best volumes of suspension and methylene blue solution to use were in the ratio of 20 : 1. He also realised that rate of respiration was at its optimum when the mixture was incubated in a water bath at a temperature of between 40 °C and 45 °C.

(i) Use the results of the preliminary trials to plan and carry out an investigation to provide results that will enable you to support or reject this hypothesis.

You are provided with:

- 15 cm³ of baker's yeast suspension, in a specimen tube labelled **BA**
- 15 cm³ of brewer's yeast suspension, in a specimen tube labelled **BR**
- 5 cm³ of methylene blue solution, in a specimen tube labelled **G**.

Using the test tubes and the other apparatus provided, plan **and** carry out a method to obtain results to support or reject the student's hypothesis. Do **not** plan to carry out repeats. The results are to be recorded in a suitable format in **(b)(ii)**.

Your planned method should:

- have a clear and helpful structure so that the method described could be repeated by anyone reading it
- identify the independent and dependent variables
- include details to ensure that results are as accurate and repeatable as possible
- use the correct technical and scientific terms
- include reference to safety measures to minimise any risks associated with the proposed experiment.
- only make use of the apparatus and materials provided.

*no need for state and explain hypothesis since explanation already provided, no control expt, Q mentioned not to plan repeats

[5]

- state and describe independent and dependent variable;
- the independent variable is type of yeast •
- the dependent variable is time taken for blue solution to turn colourless/decolourise • methylene blue solution;

(b) controlled/fixed/standardised, variables to improve accuracy or reliability

- identify two variables to be controlled ; i.
- describe how two identified variables are controlled ; ii.
 - volume of BA/BR
 - kept constant at 10 cm³ of BA/BR using syringe -
 - volume of G
 - kept constant at 0.5 cm³ of G using syringe -
 - Equilibration time
 - kept constant at 2 min using stopwatch
 - temperature •
 - kept constant at 40 °C (A: 40-45) using thermometer

(c) Describe the experimental procedure (method)

- BA/BR : G is 20 : 1 ratio ; *i*.
- ii. Use of water-bath at temperatures between 40 °C to 45 °C;
- iii. Calculate rate of respiration using 1/t or 1000/t;
 - 1. Label a test tube BA and a test tube G.
 - 2. Put 10 cm³ of BA into tube BA and 0.5 cm³ of G into tube G.
 - 3. Put both tubes into a (R: thermostatically-controlled because can ONLY make use of apparatus provided) water bath at 40 °C for 2 minutes for equilibration time.
 - 4. Pour G into tube BA, mix well and start the stopwatch.
 - 5. Record time taken for the blue solution to turn colourless.
 - 6. Calculate rate of respiration using 1/t.
 - 7. Repeat steps 1-6 for BR with G.

(d) Risks/safety

refer to hazards and precaution ;

Risks	Safety Precautions
Yeast/methylene blue solution are irritants to the skin and eyes.	
Glass wares are breakable and may cut.	Keep glass wares away after use to prevent breakage.
Saccharomyces cerevisiae is a microorganism which may cause infection.	 To prevent infection by : Covering all cut or broken skin with a waterproof dressing Wearing tightly fitting disposable gloves and clean laboratory coat Cleaning the bench surface with disinfectant and use a Bunsen burner which creates a sterile environment. One should work as close as possible to the flame. Swap any spillages with disinfectant Proper disposal / treatment of contaminated materials or equipment using steriliser/autoclave

Before proceeding further, use the beaker labelled **hot water** to collect approximately 400 cm³ of hot water from where it is provided in the laboratory.

The beaker labelled **tap water** can be filled as necessary from the tap.

Carry out your method described in **(b)(i)** to collect results. You should not spend more than 5 minutes on the experiment. If there are no changes after 300 seconds, record 'more than 300'.

(ii) Record your results in a suitable format in the space provided. [4]

Table showing the time taken for first colour change of Benedict's solution for

BA and BS

suspension	time taken for blue solution to turn colourless / s	
BA	200	
BR	more than 300	

- 1. (headings) suspension, time taken for blue solution to turn colourless / s ;
- 2. collects results for both suspension ;
- 3. correct trend positive results for BA, no results for BR ;
- 4. records all results using whole seconds ;
- (iii) State whether or not your results in (b)(ii) support the student's hypothesis. Give a reason for your decision.

......[1]

1. <u>Yes</u>, results support the student's hypothesis because <u>blue solution did not turn</u> <u>colourless even after 300s for BR / time taken for blue solution to turn colourless</u> <u>is longer for BR</u>, thus brewer's yeast has a lower rate of respiration ;

[Total: 19]