H

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

CANDIDATE NAME			
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	17
2	22
3	16

Total	55

This document consists of **20** printed 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**
- distilled water
- 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.

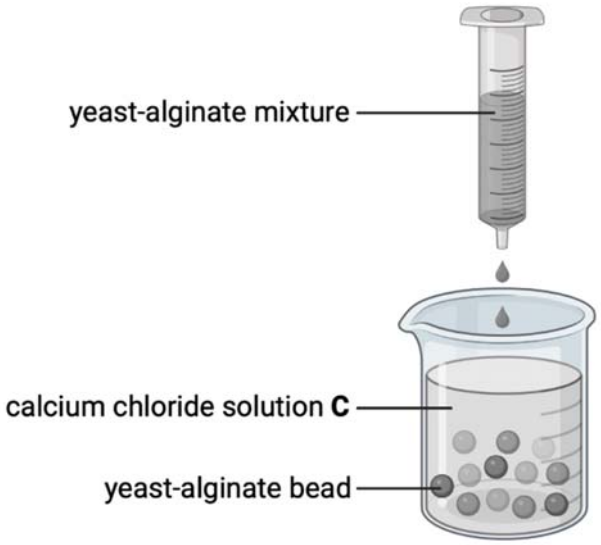


Fig. 1.1

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 hydrogencarbonate indicator 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.

[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 ☐ **S2** ☐ **S3** ☐

explanation _____

[3]

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

control _____

_____

purpose _____

_____

_____ [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 _____

_____

_____ [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)		

(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.

value of t = _____

number of degrees of freedom = _____
[2]

(iii) State the null hypothesis.

.....

.....

..... [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 _____

_____

_____

_____ [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				
percentage concentration of the ethanol solution to be diluted					
volume of the ethanol solution to be diluted / cm^3					
volume of distilled water to make the dilution / cm^3					

[3]

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 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.

[4]

(c) A student decided 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 needs 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.

[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 = _____ 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.

_____

_____

_____

_____

_____ [2]

Question 2 continues on page 14.

(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.

[6]

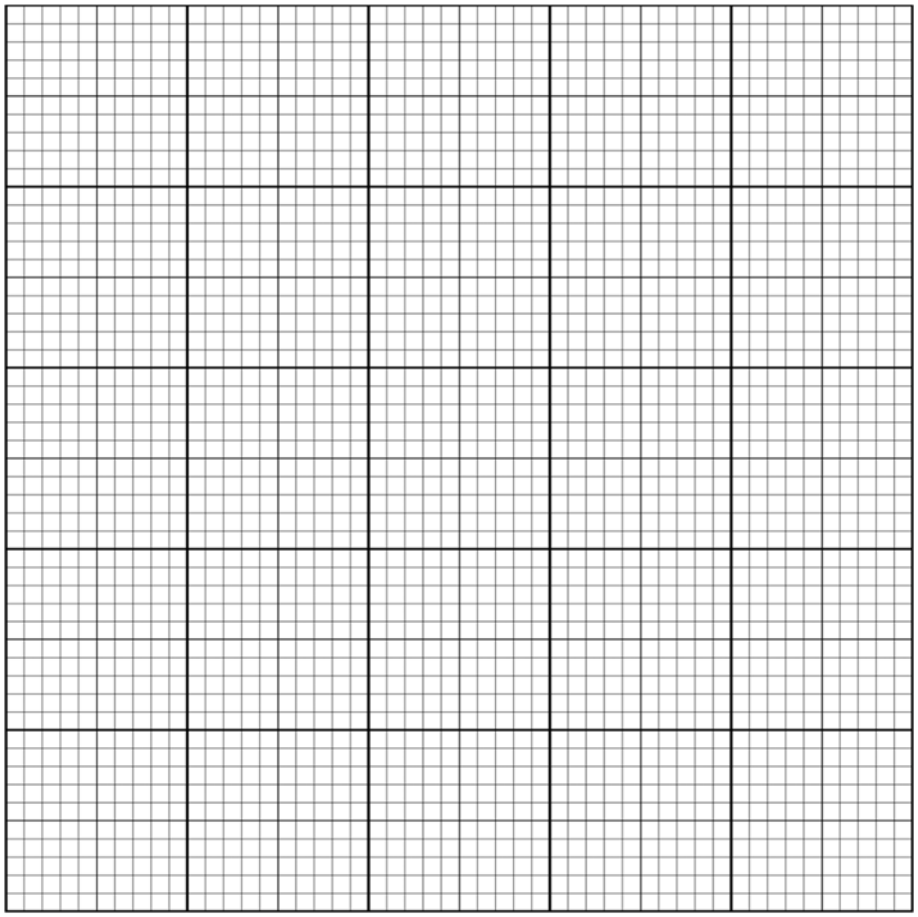
(f) The student carried out the investigation to compare the effect of the concentration of ethanol on the rate of respiration in yeast suspension and yeast-alginate beads.

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.



[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)**.

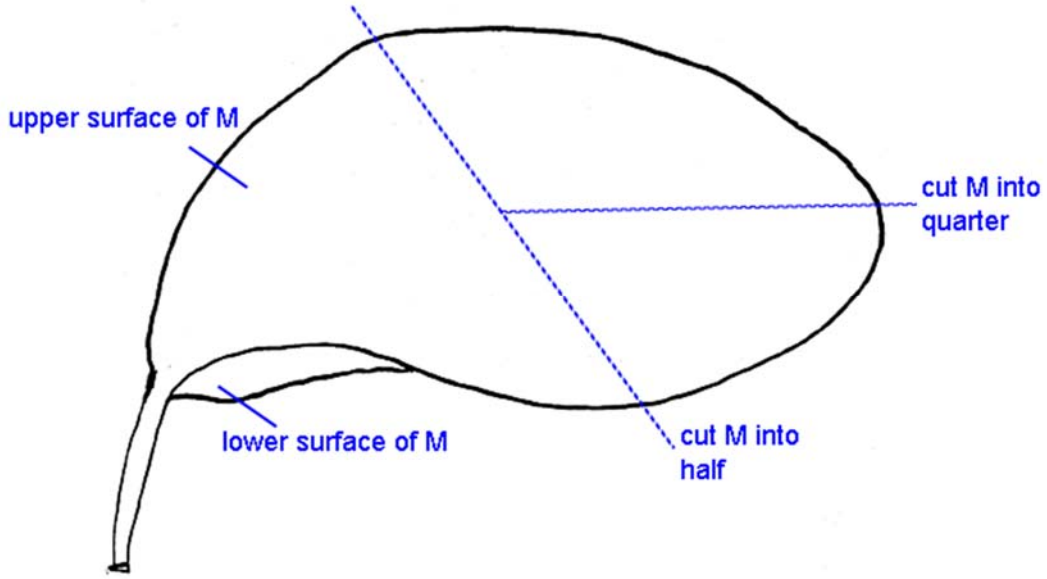


Fig. 3.1

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.

[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 facing up and the other piece has the lower surface of the leaf facing 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]

(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.

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

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

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 = × _____

_____

_____ [1]

- Make a large drawing of the stoma that you have selected and **three** surrounding cells.

Labels are **not** required.

[4]

(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
×10	9
×40	38

Show your working.

1 eyepiece graticule unit = ... μm

mean actual length = ... μm
[2]

(iv) Calculate the stomatal density of the surface 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
×10	2.0
×40	0.5

Show your working.

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

[Total: 16]