H

NATIONAL JUNIOR COLLEGE, SINGAPORE
Senior High 2
Preliminary Examination
Higher 2

CANDIDATE NAME	**MARK SCHEME**		
BIOLOGY CLASS	2bi2_____	REGISTRATION NUMBER	

Biology

9744/04

Paper 4 Practical

26 August 2024

2 hours 30 minutes

READ THESE INSTRUCTIONS FIRST

Write your name, Biology class, and registration number on all the work you hand in.
Give details of the practical shift and laboratory in the boxes provided.
Write in dark blue or black pen on both sides of the paper.
You may use a soft pencil for any diagrams, graphs.
Do not use staples, paper clips, highlighters, 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 workings 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 the brackets [ ] at the end of each question or part of question.

Shift				
1	2	3		
Laboratory				
BI23	BI24	CM42	CM43	CM44

For Examiner's Use	
1	20
2	20
3	15

Total	55

This document consists of **19** printed pages and **1** blank pages.

Answer **all** questions.

1 Yeast can convert different respiratory substrates into carbon dioxide via cellular respiration.

You will investigate the effect of different respiratory substrates on the rate of respiration in yeast.

Hydrogencarbonate indicator is a pH indicator which can be used to measure the amount of carbon dioxide produced by the respiring yeast cells. When carbon dioxide dissolves in water, carbonic acid is formed, reducing the pH and changing the colour of the indicator solution.

The initial pH of the reaction mixture containing yeast and respiratory substrate may be slightly different depending on the type of respiratory substrate. This can be standardised by using sodium bicarbonate solution to adjust pH so that the starting colour corresponds to the colour number 6 on the hydrogencarbonate indicator colour chart provided.

You are provided with:

- yeast suspension, labelled **Y**
- alginate solution, labelled **A**
- calcium chloride solution, labelled **C**
- respiratory substrate 1, labelled **S1**
- respiratory substrate 2, labelled **S2**
- respiratory substrate 3, labelled **S3**
- hydrogencarbonate indicator, labelled **H**
- sodium bicarbonate solution, labelled **B**
- hydrogencarbonate indicator colour chart

Read steps **1–12** before starting the investigation.

Proceed as follows.

1 Add about 30cm^3 of **C** into a 50cm^3 beaker.

2 Stir **Y** to suspend the yeast cells. Transfer 3.0cm^3 of **Y** into another 50cm^3 beaker.

3 Add 6.0cm^3 of **A** into the beaker containing **Y**, taking care not to introduce air bubbles into the mixture.

Stir the resulting yeast-alginate mixture gently using a glass rod. Do not mix vigorously as this may introduce air bubbles into the mixture.

4 Remove the plunger of a 5.0cm^3 syringe. Hold the empty syringe barrel with the nozzle facing down above the beaker containing **C**, as shown in Fig. 1.1.

5 Pour the yeast-alginate mixture prepared from step **4** into the syringe barrel and allow the yeast-alginate mixture to drip into **C**, as shown in Fig. 1.1. As it drips, gently swirl the beaker to prevent the beads from aggregating with each other. The mixture will form a bead upon contact with calcium chloride.

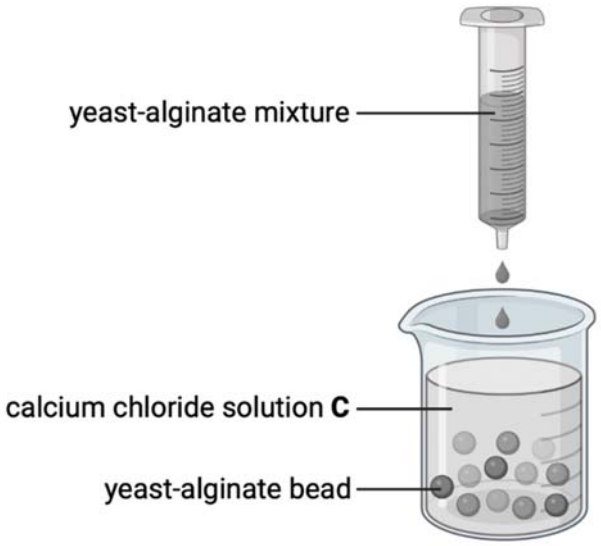


Fig. 1.1

6 Transfer only the yeast-alginate beads into a Petri dish. Rinse the beads with distilled water. Keep the yeast-alginate beads in distilled water. Remove and discard any bead that is obviously different in size, distorted in shape, or floating.

You will need to use 15 yeast-alginate beads for **Question 1**.

Keep 25 yeast-alginate beads for **Question 2**.

7 Add 2.0cm^3 of **S1** and 0.2cm^3 of **H** into a test tube. Mix the tube contents well.

8 Check if the colour of the mixture matches colour 6 on the hydrogencarbonate indicator colour chart.

If not, using a 1cm^3 Pasteur pipette, add **B** dropwise to the mixture until colour 6 is achieved.

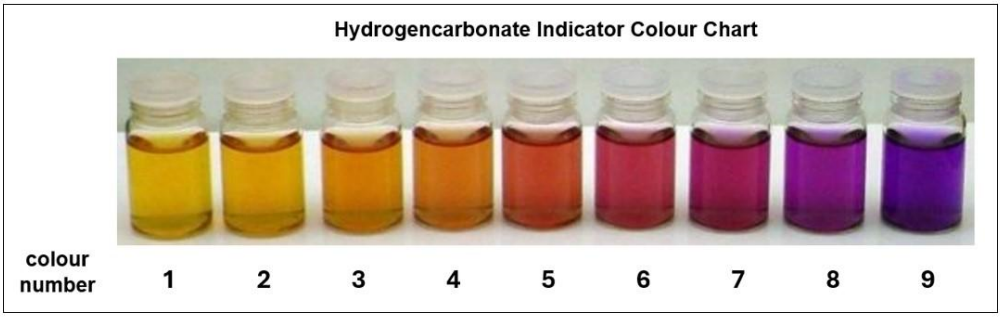
9 Add five yeast-alginate beads into the test tube. You should ensure that as little distilled water is transferred along with the beads as possible. Start timing immediately.

10 At the end of three minutes, mix the tube contents well.

11 Record, in **(a)(i)**, the final colour of the mixture, using the colour number which it matches with on the colour chart.

12 Repeat steps **7–11** with each of the other respiratory substrates, **S2** and **S3**.

(a) (i) Record your results in an appropriate table.



respiratory substrate	final colour number of the mixture
S1	1
S2	1
S3	5

Headings;

(IV) respiratory substrate

(DV) final colour number of the mixture

Trend;

S3 has the highest colour number (6 or below)

Complete;

3 final colours recorded in the table [3]

(ii) All three respiratory substrates, **S1**, **S2** and **S3**, are carbohydrates but only one of them is a polysaccharide.

Based on your results obtained in **(a)(i)**, put a tick (✓) in the appropriate box to indicate the respiratory substrate that is a polysaccharide and explain your answer.

S1 ☐ **(sucrose)** **S2** ☐ **(glucose)** **S3** ✓ **(starch)**

explanation

Compulsory:
smallest decrease / change in colour number / pH in the presence of S3;
OR
least amount of carbon dioxide produced by the respiring yeast cells in the presence of S3;

Enzyme:
Yeast lacks the enzyme to utilise the polysaccharide as a respiratory substrate;
OR
The amount of yeast enzyme that can break down S3 for use in respiration is lower than the amount of yeast enzyme that can break down S1/S2 for use in respiration;
OR
Longer time needed for yeast enzyme to break down S3 for use in respiration;

[3]

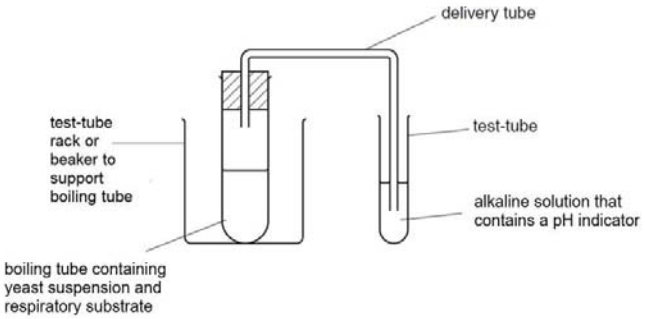
(iii) Describe a suitable control for this investigation and explain its purpose.

control	purpose
In step 2, replace the yeast suspension with equal volume ($3.0cm^3$) of boiled and cooled yeast suspension while keeping all other experimental conditions the same;	**to show that the change in colour number / pH of the mixture containing the yeast cells and the respiratory substrate is due to the carbon dioxide produced by the respiring yeast cells;**
In step 7, replace the respiratory substrate with equal volume ($2.0cm^3$) of distilled water while keeping all other experimental conditions the same;	**to show that the change in colour number / pH of the mixture containing the yeast cells and the respiratory substrate is due to the presence of the respiratory substrate;**

[2]

(iv) Identify **one** significant source of error in this investigation and suggest an improvement to the procedure that will reduce the effect of the error.

error	improvement
Determination of final colour of the mixture by comparison with the colour chart is subjective;	**Use a colorimeter / spectrophotometer / carbon dioxide sensor / pH meter / pH probe + datalogger / describe different set up + count the number of bubbles produced in one minute;**
Size of yeast-alginate beads may vary;	**Use a syringe to add known volume of the yeast suspension directly to the tube containing the respiratory substrate;**

	
Ambient temperature may fluctuate;	**Use a thermostatically controlled water bath;**

[2]

(b) Microalgae synthesise various carbohydrates to support cellular functions. Cellulose and starch are the most abundant polysaccharides found in microalgae. However, microalgae have also been reported to synthesise two novel carbohydrates, **N1** and **N2**.

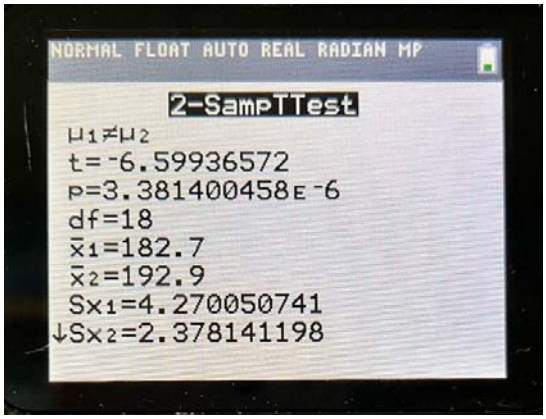
A student carried out an investigation with **N1** and **N2** to find out their effect on the rate of respiration in yeast. Methylene blue was used to monitor the rate of respiration. It acts as an artificial hydrogen acceptor. When this dye is reduced by accepting hydrogen atoms it turns from blue to colourless.

Table 1.1 shows the results of the investigation.

Table 1.1

sample number	time taken for decolourisation of methylene blue / s	
	N1	**N2**
1	181	190
2	182	191
3	190	192
4	178	193
5	184	194
6	183	194
7	181	196
8	179	197
9	190	190
10	179	192
mean ($\underline{x}$)	182.7	192.9
standard deviation (s)	4.27	2.38

variance (s^2)	**18.23 (2dp) or 18.2 (3sf)**	**5.66 (2dp / 3sf)**



(i) Complete Table 1.1 by calculating the variance (s^2) for the time taken for decolourisation of methylene blue in the presence of each of the carbohydrates: **N1** and **N2**. [1]

(ii) A *t*-test can be used to determine whether there is any significant difference between the time taken for decolourisation of methylene blue in the presence of **N1** and **N2**.

Calculate the value of *t* and the number of degrees of freedom, using these formulae:

$$t = \frac{|\underline{x}_1 - \underline{x}_2|}{\sqrt{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)}} \qquad v = n_1 + n_2 - 2$$

key to symbols

s = standard deviation
$\bar{x}$ = mean
n = sample size (number of observations)
v = degrees of freedom

Show your working.

$$t = \frac{|\ 182.7 - \ 192.9\ |}{\sqrt{\left(\ \frac{18.23}{10}\ + \frac{5.66}{10}\ \right)}} \quad = \quad 6.60$$

W – shows figures correctly substituted into formula;

A – gives correct value of *t* and degrees of freedom;

calculated *t* = **6.60**

degrees of freedom = **18**

[2]

(iii) State the null hypothesis.

There is no significant difference between the mean time taken for decolourisation of methylene blue in the presence of N1 and N2. Any differences may be due to chance or sampling error;

[1]

Table 1.2 shows the critical values for *t* at several different probabilities and degrees of freedom.

Table 1.2

degrees of freedom	probability, *p*			
	0.5	0.1	0.05	0.01
1	1.00	6.31	12.71	63.66
2	0.82	2.92	4.30	9.92
3	0.76	2.35	3.18	5.84
4	0.74	2.13	2.78	4.60
5	0.73	2.02	2.57	4.03
6	0.72	1.94	2.45	3.71
7	0.71	1.89	2.36	3.50
8	0.71	1.86	2.31	3.36
9	0.70	1.83	2.26	3.25
10	0.70	1.81	2.23	3.17
11	0.70	1.80	2.20	3.11
12	0.70	1.78	2.18	3.05
13	0.69	1.77	2.16	3.01
14	0.69	1.76	2.14	2.98
15	0.69	1.75	2.13	2.95
16	0.69	1.75	2.12	2.92
17	0.69	1.74	2.11	2.90
18	0.69	1.73	2.10	2.88
19	0.69	1.73	2.09	2.86
20	0.69	1.72	2.09	2.85

(iv) Use Table 1.2 and your answers to **(b)(ii)** to decide whether the null hypothesis should be rejected or not.

Show, with a tick (✓) in the appropriate box, whether you reject or do not reject the null hypothesis and explain your answer.

reject ✓ do not reject ☐

explanation

calculated *t*-value (6.60) > critical *t*-value (2.10) at *p*=0.05 (*p*<0.01);

There is significant difference between the mean time taken for decolourisation of methylene blue in the presence of N1 and N2. Any differences may be due to chance or sampling error;

[3]

[Total: 17]

2 Breweries make use of yeast to produce ethanol under anaerobic conditions. However, high ethanol concentration affects the survival of yeast in brewery. Researchers hypothesised that immobilisation of yeast cells increases the tolerance of yeast to ethanol.

You will investigate the effect of the concentration of ethanol on the rate of anaerobic respiration in immobilised yeast in alginate beads, using hydrogencarbonate indicator.

In addition to the materials from **Question 1**, you are provided with:

- 50.0% ethanol solution, labelled **E1**

E1 contains ethanol, which is harmful and flammable. Suitable eye protection should be worn. The lid on the plastic vial should be kept on, when not in use.

(a) You are required to carry out a serial dilution of **E1** to reduce the concentration of ethanol solution by a factor of two between each of four successive dilutions, **E2**, **E3**, **E4**, and **E5**.

You will need to make up 10.0cm^3 of each solution. Some of this will be used to make the next solution.

Complete Table 2.1 to show how you will make the concentrations of the ethanol solutions **E2**, **E3**, **E4**, and **E5**.

Table 2.1

	E1	E2	E3	E4	E5
percentage concentration of ethanol solution	50.0	25.0	12.5	6.25	3.125
percentage concentration of the ethanol solution to be diluted		50.0	25.0	12.5	6.25
volume of the ethanol solution to be diluted / cm^3		5.0	5.0	5.0	5.0
volume of distilled water to make the dilution / cm^3		5.0	5.0	5.0	5.0

[3]

1m – C: correct percentage **C**oncentration of ethanol solution, correct percentage **C**oncentration of ethanol sol to be diluted

1m – R: correct volume **R**atio and total volume of 10.0cm^3

1m – P: correct **P**recision

Read steps **1–9** before starting the investigation.

Proceed as follows.

1 Prepare ethanol solutions **E1**, **E2**, **E3**, **E4**, and **E5** in the plastic vials provided, as shown in your completed Table 2.1.

2 Add 0.8cm^3 of **S1** and 0.2cm^3 of **H** into a test tube.

3 Add 1.0cm^3 of **E1** into the test tube. Mix the tube contents well. Label the test tube with the final ethanol concentration, which is half of the prepared concentration.

4 Repeat steps **2** and **3** with each of the other concentrations of ethanol that you prepared in step **1**.

5 Check if the colour of the mixture matches colour 6 on the hydrogencarbonate indicator colour chart.

If not, using a 1cm^3 Pasteur pipette, add **B** dropwise to the mixture until colour 6 is achieved.

6 Add five yeast-alginate beads into the test tube containing **E1**. You should ensure that as little distilled water is transferred along with the beads as possible. Seal the test tube with masking tape. Start timing immediately.

7 After 30 seconds, and every 30 seconds from then on, mix the contents of the test tubes to ensure that the colour of the solution is homogenous, until the solution turn yellow colour, matching colour 1 on the colour chart.

8 Record, in **(b)**, the final ethanol concentration in the test tube, the time taken for the hydrogencarbonate indicator to reach colour 1, and the colour of the mixture in terms of the colour number on the colour chart which it matches to.

If the colour 1 has not been reached after 180 seconds, record the time taken as 'more than 180'.

9 Repeat steps **6–8** with each of the other ethanol concentrations that you prepared in step **1**: **E2**, **E3**, **E4** and **E5**.

(b) Record your results in an appropriate table.

Final ethanol concentration / %	Time taken for hydrogencarbonate indicator to reach colour 1 / s	Colour of mixture
25.0	more than 180	2
12.5	more than 180	2
6.25	150	1
3.125	120	1
1.5625	90	1

[4]

H – headings with units (final ethanol conc, not initial)

T – correct trend, decreasing ethanol conc, decreasing time taken and colour no.)

P – correct precision of all values (time max up to 1dp, colour in number)

C – complete data set (correct final ethanol conc and complete data for time taken and colour)

(c) A student suggested to compare the effect of the concentration of ethanol on the rate of respiration in yeast suspension and yeast-alginate beads to test the hypothesis that immobilisation of yeast cells increases the tolerance of yeast to ethanol.

(i) An equivalent volume of yeast suspension need to be used to replace the yeast-alginate beads for this investigation.

You are required to decide on the method that you will use to determine the volume of five yeast-alginate beads.

The method should use the apparatus available, take no longer than five minutes and allow an assessment of the degree of confidence in the results to be made.

Describe the method that you plan to use.

Ref to measurement of displacement / volume difference using syringe OR measure diameter using ruler and calculate volume of sphere by $4/3\pi r^3$ then multiply by 5;
Ref to calculating mean ;

[2]

(ii) Carry out the method that you have described in **(c)(i)** and determine the volume of five yeast-alginate beads.

volume of five yeast-alginate beads = 0.1 – 0.3 cm^3
[1]

Read steps **10–14**.

Proceed as follows.

10 Repeat steps **2** and **3** and place the test tube.

11 Add 6.0cm^3 of distilled water and 3.0cm^3 of **Y** into a 50cm^3 beaker to dilute the yeast suspension to the same concentration as the yeast-alginate beads.

12 Carefully observe the colour changes in steps **13** and **14**.

13 Add the required volume of diluted yeast suspension from step **11** into the test tube from step **10**. The required volume is the volume that you have determine in **(c)(ii)**.

14 Using a 1.0cm^3 syringe, add 1.0cm^3 of **B** dropwise, mixing after each drop.

(d) Based on your observations in steps **13** and **14**, explain why the method described in steps **1–9** cannot be used to determine the effect of the concentration of ethanol on the rate of respiration in yeast suspension.

The colour of the mixture **changed to colour 1 immediately / rapidly** after the addition of diluted yeast suspension**;**

The colour of the mixture **remains at colour 1 / quickly turn to colour 1** after the addition of 1.0cm^3 of B dropwise;

Ref to unable to determine rate of respiration which is calculated from the inverse of time taken for colour change;

Ref to unable to determine effect of the concentration of ethanol as the difference in time taken for different concentration of ethanol cannot be accurately measured / is negligible;

[2]

(e) Instead of adding the hydrogencarbonate indicator into the test tube containing yeast suspension and observing colour change in the same reaction tube, carbon dioxide can be delivered into a second test tube containing hydrogencarbonate indicator.

Design an experiment to compare the effect of the concentration of ethanol on the rate of respiration in yeast-alginate beads and in yeast suspension.

In your plan, you must use:

- yeast-alginate beads
- yeast suspension of the same concentration as yeast-alginate beads
- ethanol concentrations in Table 2.1
- hydrogencarbonate indicator
- delivery tube with bung to fit a test tube.

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

- normal laboratory glassware, e.g. test-tubes, boiling tubes, beakers, measuring cylinders,
- graduated pipettes, glass rods, etc.
- thermostatically controlled water-bath
- syringes
- timer, e.g. stopwatch

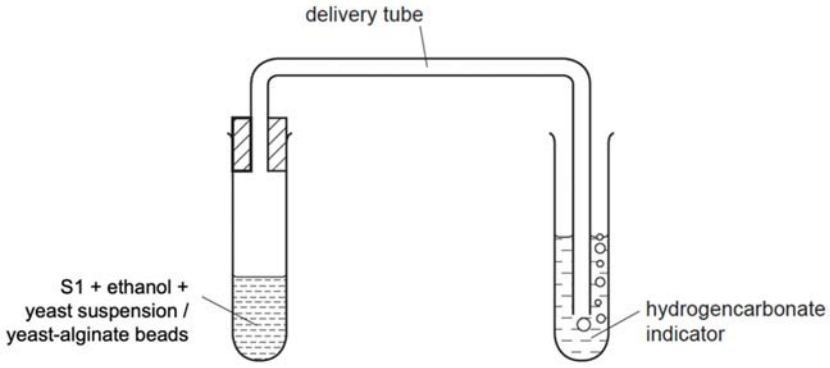
Your plan should:

- include a prediction and the justification for the prediction
- 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 variables you will need to control
- use the correct technical and scientific terms

You do not need to include details of dilution of ethanol or how to make the yeast-alginate beads.

Answer	Mark Scheme
Hypothesis and explanation: • At **low** ethanol concentration, yeast suspension will have **higher** rate of respiration and reach colour 1 in a shorter time than yeast-alginate beads; • Yeast cells are free in a suspension, hence have a **higher surface area-to-volume ratio** than yeast cells immobilised in alginate beads, for efficient access to respiratory substrates / **alginate matrix slows down the diffusion** of respiratory substrate into the bead and carbon dioxide out of the bead • At **high** ethanol concentration, yeast suspension will have **lower** rate of respiration and reach colour 1 in a shorter time than yeast-alginate beads OR **Rate** of respiration will **decrease more quickly for yeast suspension compared to yeast-alginate beads** as ethanol concentration increases; • Yeast cells in a suspension will be **directly exposed to ethanol which can dissolve cell membranes by disrupting hydrophobic interactions between phospholipid tails**, killing the cells; • Alginate beads provide **structural support that helps maintain cell membrane integrity** hence reducing the membrane damage caused by ethanol; • **Alginate matrix slows down the diffusion** of ethanol into the bead, hence immobilised yeast are less affected by ethanol;	Hypothesis and explanation [max 4m] – H
Independent variable: concentrations of ethanol (from serial dilution), and form of yeast preparation (yeast-alginate beads and yeast suspension) (note: there are two independent variables in this qn)	Identify independent variable [1] – IV
Dependent variable: time taken for hydrogencarbonate indicator in second test tube to change to colour 1 Accept colour change of hydrogencarbonate indicator in second test tube at the end of 1min (but this DV is not preferred as it cannot be used to calculate rate) (note: DV is the variable that is **measured**, NOT amount of CO_2 released or rate)	No mark
Controlled variable: • volume / concentration of hydrogencarbonate indicator in second test tube • starting colour of hydrogencarbonate indicator (colour 6) • volume / concentration of yeast suspension and yeast-alginate beads • volume and type of respiratory substrate (S1) • volume of different ethanol concentrations • temperature	Identify at least two controlled variables [1] – CV

Procedure		[6]

1. Conduct a serial dilution to obtain 5 different concentrations of ethanol according to Table 2.1.	No mark (same step as qn)
2. Label 10 test tubes with 2 test tubes for each ethanol concentration.* 3. Using a 1.0cm^3 syringe, add 1.0 cm^3 of the different ethanol concentrations into the respective labelled test tubes.* 4. Using a 1.0cm^3 syringe, add 1.0 cm^3 of S1 into each of the test tubes from step 6.*	No mark (same steps as qn)
5. Place the 10 test tubes in a thermostatically controlled water bath set at 35°C (accept temperature range 30 to 37°C) for 3 minutes (for equilibration).	Describe procedure to keep controlled variable constant (temperature) [1] – CV CV max 2m for procedure
6. Using a 5.0cm^3 syringe, add 2.0cm^3 of distilled water into 10 separate test tubes.* 7. Using a 1.0cm^3 syringe, add 0.2 cm^3 of hydrogencarbonate indicator.* 8. Check if the colour of the mixture matches colour 6 on the hydrogencarbonate indicator colour chart. If not, using a 1cm^3 Pasteur pipette, add sodium bicarbonate solution dropwise to the mixture until colour 6 is achieved. (accept variation in volumes with a **minimum of 2.0cm^3 solution** in second test tube so that delivery tube can be put into the solution)	Describe procedure to keep controlled variable constant (volume of hydrogencarbonate indicator) [1] – CV No mark for correct start colour (same steps as qn) *Describe procedure to use appropriate volumes of **S1, ethanol, yeast & H** with apparatus of appropriate precision [1] – V
9. To one test tube with E1, add five yeast alginate beads. Tightly fit the rubber bung end of a delivery tube to this test tube, and place the other end of the delivery tube into a test tube with hydrogencarbonate indicator from step 10. Start timing immediately.  Delivery tube must be in the hydrogencarbonate indicator solution.	Describe procedure on how to connect delivery tube / draw setup [1] – S
10. Record the time taken for the hydrogencarbonate indicator to reach colour 1 on the colour chart.	Describe procedure to monitor

Accept: At the end of 1 minute, mix the tube contents well and record the colour change of hydrogencarbonate indicator.	dependent variable [1] – DV
11. Repeat step 9 and 10, replacing the yeast alginate beads with 0.2cm^3 of yeast suspension of the same concentration as yeast-alginate beads (from page 12 step 11).	Describe procedure to keep controlled variable constant (volume of yeast must be same as volume of yeast-alginate beads) [1] – CV
12. Calculate the rate by taking the inverse of the time taken for hydrogencarbonate indicator to reach colour 1.	Describe procedure to determine rate [1] – R

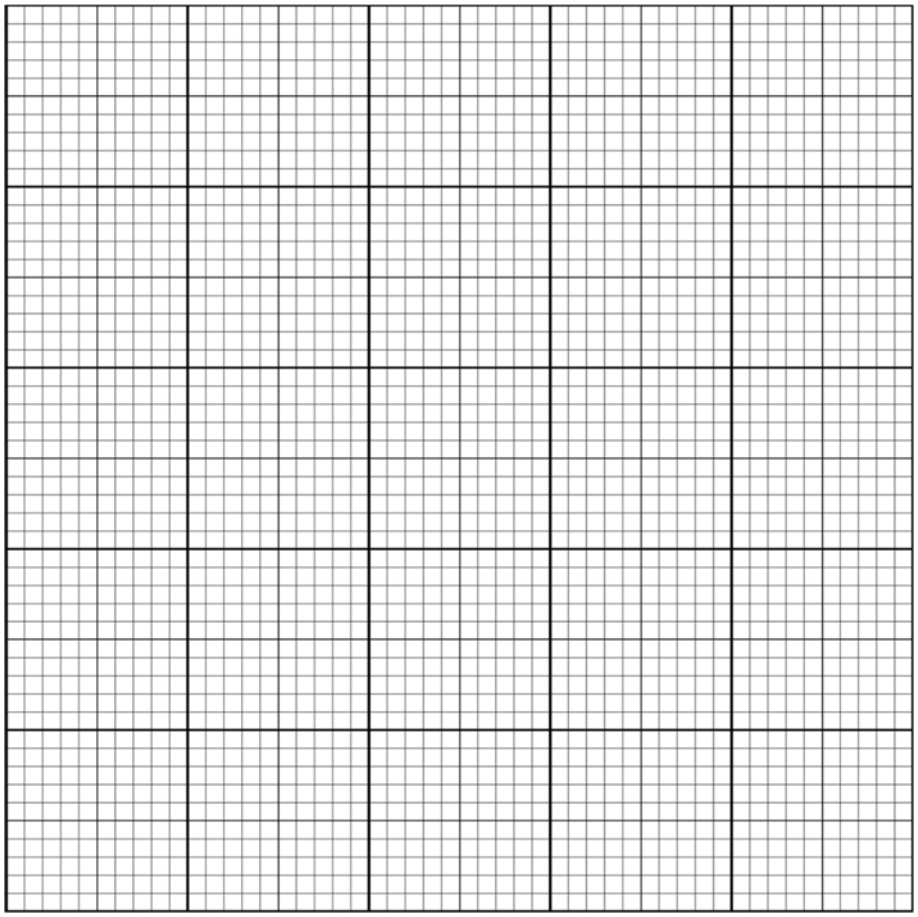
(f) The student carried out the investigation to compare the effect of the concentration of ethanol on the rate of respiration in yeast suspension and yeast-alginate beads.

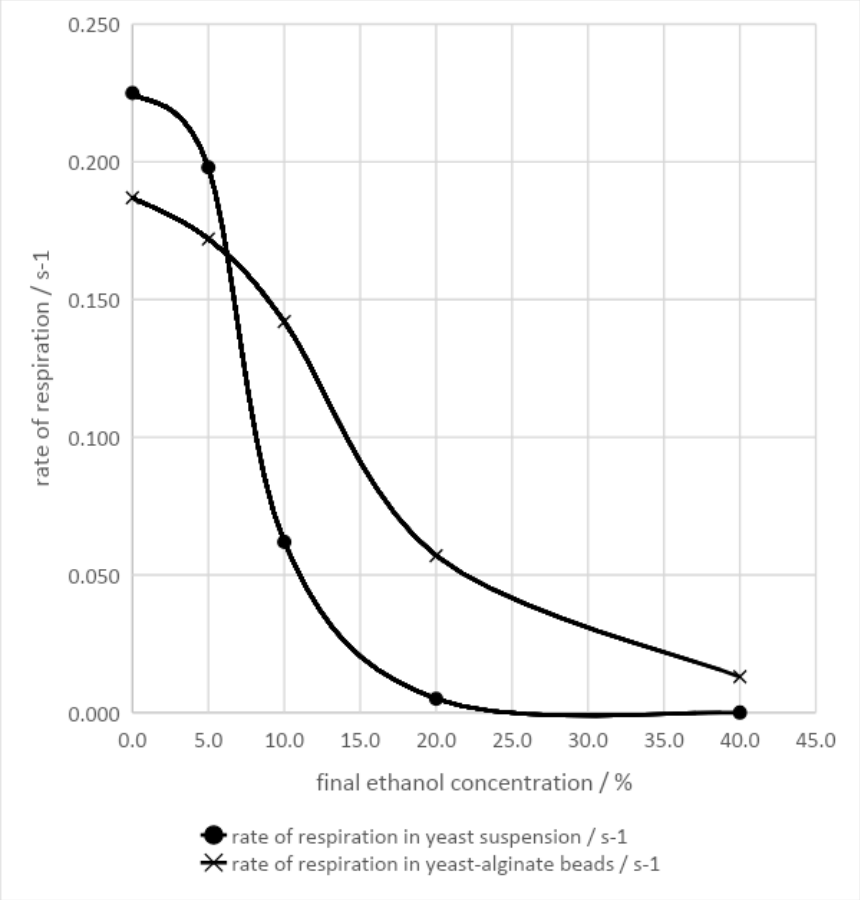
Table 2.2 shows the results of this investigation.

Table 2.2

final ethanol concentration / %	rate of respiration in yeast suspension / s^{-1}	rate of respiration in yeast-alginate beads / s^{-1}
0.0	0.225	0.187
5.0	0.198	0.172
10.0	0.062	0.142
20.0	0.005	0.057
40.0	0.000	0.013

Use the grid provided to display the results shown in Table 2.2. Draw the line of best fit.





G – best-fit curve, pass through all points (accept if points on both sides of the curve are equal distance, and not far away from line)

A – correct axis label with units, correct precision (label every 10 squares)

P – all points plotted correctly

S – appropriate scale

[4]

[Total: 22]

3 During this question you will require access to a microscope.

You are provided with a leaf specimen, **M**, in a petri dish labelled **M**.

You are not expected to be familiar with this specimen.

(a) You are required to:

- prepare a microscope slide of transverse sections of **M**
- record observations of the tissues.

Read steps **1–5**.

Proceed as follows.

1 Cut **M** into two halves as shown in Fig. 3.1.

Use the half of the leaf attached to the leaf stalk for this part of the question.

Keep the other half for part **(b)**.

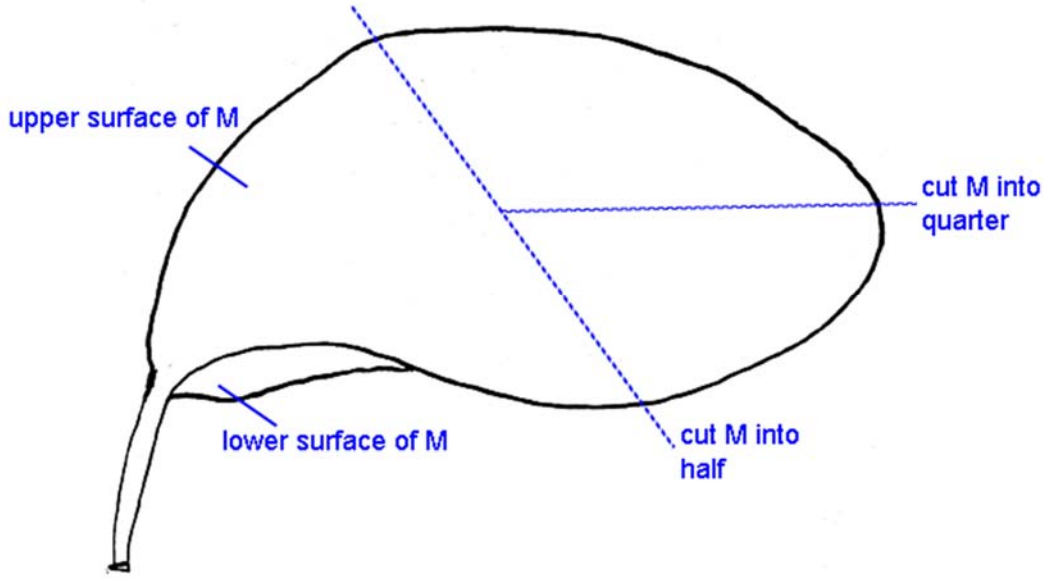


Fig. 3.1

2 Cut several transverse sections of **M** as thin as possible. Each transverse section should be **no more than 0.5mm** in thickness. It is important to make very thin sections of the leaf for details of the tissues to be clearly observable under the microscope.

3 Select three thinnest and complete transverse sections. Place the cut side of the transverse sections facing down on a clean slide. Arrange the three transverse sections so that there is a small space in between each section and all three sections are in close proximity such that they can be covered by a single coverslip.

4 Add a drop of distilled water on the sections and place a coverslip over them. Use a paper towel to remove any excess water that is outside the coverslip.

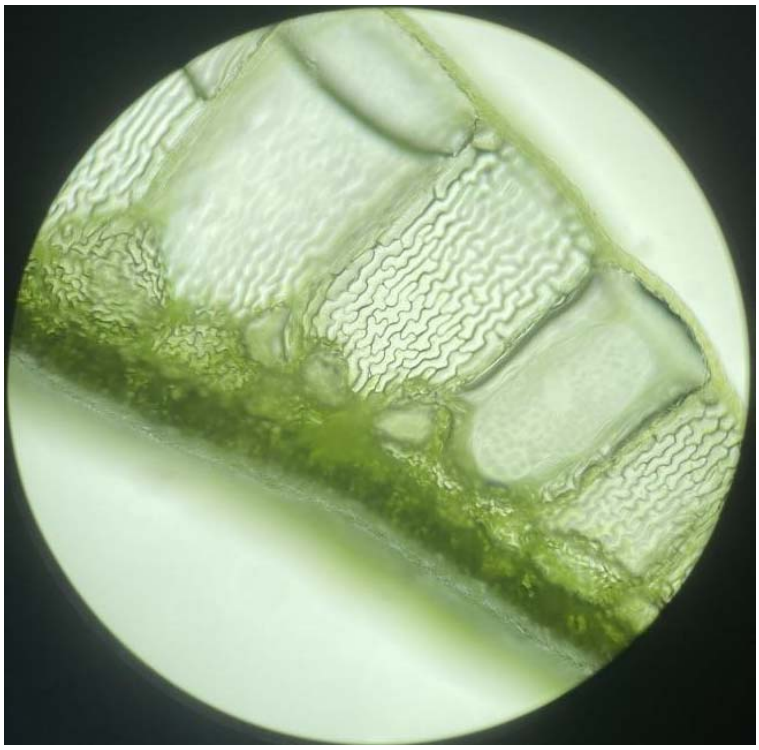
5 Observe the transverse sections using your microscope and focus on a region where a vascular bundle is observable.

Draw a large plan diagram of the part of a transverse section containing a vascular bundle.

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

No cells should be drawn.

Labels are **not** required.



S – size of drawing occupies at least ¾ of space (height > 6.7cm, length > 11.2cm);

P – vascular bundle size is similar to thickness of green tissue layer; [not assessed: green tissue layer varies from 1/20 of the thickness of leaf TS (near the center of leaf) to ½ of the thickness of leaf TS (near the leaf margin);]

D – shows (oval-shaped) vascular bundle at junction between two tissue layers;

[3]

(b) You are required to determine the relative stomatal density of the upper and lower surfaces of the leaf, using the other half of the leaf specimen, **M**.

Read steps **1–5**.

Proceed as follows.

1 Place the remaining half of **M** from step **1** on a clean glass slide.

2 Cut the remaining half of **M** into two halves, as shown in Fig. 3.1.

3 Arrange the two quarter pieces of **M** so that one piece has the upper surface of the leaf faced up and the other piece has the lower surface of the leaf faced up.

4 Add a drop of distilled water on the sections and place a cover slip over the specimen. Label on the cover slip, without blocking the leaf specimen, **U** for the upper surface and **L** for the lower surface.

5 Observe the upper and lower surfaces of the leaf and compare their stomatal density.

(i) Put a tick (✓) in the appropriate box to identify the surface with higher stomatal density.

upper surface ✓ lower surface ☐ [1]

reject lower epidermis, stomata not observable on lower epidermis

(ii) Choose the side of the leaf that has higher stomatal density.

- Observe the specimen with a microscope.
- Measure and record the **mean** length of a stoma in eyepiece graticule units.

R – shows replicates, at least three stomata;

M – calculated mean falls within reference ranges (accept final answer up to 1dp);

If 10x objective, then accept range from 3 to 5 eyepiece graticule units;

If 40x objective, then accept range from 15 to 19 eyepiece graticule units;

mean length of stoma = _____ eyepiece graticule units [2]

- Count the number of stomata in a field of view.

If 10x objective, then accept range from (30 – 65) x 4 stomata;

If 40x objective, then accept range from 7 – 19 stomata;

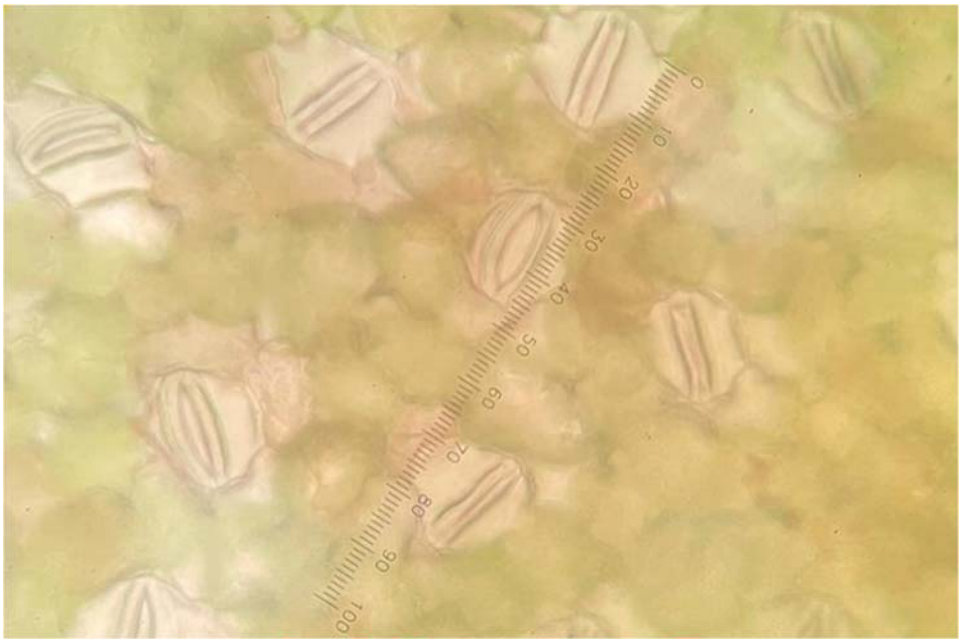
number of stomata = _____

- Record the magnification of the objective lens that you used when measuring the length of the stoma with the eyepiece graticule. Justify your choice.

magnification = æ 10 or 40

+ ref any logical justification relevant to choice of magnification [1]

- Make a large drawing of the stoma that you have selected and **three** surrounding cells. Labels are **not** required.



C – clean, continuous lines

S – size of drawing occupies at least ¾ of space (height > 6.0cm, length > 10.5cm);

P – shows appropriate proportion of length:width of guard cells;

D – shows two guard cells and one other cell;

(iii) Using the measurement of length in eyepiece units recorded in **(b)(ii)**, calculate the actual length, in micrometres (μm), of the stoma that you have drawn.

You can assume that calibration of the eyepiece graticule using stage micrometer gives the results shown in Table 3.2.

Table 3.2

objective lens used	number of eyepiece graticule units in a 0.1mm division of a stage micrometer
æ10	9
æ40	38

Show your working.

W1 – shows working for determining the length of 1 eyepiece graticule unit;

objective lens used	number of eyepiece graticule units in a 0.1mm division of a stage micrometer	length of 1 eyepiece graticule unit
æ10	9	= 100 μm/ 9 = 11.1
æ40	38	= 100 μm/ 38 = 2.6

1 eyepiece graticule unit = _____ μm

W2 – shows working for determining the length of 1 eyepiece graticule unit;

objective lens used	mean length / μm	Mean actual length / μm
æ10	3.0 – 5.0	3 x 11.1 = 33.3 5 x 11.1 = 55.5
æ40	15.0 – 19.0	15 x 2.6 = 39.0 19 x 2.6 = 39.4

mean actual length = ... μm
[2]

(iv) Calculate the stomatal density of the side of the leaf with higher stomatal density, using these formulae:

$$stomatal\ density = \frac{number\ of\ stomata}{area} \qquad area\ of\ circle = \pi r^2,$$

where r is the radius of a circle

You can assume that the diameter of a field of view is given in Table 3.3.

Table 3.3

objective lens used	diameter of a field of view / mm
æ10	2.0
æ40	0.5

Show your working.

1 – shows how area is calculated;

objective lens used	diameter of a field of view / mm	area of field of view (πr^2) / mm^2	area of field of view ($2\pi r^2$) / mm^2
æ10	2.0	3.141	6.282
æ40	0.5	0.1963	0.3926

2 – shows how density is calculated;

objective lens used	stomatal count	density based on πr^2 / mm^2	density based on $2\pi r^2$ / mm^2
æ10	(30 – 65) x 4	38 – 83	19 – 41
æ40	7 – 19	36 – 97	18 – 48

3 – gives answer in whole number;

density = ... stomata per mm^2
[3]

[Total: 16]