

NANYANG JUNIOR COLLEGE
PRELIMINARY EXAMINATIONS
Higher 2

CANDIDATE
NAME

ANSWERS

CLASS

BIOLOGY

9744/04

Paper 4 Practical

28 August 2025

Candidates answer on the Question Paper

Additional Materials: As listed in the Confidential Instructions

2 hour 30 minutes

READ THESE INSTRUCTIONS FIRST

Write your name and CT 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, highlighters, glue or correction fluid.

DO NOT WRITE IN 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	29
2	26
Total	55

This document consists of **18** printed pages.

[Turn over

Answer **all** the questions.

- 1** Yeast cells contain enzymes that break down sugars to release energy. In these reactions carbon dioxide is released. Some of this carbon dioxide dissolves in water forming carbonic acid.

The progress of these enzyme-catalysed reactions can be followed by measuring the time taken for an indicator to change colour as carbonic acid is formed. You will investigate the effect of changing the concentration of yeast suspension on the time taken for the indicator to change colour.

You are provided with the materials shown in Table 1.1.

Table 1.1

labelled	contents	hazard	volume/ cm ³
Y	10% yeast suspension	none	100
H	hydrogencarbonate indicator solution	none	50
W	distilled water	none	100

Note that it is normal for **Y** to froth over time and the froth may overflow from the container.

If any solution comes into contact with your skin, wash off immediately under cold water. It is recommended that you wear suitable eye protection.

Read step 1 to step 4.

- (a) (i)** Using Table 1.1 **and** step 1 to step 4, assess the risk of this procedure as **low**, **medium** or **high**. Give **one** reason for your answer.

risk **low or medium**

reason **ref to no hazards listed in table 1.1 ;**

[1]

The hydrogencarbonate indicator solution, **H**, will change colour from red-brown to yellow as carbon dioxide passes through it.

To help you identify the end-point (yellow) carry out step 1 to step 4.

- Put 5cm³ of **H** into a test-tube.
- Put a drinking straw into this test-tube.
- Gently breathe **out** through the straw so that you are blowing air bubbles through **H**.
- Stop blowing when the colour changes to yellow. This is the end-point.

You will need to use this test-tube for colour comparison in step 14.

You will use proportional dilution to make different concentrations of yeast suspension, **Y**.

You will need to prepare 20cm³ of each concentration of yeast suspension.

- (ii) Complete Table 1.2 to show how you will prepare the concentrations of yeast suspension you will use.

Table 1.2

percentage concentration of yeast suspension	volume of Y / cm ³	volume of W / 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

[2]

1. four additional concentrations of yeast with regular interval (rej: 0.0);
2. correct volumes of yeast and water + dp;

Carry out step 5 to step 18.

- 5 Use a glass rod to stir the yeast suspension, **Y**. Carefully decant the yeast suspension into a clean beaker, leaving the froth behind.
- 6 Prepare the concentrations of yeast suspension as decided in **(a)(ii)** and shown in Table 1.2, using the small beakers provided.
- 7 Label a small test-tube for each of the concentrations of yeast suspension you have prepared in step 6.
- 8 Put 5cm³ of **H** into each labelled test-tube.
- 9 Using the container and beakers labelled **water-bath**, **hot water** and **cold water**, set up and maintain a water-bath with water between 35°C and 40°C.

Fig. 1.1 shows the apparatus you will set up for the investigation.

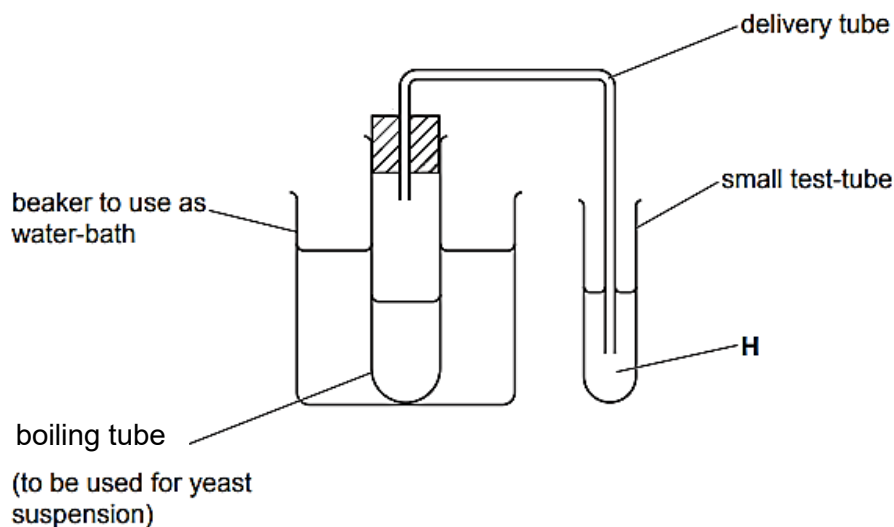


Fig. 1.1

- 10 Use a glass rod to stir the 10% yeast suspension, then pour the prepared 20cm³ of the 10% yeast suspension into the boiling tube.
- 11 Put the boiling tube in the water-bath.
- 12 Put the bung in the boiling tube, and make sure it covers the mouth of the boiling tube tightly. Ensure the end of the delivery tube is in **H**, as shown in Fig. 1.1.
- 13 Start timing.
- 14 Stop timing when the end-point is reached.

The test-tube from step 4 can be used to confirm the end-point.

Record the result in **(a)(iii)**. If the time taken is more than 240 seconds, record the result as 'more than 240'.

- 15 Put the contents of the boiling tube into the container labelled **For waste**.
- 16 Rinse the boiling tube. You may use water from the beaker labelled **For washing**.
- 17 Put another test-tube of **H**, prepared in step 7, into the apparatus shown in Fig. 1.1.
- 18 Repeat step 10 to step 17 for the other concentrations of yeast suspension you prepared in step 6.

(iii) Record your results in an appropriate table.

percentage concentration of yeast suspension	Time taken to reach end point color / s
10.0	75
8.0	96
6.0	113
4.0	210
2.0	More than 240

1. heading for IV + no units in body of table (rej "/%")
2. heading for DV + no units in body of table
3. overall trend - time for the highest conc of yeast shorter than for the lowest conc
 - Accept "more than 240" at most twice (for 4.0 and 2.0 percent yeast)
4. results recorded to nearest whole second ;

If table border is not drawn, overall minus 1m

(iv) Describe **two** significant sources of error in this investigation.

1. difficulty of judging when the indicator changed to yellow /
2. temperature of water-bath fluctuates between 35°C and 40°C
3. delay putting the bung into the large test-tube which allowed some carbon dioxide to be lost

.....
[2]

- (b) Baker's yeast, *Saccharomyces cerevisiae*, has two metabolic pathways to produce ATP, aerobic respiration and fermentation. Oxygen is **not** used in fermentation.

These pathways are summarised in Fig. 1.2.

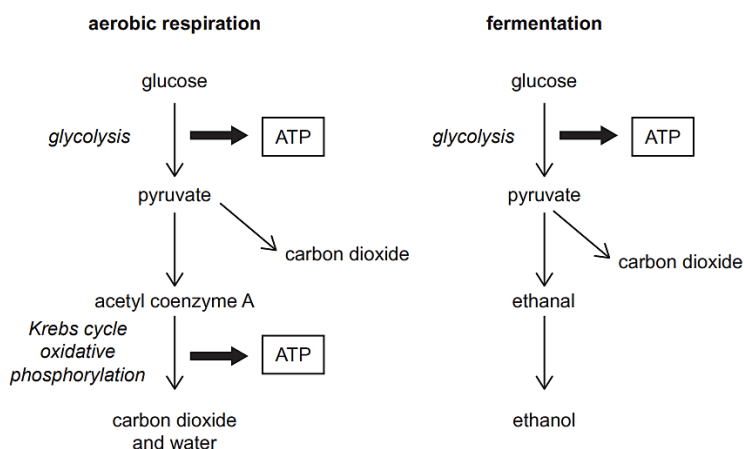


Fig. 1.2

Some students were researching details of the respiration of yeast. In their research, the students found information about the Crabtree effect. This effect occurs when yeast is kept in high concentrations of glucose. The yeast gains sufficient ATP from substrate-level phosphorylation during glycolysis. Yeast does not carry out the Krebs cycle or oxidative phosphorylation and therefore does not use oxygen.

The students wanted to investigate the effect of temperature on the activity of yeast and decided to use yeast solutions in which the yeast obtained all its ATP by fermentation.

- The students made a suspension of yeast from 1g of dried yeast and 25cm³ of water.
- The suspension was left for 2 hours at 20°C.
- After 2 hours, 25cm³ of glucose solution was added to the yeast suspension and the mixture was stirred.

The students measured the volume of carbon dioxide produced by yeast fermentation.

Plan an investigation to find the temperature at which yeast carries out fermentation at its maximum rate.

You can assume that you have access to all standard laboratory equipment.

Your plan should:

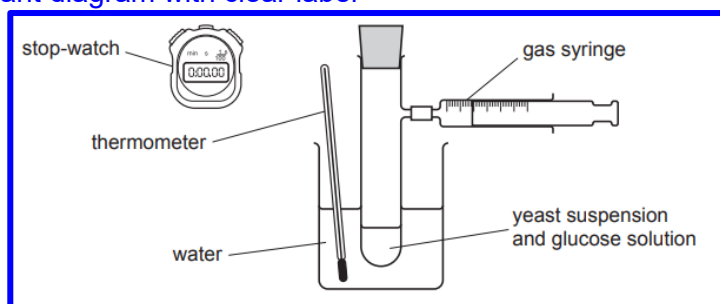
- have a clear and helpful structure such that the method you use is able to be repeated by anyone reading it
- be illustrated by relevant diagrams, if necessary
- identify the independent variable and dependent variable
- identify the variables you will need to control
- use the correct technical and scientific terms
- indicate how the results will be recorded and analysed.

You do **not** need to include details of how to make the yeast suspension or reference to any safety measures in your plan.

[7]

- 1 **[compulsory] Independent variable:** Temperature **/°C**: 20, 30, 40, 50, 60°C (accept any suitable range, *reject temp lower than 20°C*)
- 2 **[compulsory] Dependent variable:** volume of CO₂ produced at **3 min** (*a fixed time*) / **cm³** (accept time taken to collect **5cm³** (*a fixed volume*) of CO₂/s)
- 3 **[compulsory] Variables to be controlled and how to control** [max 2]
 - (a) volume of yeast-glucose mixture used- use a **syringe** to draw **5 cm³**
 - (b) time taken for CO₂ to be collected- use a **stopwatch** to time **3 mins**
or
volume of CO₂ to be collected – i.e. **5cm³** using **gas syringe** (*depends on the dep variable stated in point 2*)
 - (c) constant pH – use same pH buffer solution at pH7
- 4 Set up **5 thermostatically controlled water baths** at 20, 30, 40, 50, 60°C
OR
Use hot and ice water to set up 5 water baths maintained at the 5 different temperatures at 20, 30, 40, 50, 60°C. Use a **thermometer** to monitor the temperature
- 5 Label 5 test tubes 1 – 5. Stir yeast mixture using glass rod. Using a **syringe** add **5cm³** of yeast-glucose mixture into the test tube (*mark once only for variables to be controlled*).
- 6 *Ref to* **equilibration in the respective water bath for 2 minutes**
- 7 Immediately attach the gas syringe and rubber bung and start **timing for 3mins using stopwatch** (*mark once only for variables to be controlled*), record the volume of CO₂ produced after 3min
- 8 Repeat step 5-7 to perform **2 more replicates and calculate average** to ensure **accuracy** of the results
- 9 *idea of* repeat investigation with **narrower range** of temperatures (around that which seems the one giving maximum rate)
- 10 **Control:** use boiled yeast-glucose mixture / replace equal volume of yeast with distilled water
- 11 **[compulsory] Plot a graph of** mean volume of CO₂ produced for each temperature and determine the **temperature which has the highest CO₂ produced** using the graph
- 12 Ref to theory: Link specific IV to DV [max 1]

As **temperature increases** towards the optimum, increase in **kinetic energy** leads to **higher frequency of effective collision** between enzyme and substrate, **higher rate of formation of E-S complexes** and hence **higher rate of CO₂ produced**
- 13 Relevant diagram with clear label



- (c) A student carried out an investigation into the effect of temperature on the activity of the enzymes in Y.

The student immobilised the yeast cells in alginate beads which were then dropped into a solution of the substrate at different temperatures.

As carbon dioxide collected in the beads, they rose to the surface of the solution of the substrate as shown in Fig. 1.3.

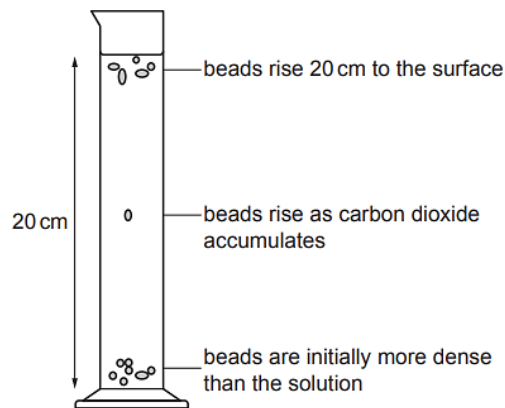


Fig. 1.3

The student measured the time taken for 5 beads to rise to the surface, a distance of 20cm. The processed results are shown in Table 1.3.

Table 1.3

temperature / °C	mean time to rise / seconds
10	72
25	19
40	11
50	36
55	109

- (i) Identify the temperature at which the beads rise the fastest.40°C

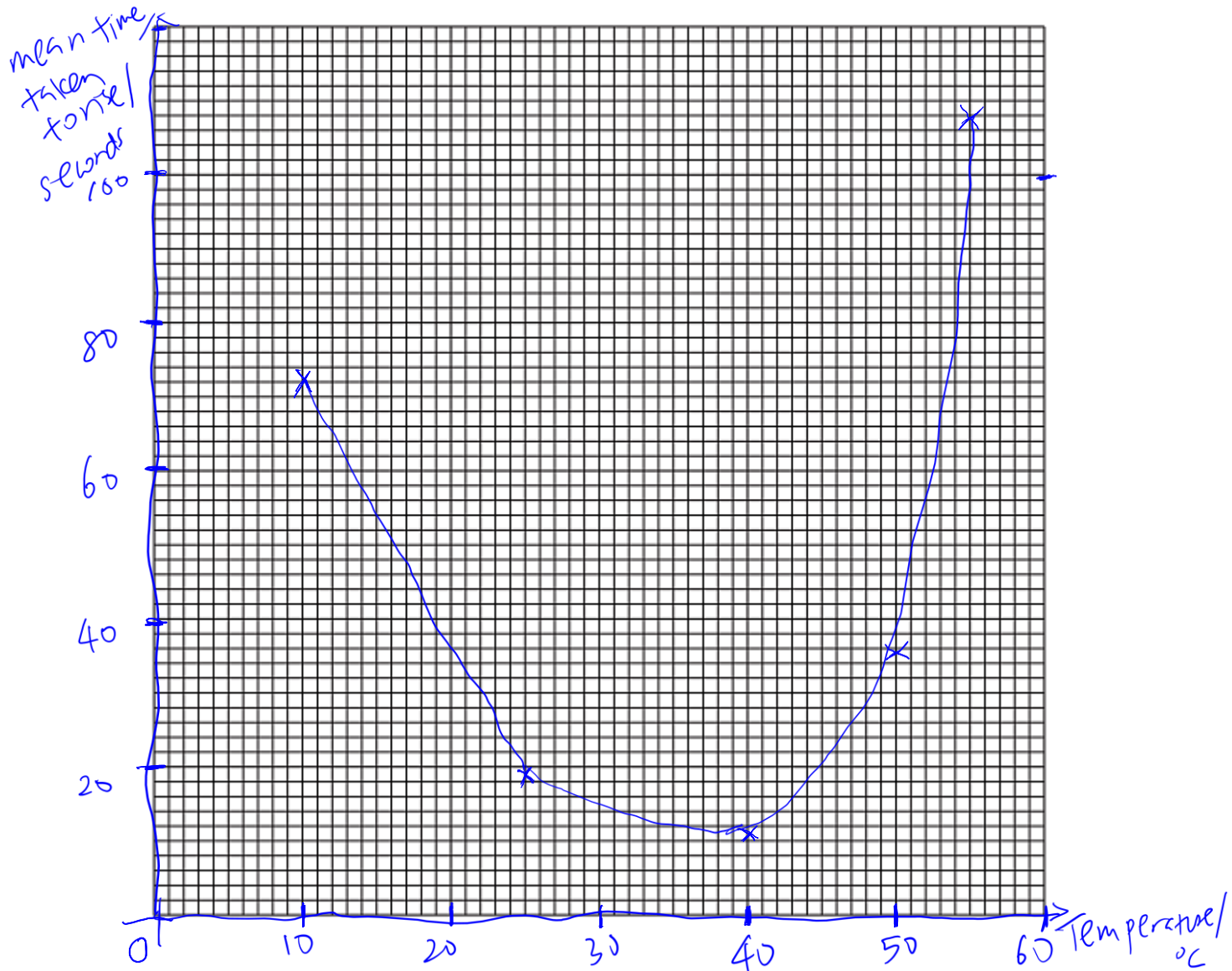
For this temperature calculate the rate at which these beads rise 20cm.

$$20 \div 11 = 1.82 / 1.8 \text{ cm s}^{-1}$$

rate = [2]

(ii) Plot a graph of the data shown in Table 1.3.

Use a sharp pencil for drawing graphs.



[4]

Ax: x axis: temperature / °C + y axis: mean time to rise / seconds + origin labelled;

S: (xaxis) 10°C to 2cm labelled each 2cm + (yaxis) 20 seconds to 2cm labelled each 2cm;

- Scale needs to be **marked out at regular intervals**
- Awkward scale will affect accuracy of plot points

P: Correct plotting of five points as small cross or dot in circle or cross + marked clearly;

G: ruled sharp lines exactly point-to-point or ruled line of best fit + smooth line (accept point to point ruled line or best fit curve);

(iii) Explain the shape of the graph:

between 10°C and 40°C:

enzyme and substrate have more kinetic energy ;

more / higher frequency of successful / effective collisions

or more enzyme substrate complexes formed ;

Minimum 1 mark for this sub-question

between 40°C and 55°C:

Thermal agitation ; disrupt (weak) bonds e.g. hydrogen, etc.. ;

shape of the active site distorted ;

or active site no longer complementary to substrate

substrate prevented from binding to active site ;

or fewer enzyme substrate complexes formed ; **[same point]**

or enzyme denatures

Max 2 marks

[3]

- (d) Ethanol is a renewable alternative to fossil fuels and is increasingly used as a biofuel or blended fuel in transportation.

Recent research has demonstrated that immobilised yeast cells can produce greater quantities of ethanol than free yeast cells under the same fermentation conditions. Immobilisation typically involves enclosing yeast in a matrix, such as calcium alginate beads, which allows substrates like glucose to diffuse in while ethanol and carbon dioxide diffuse out.

A student conducted an experiment to compare the percentage ethanol yield between free yeast and immobilised yeast over 6 days of fermentation. He carried out a statistical test to determine whether there was a significant difference between these means.

- (i) State a statistical test that could have been used to determine whether the difference in percentage ethanol yield between free yeast and immobilized yeast is significant.

t-test;

[1]

- (ii) A summary of the student's results is shown in Table 1.4.

Table 1.4

percentage ethanol yield on day 6 of fermentation		significance of difference
immobilised yeast	free yeast	
82.73	68.79	$p < 0.05$

Comment on what these results show and suggest an explanation for any pattern.

What these results show:

Percentage ethanol yield was higher for immobilised yeast than free yeast on day 6 of fermentation;

Since $p < 0.05$, difference between the two means is **significant and not due to chance;**

Explanation:

Ref to higher cell density: immobilisation allows for a higher conc of yeast cells per unit volume of fermentation medium ;

Ref to greater cell stability → immobilised yeast cells can tolerate higher levels of ethanol concentration produced in the process;

Increase s.a. blue volume ratio boost nutrients / substrate contact **or** immobilised yeast more ethanol due to lack of oxygen in beads **or** ensure correct orientation

® no explanation / reject or don't reject without reference to null hypothesis

[3]

[Total: 29]

2 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.

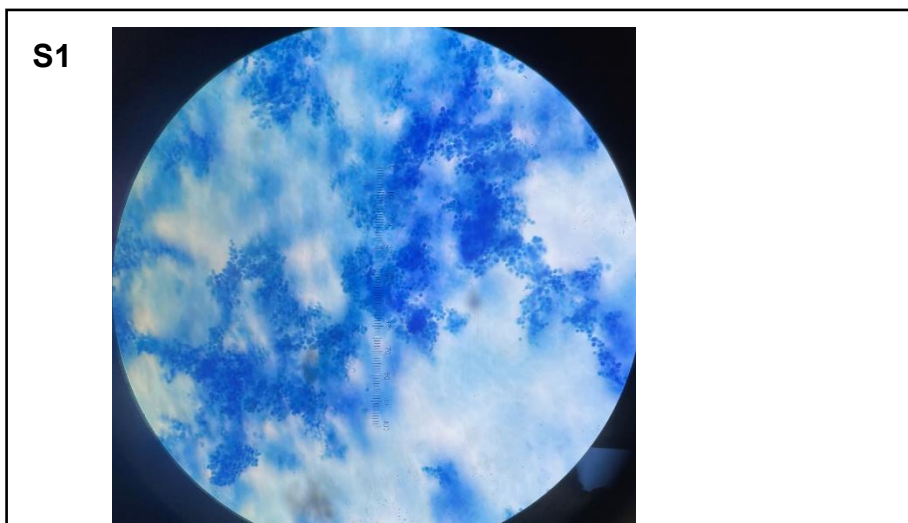
You are required to:

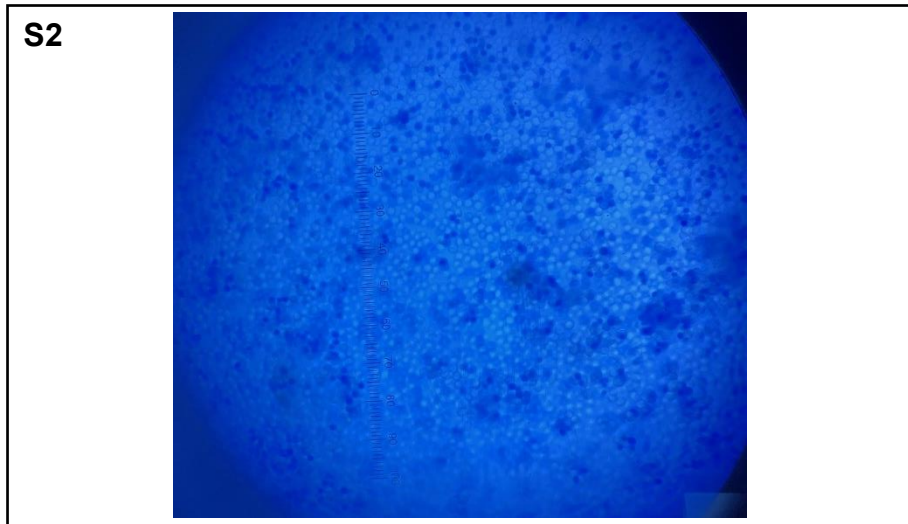
- use the microscope to observe the colour of the yeast cells from **S1**, **S2** and **S3**, after **M** has been added
- record your observations by using annotated drawings of three yeast cells from each of **S1**, **S2** and **S3**
- identify the temperature at which each of **S1**, **S2** and **S3** was heated.

1. Label three microscope slides **S1**, **S2** and **S3**.
2. Place **one small drop** of **S1** onto slide **S1** and add **one small 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.





S3

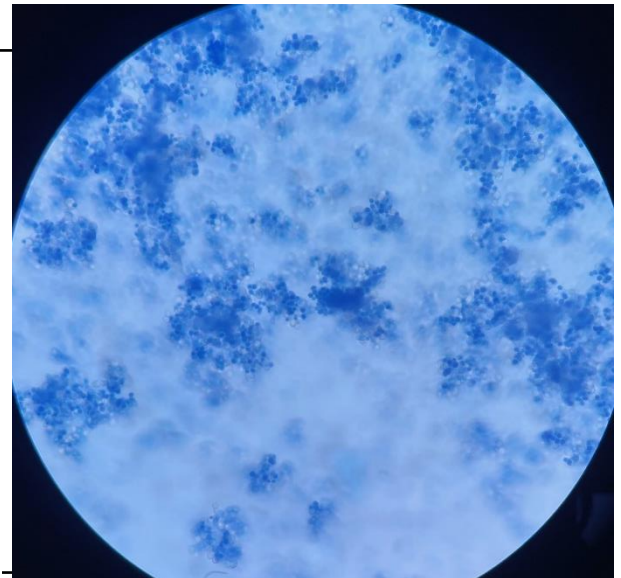
S: size at least 10mm across largest cell, in any box + smooth lines + correct cell shape;

A: From 2 to 6 whole cells (not overlapping) drawn in each of the boxes S1, S2, and S3 ;

T: Drawn only 3 cells in each of the three boxes ;

C: Annotation: At least one colour stated for each of the cells in the boxes S1, S2 and S3;

*Can ignore cell wall for this qn as it will be marked for in (ii) ® **NO SHADING + No mention of colour, simply label "M" but note that we need to describe effect of Methylene Blue.***



[4]

- (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

[1]

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

Most number of Yeast cells blue + therefore inactive or dead ;

® Dead yeast cells are stained blue but question is asking how do you know it was heated at 100°C.

[1]

- (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.

Heat to at least two named temperatures, at least 10°C apart, between 45°C and 80°C;

Count dead / blue yeast cells (from sample of yeast cells);

Plot graph to find unknown temperature;

® **colourimeter / count bubbles**

[3]

Fig. 2.1 is a photomicrograph of yeast cells.

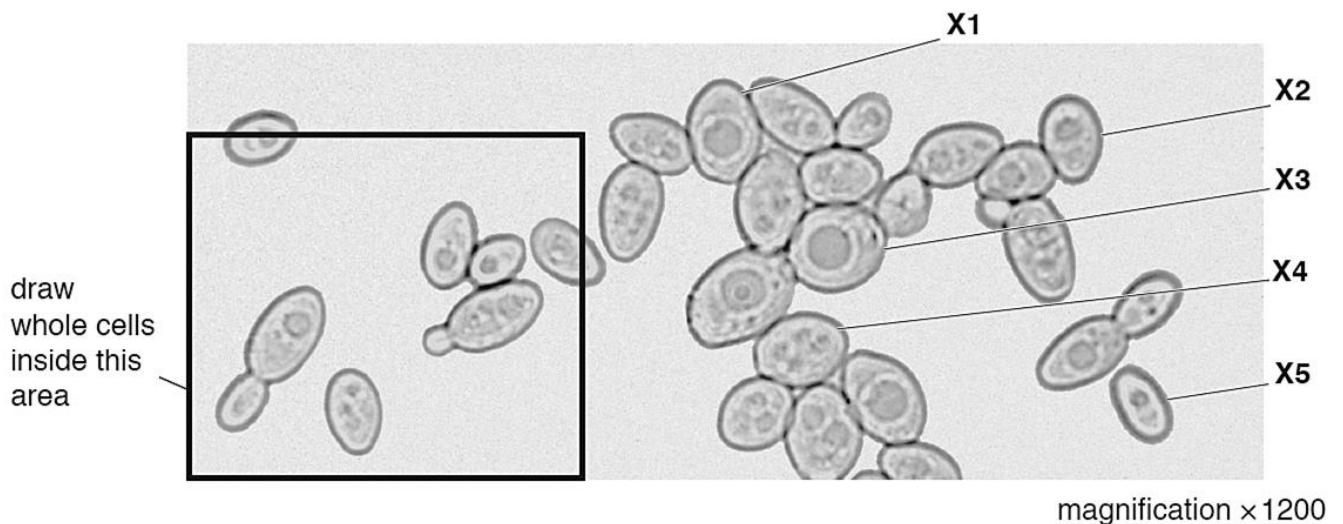


Fig. 2.1

- (b) (i) Make a large drawing of the whole cells shown in the area on Fig. 2.1.

On your drawing, use a label line and label to show one observable feature of these cells which identify them as being similar to animal cells.

W: only 5 whole cells (non overlapping) + no shading + size at least 20mm across cell with greatest width + sharp and continuous outer line;

CW: Cell wall drawn as two lines;

S+P: correct shape of cells + proportion + relative position + no other internal structures except nucleus in 3 of the cells;

B: only 2 cells with buds (budding cells);

L: Uses label line and correct label of nucleus (only distinct in 3 cells) or cytoplasm or cell surface membrane ;

- (ii) Use the magnification to calculate the mean maximum actual length, in μm , of yeast cells, **X1**, **X2**, **X3**, **X4** and **X5**. [5]

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

- Five measurements written clearly, to $\pm 0.1\text{cm}$ for each measurement: X1= 1.4cm, X2 = 1.2cm, X3 = 1.3cm, X4= 1.25/1.3cm, X5 = 1.1cm;
- Shows addition of measurements + shows division by 5 ;
- Show division by 1200;
- Shows conversion of mm to μm as $\times 1000$ + units; @ conversion of cm to μm as $\times 10\,000$ + units + final answer to 2sf;

..... μm [4]

- (c) Rhizophagy, derived from "rhizo" (root) and "phagy" (eating), refers to a recently discovered nutritional symbiosis where plants actively take up microbes such as yeast into their roots, extract nutrients from them, and then release them back into the soil. This cycle is thought to be a way for plants to obtain essential nutrients, particularly micronutrients, from soil microbes.

Yeast is a waste product of the brewing industry and can be used as biofertilizer. The addition of live or dead yeast to fertilised soil has been shown to improve plant growth and health of crops like corn (*Zea mays*), sugarcane (*Saccharum officinarum*) and tomato (*Solanum lycopersicum*).

K1 is a slide of a stained transverse section through a *Zea mays* root.

You are not expected to be familiar with this specimen.

Use a sharp pencil for drawing.

You are expected to draw the correct shape and proportions of the different tissues.

- (i) Draw a large plan diagram of the whole root.

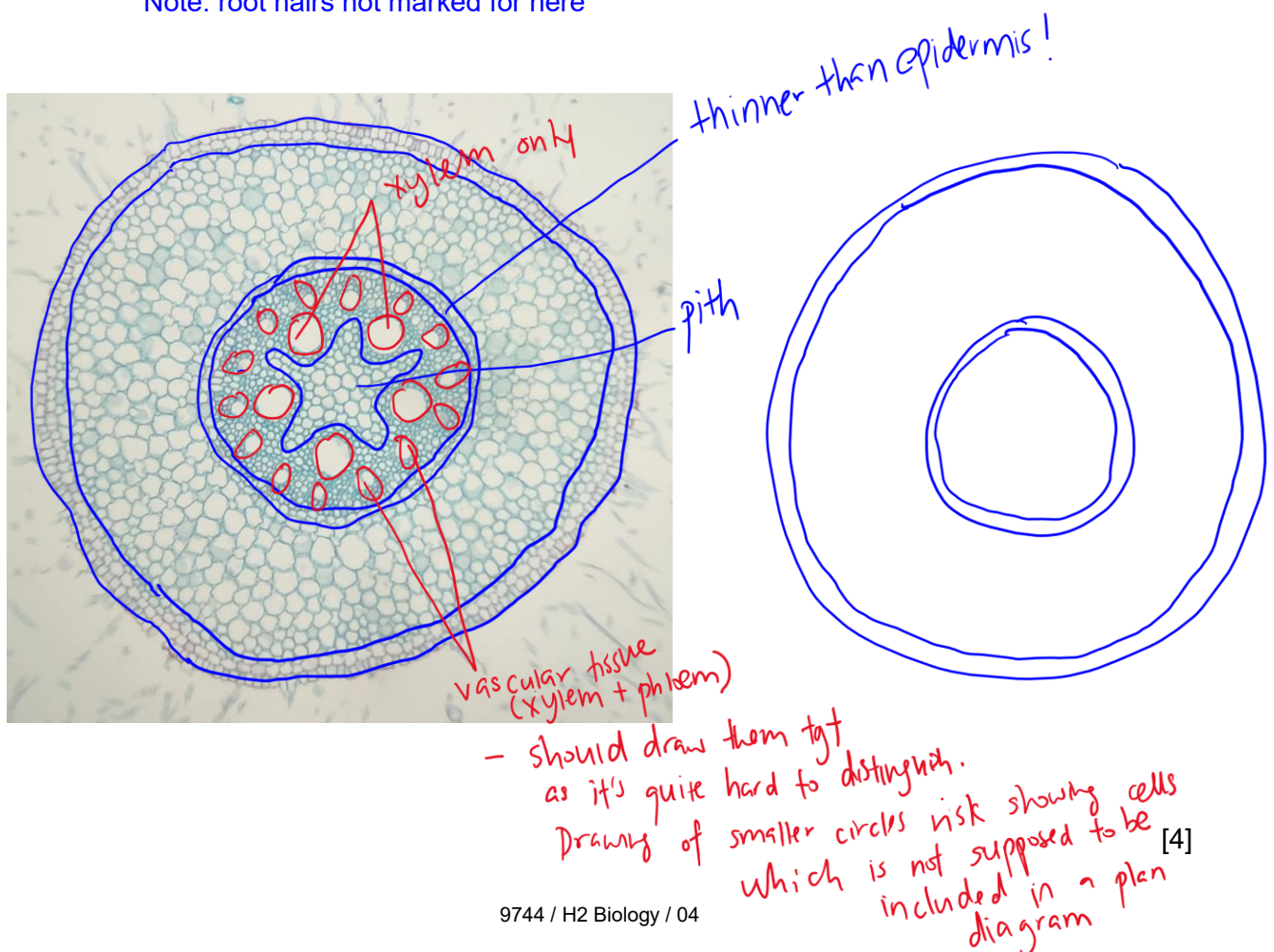
S: suitable size and no shading and smooth continuous lines;

L: at least 5 regions + 2 lines for epidermis + (bigger) xylem OR pith tissue shown;

W: whole root + **no cells** (teach: individual smaller xylem not drawn)

S+P: correct shape and proportion of the different tissue regions;

Note: root hairs not marked for here



- (ii) Fig. 2.2 is a photomicrograph of a stained transverse section through the root of a *Solanum lycopersicum* tomato plant. You are not expected to be familiar with this specimen.

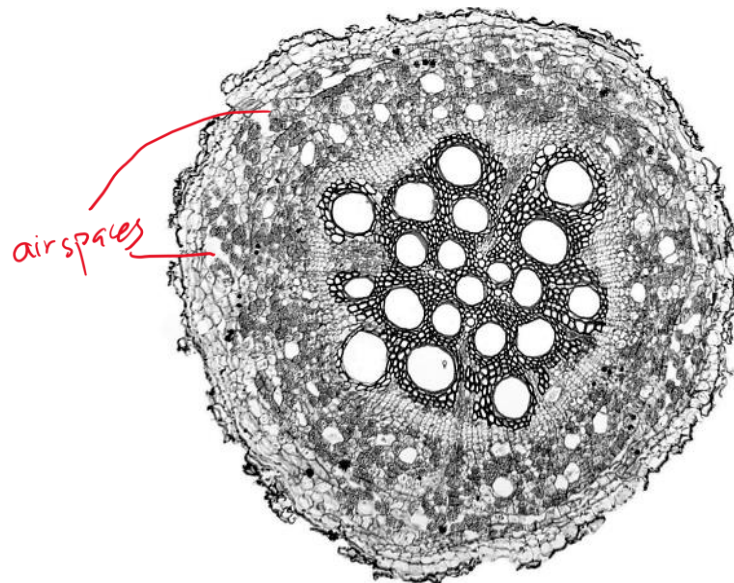


Fig. 2.2

Prepare an appropriate table so that it is suitable for you to record the observable differences between the root on **K1** and the root in **Fig. 2.2**.

Record the observable differences in your table.

feature	K1	Fig. 2.2 (not K2!)
Number of large xylem vessels Rej: vascular bundles / phloem (harder to count)	Few(er)	Many / more
Arrangement of xylem @phloem	Arranged in a ring	Located / scattered in centre part of root tissue
Presence of pith	Present	Absent
Presence of air spaces	None	Present
Presence of root hairs	Present	None

@ proportion of vascular tissue to whole root: K1 smaller, Fig. 2.2 bigger

@ thickness of cortex relative to whole root: K1 bigger, Fig. 2.2 smaller

@ number of different tissue regions: K1 has 6 while Fig. 2.2 has 5

@ number of layers of epidermis: K1 fewer while Fig. 2.2 more

Rej any comparison of absolute sizes as both are not of the same magnification.

Rej comparison of epidermis texture as K1 has root hairs which makes it not smooth either

Rej mention of cell structures like cell wall as the images are whole root tissues

Rej any mention of leaf structures such as mesophyll

[4]

[Total: 26]