HWA CHONG INSTITUTION
JC2 Preliminary Examinations
Higher 2

CANDIDATE NAME | CT GROUP 23S7__

CENTRE NUMBER | INDEX NUMBER

BIOLOGY

9744/04

Paper 4 Practical

26 August 2024

Candidates answer on the Question Paper.

2 hours 30 minutes

Additional Materials: As listed in the Confidential Instructions.

INSTRUCTIONS TO CANDIDATES

There are **two** question booklets (**I** and **II**) to this paper. Write your **name**, **CT group**, **Centre number** and **index number** in the spaces provided at the top of this cover page and on the lines provided at the top of the cover page of Booklet **II**.

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.

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

Shift
Laboratory

INFORMATION FOR CANDIDATES

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.

The number of marks is given in brackets [ ] at the end of each question or part question.

You are reminded of the need for good English and clear presentation in your answers.

For Examiners' Use	
1	/ 30
2	/ 25
Total	/ 55

This document consists of **16** printed pages.

QUESTION 1

A group of students investigated the growth of different varieties of yeast.

The students learnt that the rate of respiration can be used as a measure of the growth of a yeast culture, which depends on a variety of factors.

One such factor is the activity of enzymes that are responsible for hydrolysing sucrose into reducing sugars to be used as respiratory substrate.

You will investigate the activity of the enzymes in yeast cells, which will be immobilised in sodium alginate beads.

You are provided with the materials shown in Table 1.1.

Table 1.1

labelled	contents	hazard	volume / cm^3
Y	yeast cell suspension	none	40
A	sodium alginate solution	harmful irritant	20
C	calcium chloride solution	harmful irritant	30
S	sucrose solution	none	100
B	Benedict's solution	harmful irritant	30

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

You will investigate the activity of yeast enzymes by using different numbers of beads of immobilised yeast cells in sucrose solution, **S**.

Carry out steps **1** – **9** to immobilise the yeast cells in sodium alginate beads.

1 Put 10 cm^3 of **A** into a beaker.

2 Stir the yeast cell suspension **Y** with a glass rod.

3 Put 10 cm^3 of **Y** into the beaker containing **A** and mix well. Do **not** introduce bubbles into the mixture.

4 Put 20 cm^3 of **C** into another beaker.

5 Use a 10 cm^3 syringe to collect 10 cm^3 of the mixture of **A** and **Y**.

6 Hold this syringe over the beaker containing 20 cm^3 of **C** (step 4), as shown in Fig. 1.1.

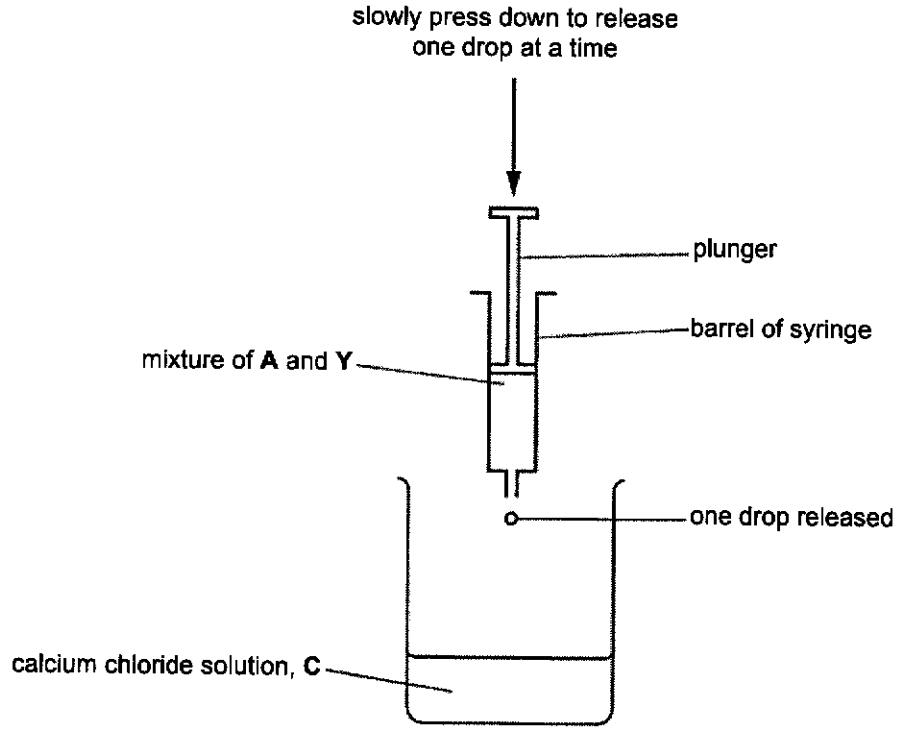

Fig. 1.1

7 Hold the barrel of the syringe with one hand while slowly pressing down on the plunger with the other hand so that a drop of the mixture is released into solution **C**. The drop will form a bead.

8 Repeat step 7 to make at least 31 beads. The immobilised yeast beads must be left in the beaker for 5 minutes.

9 After 5 minutes tip the beads and the solution into a Petri dish.

You will test the activity of the yeast enzymes by using different numbers of beads (1, 2, 4, 8 and 16) in sucrose solution.

Carry out steps **10 – 20**.

10 Label five beakers 1, 2, 4, 8 and 16 **and** label five test-tubes 1, 2, 4, 8 and 16.

11 Put 1, 2, 4, 8 or 16 beads into each of the appropriately labelled beakers, as shown in Fig. 1.2.

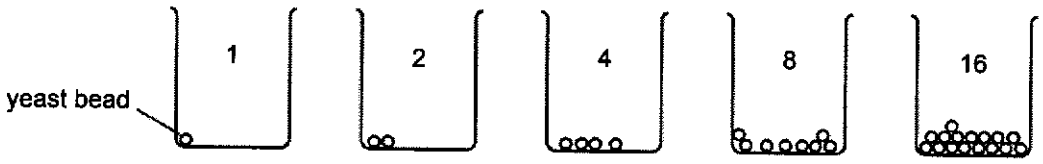

Fig. 1.2

12 Put 10 cm^3 of sucrose solution, **S**, into each of the beakers containing the beads.

13 Start timing and leave for 5 minutes. While you are waiting set up a water-bath ready for step 14 and step 19.

In step 19 you will use the water-bath to carry out the test for reducing sugars using Benedict's solution, **B**.

14 Heat the water-bath to around 90 °C to 100 °C.

15 At the end of 5 minutes (step 13) stir the contents of each beaker.

16 Use a syringe to transfer 2 cm^3 of the solution from beaker 1 into the test-tube labelled 1.

17 Repeat step 16 for each of the beakers and test-tubes labelled 2, 4, 8 and 16.

18 Put 2 cm^3 of Benedict's solution, **B**, into each of the test-tubes labelled 1, 2, 4, 8 and 16.

19 Put test-tube 1 into the water-bath and time how long before the appearance of the first colour change. If there is no colour change after 2 minutes, stop timing and record as 'more than 120'.

Record your result in **(a)(ii)**.

20 Repeat step 19 for the other test-tubes, 2, 4, 8 and 16.

(a)(i) State the independent variable in this investigation.

.....

[1]

(ii) Record your results in an appropriate table.

[4]

(iii) Explain your results in **(a)(ii)**.

.....

.....

.....

.....

..... [2]

(iv) Other than lack of replicates and repeats, identify **one** main source of error in this investigation and suggest an **improvement** to reduce the effect of this error.

source of error: .....

.....

.....

improvement: .....

.....

..... [2]

(v) A student set up a beaker as a control experiment. The result of the control experiment showed that the sucrose was hydrolysed by an enzyme.

Suggest what substances the student put in the beaker for the control experiment.

.....

..... [1]

(vi) The procedure described in step 1 to step 20 investigated the effect of changing the number of yeast beads on the rate of hydrolysis of sucrose.

Describe how you would modify the procedure to investigate the effect of changing the concentration of the sucrose solution on the rate of hydrolysis of sucrose.

.....

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..... [2]

Another factor which could affect the rate of respiration is the variety of yeast.

Respiration rates can be measured using the redox indicator TTC.

- During respiration, hydrogen ions are removed from reducing sugars to reduce hydrogen carriers such as NAD and FAD.
- A redox indicator can be used as a hydrogen carrier in experimental conditions instead of NAD or FAD.
- The colour change of the redox indicator can be measured using a colorimeter.

Carry out a preliminary experiment to determine how the colour change of TTC would be like.

Using a 10 cm^3 syringe, add 5 cm^3 of yeast cell suspension **Y** into a conical flask, followed by 10 cm^3 of sucrose solution, **S** and 1 cm^3 of TTC, **T** as shown in Fig. 1.3. Observe the colour of TTC.

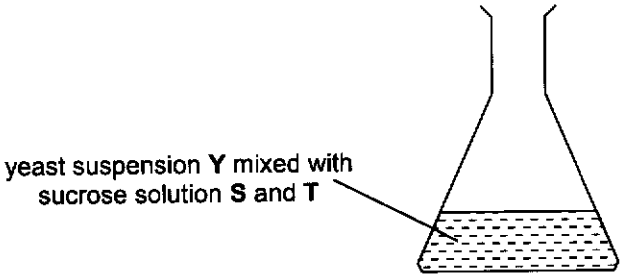

Fig. 1.3

(b)(i) Record the colour change of TTC after 10 minutes.

.....

..... [1]

(ii) Explain why the colour change of TTC in **(b)(i)** can only be observed after 10 minutes and not immediately upon addition.

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.....

.....

..... [2]

(c) Three different varieties of yeast, commonly used in food manufacture, are compressed yeast, active dry yeast and instant yeast.

The students decided to compare the growth rates of the three different varieties of yeast by measuring their respiration rates. They decided to use TTC as the redox indicator.

Describe a method that students could use to compare the respiration rates of the three varieties of yeast.

In your method, you must use:

- 10% suspension of the three varieties of yeast (compressed yeast, active dry yeast and instant yeast)
- 5% sucrose solution
- redox indicator TTC
- distilled water
- 3 cm^3 cuvettes for measuring absorbance using the colorimeter.

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

- normal laboratory glassware e.g. test-tubes, boiling tubes, beakers, conical flasks, measuring cylinders, glass rods etc.
- syringes
- timer, e.g. stopwatch
- water-bath

Your method should be set out in a logical order and be detailed enough to let another person follow it.

[7]

(d) The students found that compressed yeast gave the highest rate of respiration.

The students then carried out two further experiments to find the best conditions for growth of compressed yeast.

In both experiments, absorbance was measured in arbitrary units (a.u.). The higher the absorbance, the greater the respiration rate. Respiration is proportional to the growth rate of the yeast.

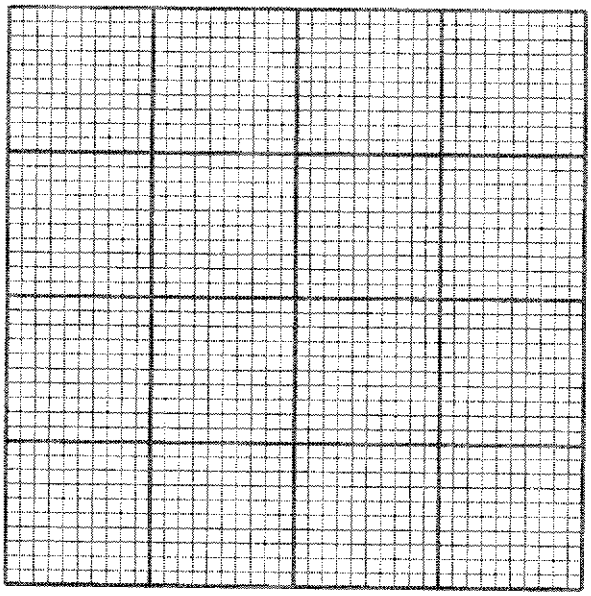
In the first experiment, they investigated the effect of changing pH and incubation time at a constant temperature of 30 °C.

The results of the first experiment are shown in Table 1.2.

Table 1.2

incubation time / hours	pH 6.0		pH 9.0	
	absorbance / a.u.	$2 \times S_M$	absorbance / a.u.	$2 \times S_M$
1	1.48	+/- 0.28	0.50	+/- 0.05
2	0.80	+/- 0.05	0.70	+/- 0.05
3	1.83	+/- 0.07	0.65	+/- 0.10
4	3.15	+/- 0.35	0.40	+/- 0.08

(i) Use the grid provided to display the results shown in Table 1.2 in an appropriate form.



[4]

(ii) State what the standard error (S_M) shows.

.....

..... [1]

(iii) The data in Table 1.2 shows the 95% confidence intervals for the data.

95% confidence interval = +/− 2 × S_M

State what this indicates about the data.

.....

..... [1]

In the second experiment the students investigated the effect of changing pH and temperature at a constant incubation time of 4 hours.

The results of the second experiment are shown in Table 1.3.

Table 1.3

temperature / °C	pH 6.0		pH 9.0	
	absorbance / a.u.	S_M	absorbance / a.u.	S_M
22	2.28	+/- 0.60	1.40	+/- 0.72
30	3.16	+/- 0.28	0.94	+/- 0.02
40	1.10	+/- 0.52	0.54	+/- 0.04
50	0.48	+/- 0.08	0.28	+/- 0.02

(iv) After completing these two experiments, the students concluded that the growth rate of yeast is highest when incubated at 30 °C and pH 6.0 for 4 hours.

State **two** ways in which the data support this conclusion.

1 .....

.....

2 .....

..... [2]

[Total: 30]

QUESTION 2

During this question you will require access to a microscope, a ruler and slide **K1**.

K1 is a slide of a stained transverse section through a plant leaf.

(a)(i) Draw a large plan diagram of the part of the leaf on slide **K1** shown by the shaded area in Fig. 2.1.

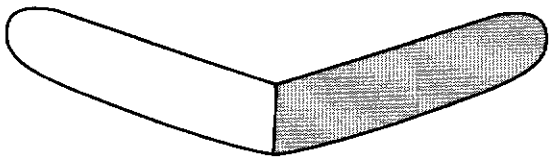


Fig. 2.1

A plan diagram shows the arrangement of different tissues. Your drawing should show the correct shapes and proportions of the different tissues and must include at least **three** vascular bundles.

No cells should be drawn.

Use **one** ruled label line and the label **S** to identify a stomatal opening.

[4]

(ii) Observe the outermost layer of cells on the upper and lower surfaces of the leaf on slide **K1**. This outermost layer is called the epidermis and is one cell thick. Select a pair of guard cells, which are epidermal cells that surround a stomatal opening, and two cells from the layer below the guard cells.

Each guard cell must touch at least one cell from the layer below it.

Make a large drawing of this group of four cells.

Labels are not required.

[4]

(b) You are required to estimate the stomatal density of the leaf on slide **K1**, for which the number of stomata per unit length of the leaf blade can be calculated.

Fig. 2.2 shows the transverse section though the leaf on slide **K1**. The lines **P** and **Q** are drawn across the length of each half of the leaf.

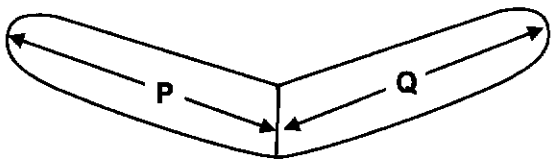


Fig. 2.2

(i) Use the ruler to measure the length of each half of the leaf, along the lines **P** and **Q**.

length of leaf along **P** = ...

length of leaf along **Q** = ...

[1]

(ii) Examine the slide **K1** using a microscope and locate the stomata. Observe the transverse section using both the low-power and high-power objective lenses and choose the lens that is most suitable for counting the number of stomata.

State which objective lens you have decided to use and give a reason for your choice.

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.....

..... [1]

(iii) Using the objective lens selected in **(b)(ii)**, determine the number of stomata on each half of the leaf blade.

Count every stoma for which the **pair of guard cells** surrounding it is visible. Record your results in Table 2.1.

Table 2.1

part of blade	number of stomata
P	
Q	

[1]

(iv) Calculate the stomatal density of the leaf on slide **K1**. The number of stomata per unit length of the leaf blade can be calculated. Show your working.

[3]

(c) Fig. 2.3 is a photomicrograph showing part of a leaf surface.

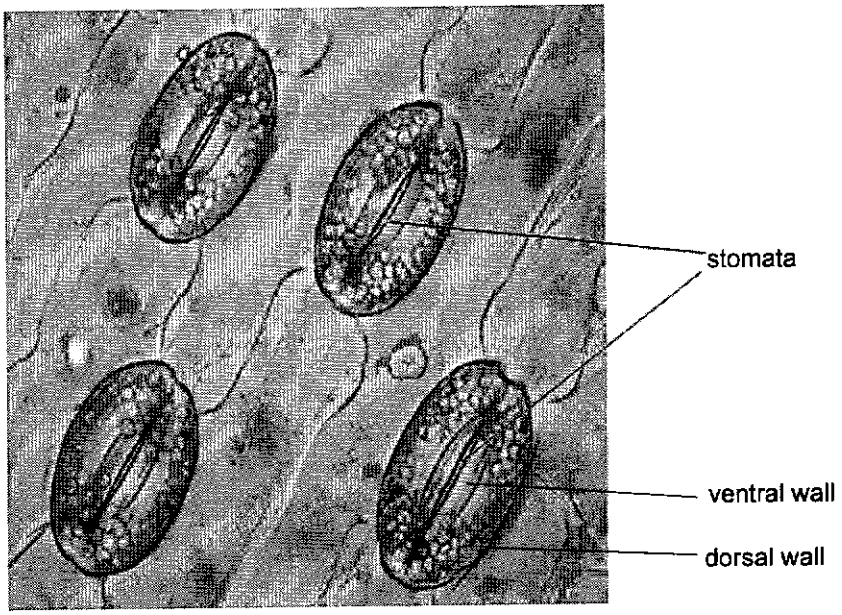

Fig. 2.3

The actual area of the photomicrograph in Fig. 2.3 is 0.04 mm^2.

The leaf from which Fig. 2.3 is taken has a total surface area of 20 cm^2.

1 cm^2 = 100 mm^2

(i) Use Fig. 2.3 to estimate the total number of stomata on the leaf.
Show your working.

number of stomata on the leaf = ...

[3]

(ii) One way to improve the accuracy of the estimate of the total number of stomata on a leaf is to use a photomicrograph with a larger area.

State **one other** way to improve the accuracy of the estimate of the total number of stomata on a leaf.

.....

..... [1]

(iii) Fig. 2.4 is a photomicrograph showing part of a leaf surface of a different type of plant.

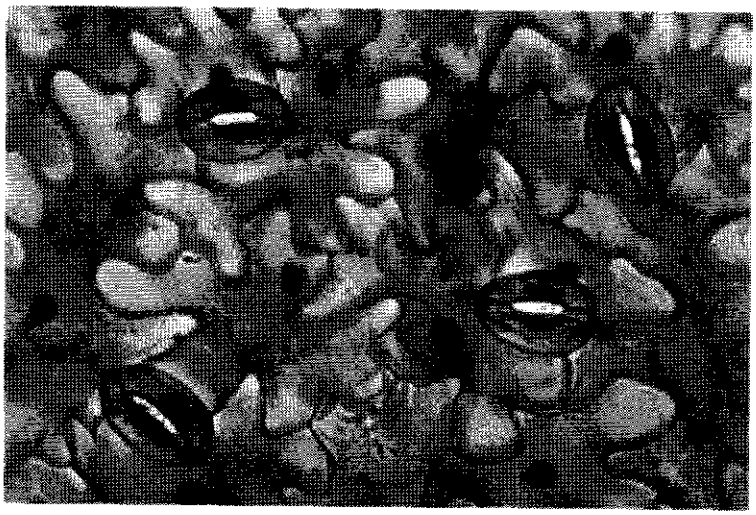

Fig. 2.4

Fig. 2.3 and Fig. 2.4 have the same magnification.

Identify the observable differences between the leaf surface shown in Fig. 2.3 and the leaf surface shown in Fig. 2.4.

Record the observable differences in Table 2.2.

Table 2.2

feature	Fig. 2.3	Fig. 2.4

[4]

Stomatal opening is crucial for gaseous exchange between a plant and the environment during daytime. Researchers working on the mechanism of stomatal opening made the following observations:

- the ventral wall of a guard cell is thicker than the dorsal wall, as shown in Fig. 2.3,
- there is active transport of potassium ions (K^+) into the guard cells from the surrounding epidermal cells during daytime.

(iv) Using the information provided, suggest how a stoma opens during daytime to facilitate gaseous exchange.

.....

.....

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.....

..... [3]

[Total: 25]

--- END OF PAPER---

HWA CHONG INSTITUTION (COLLEGE SECTION)
2024 JC2 9744 H2 BIOLOGY
PRELIMINARY EXAMINATIONS PAPER 4 MARK SCHEME

QUESTION 1

(a)(i) State the independent variable in this investigation. [1]

number of beads

(ii) Record your results in an appropriate table. [4]

number of beads	time taken for appearance of the first colour change / s
1	more than 120
2	more than 120
4	42
8	20
16	13

1 correct column headings and units
2 times recorded for five sets of beads
3 all times recorded in seconds
4 correct trend

(iii) Explain your results in **(a)(ii)**. [2]

1 ref. to more active sites available
2 ref. to more reducing sugar produced

(iv) Other than lack of replicates and repeats, identify **one** main source of error in this investigation and suggest an **improvement** to reduce the effect of this error. [2]

Source of error	Improvement
S1 ref. to beads having different sizes	**I1** ref. to use of different concentration of yeast enzymes
S2 ref. to beads not left for the same duration in sucrose	**I2** ref. to staggering start times
S3 ref. to sodium alginate and yeast may not be mixed well manually	**I3** ref. to use of magnetic stirrer

(v) A student set up a beaker as a control experiment. The result of the control experiment showed that the sucrose was hydrolysed by an enzyme.

Suggest what substances the student put in the beaker for the control experiment. [1]

boiled and cooled yeast

(vi) The procedure described in step 1 to step 20 investigated the effect of changing the number of yeast beads on the rate of hydrolysis of sucrose.

Describe how you would modify the procedure to investigate the effect of changing the concentration of the sucrose solution on the rate of hydrolysis of sucrose. [2]

1 use a set number of yeast beads

2 prepare at least five concentrations of sucrose by dilution

(b)(i) Record the colour change of TTC after 10 minutes. [1]

colourless to pink

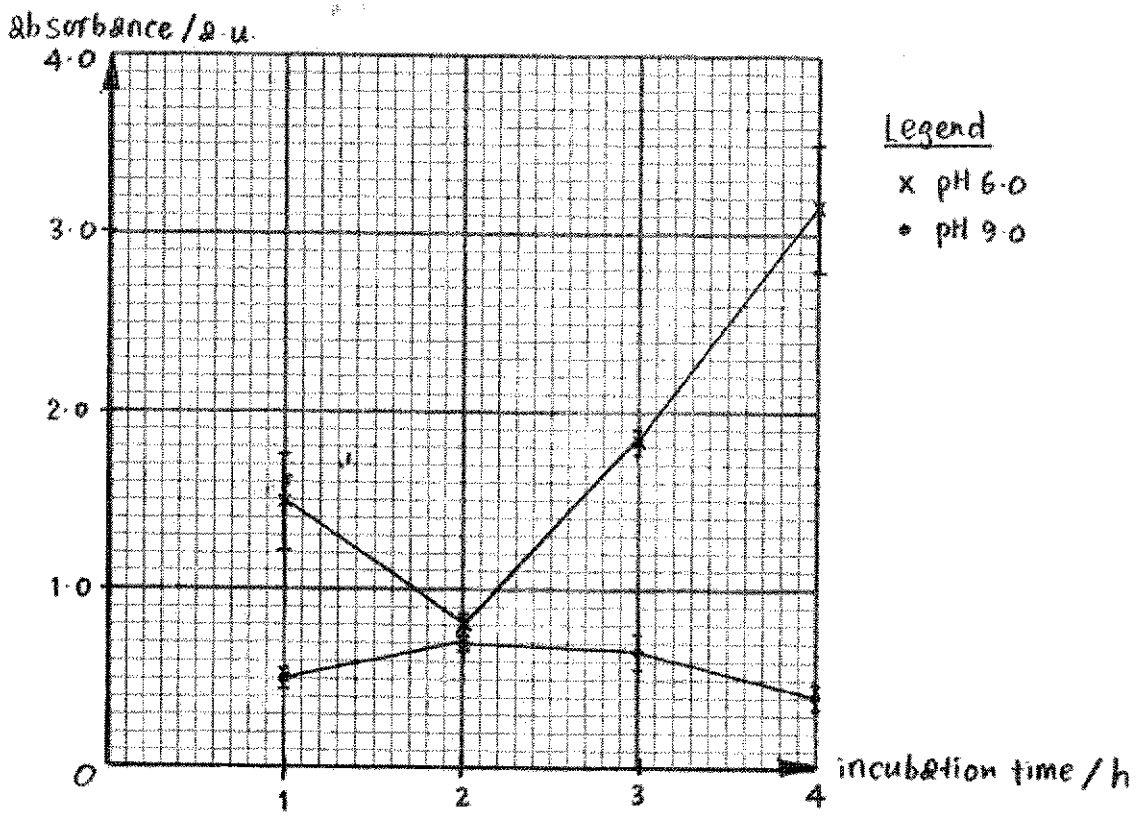
(ii) Explain why the colour change of TTC in **(b)(i)** can only be observed after 10 minutes and not immediately upon addition. [2]

1 ref. to hydrolysis sucrose into reducing sugars
2 ref. to reduction of TTC due to respiration

(c) Describe a method that students could use to compare the respiration rates of the three varieties of yeast. [7]

1 same / stated / known, volume (suspension), of each yeast (added to separate flasks)
2 same / stated / known, volume of, nutrient solution / sucrose
3 ref. to method to maintain temperature
4 suitable temperature in range 15 °C – 80 °C
5 idea of equilibration / bringing yeast suspension and nutrient solution, to temperature, before mixing
6 add TTC / redox indicator, to yeast / yeast and nutrient mixture
7 *time for recording absorbance*
8 ref. to procedure on use of colourimeter including zeroing (with distilled water)
9 ref. to method of maintaining homogeneity (of yeast)
10 use (at least) 3 replicates / repeats and find mean or identify / eliminate / remove, anomalies
11 ref. to low risk

(d)(i) Use the grid provided to display the results shown in Table 1.2 in an appropriate form. [4]



1 correct axes labels and units

2 use of appropriate scale

3 correct plotting of points

4 correct plotting of S_M

(ii) State what the standard error (S_M) shows. [1]

idea of how close the (sample) mean is to the true / population mean

(iii) The data in Table 1.1 shows the 95% confidence intervals for the data.

95% confidence interval = +/− 2 × S_M

State what this indicates about the data. [1]

1 95% of the data would be expected to lie within this range

2 at 1 / 3 / 4, hours, the (sample) mean was reliable because the confidence intervals do not overlap

(iv) After completing these two experiments the students concluded that the growth rate of yeast is highest when incubated at 30 °C and pH 6.0 for 4 hours.

State **two** ways in which the data support this conclusion. [2]

1 *from Table 1.2 / experiment 1*
pH 6.0 gives the highest absorbance at 4 hours incubation
or
from Table 1.3 / experiment 2
at 30°C at pH 6 absorbance is highest

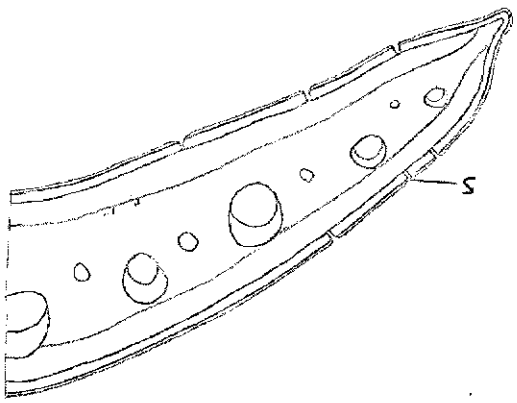
2 *from Table 1.2 / experiment 1*
the CI / error bars (for pH 6) do not overlap (at 4 hours)
or
from Table 1.3 / experiment 2
standard errors / S_M, do not overlap (with other, temperatures / pHs)

[Total: 30]

QUESTION 2

(a)(i) Draw a large plan diagram of the part of the leaf on slide **K1** shown by the shaded area in Fig. 2.1.

Use **one** ruled label line and the label **S** to identify a stomatal opening. [4]



1 no cells + clear continuous lines + correct sector

2 correct size of plan drawing + correct labelling of S

3 correct arrangement of tissues

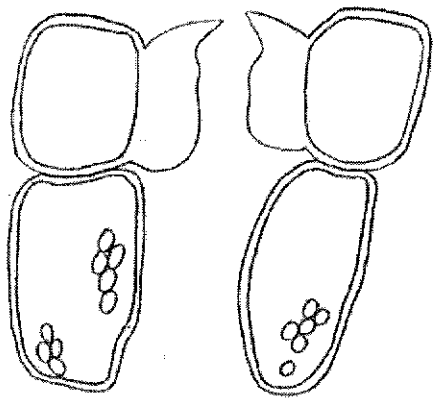
4 correct shape + correct proportion

(ii) Observe the outermost layer of cells on the upper and lower surfaces of the leaf on slide **K1**. This outermost layer is called the epidermis and is one cell thick. Select a pair of guard cells, which are epidermal cells that surround a stomatal opening, and two cells from the layer below the guard cells.

Each guard cell must touch at least one cell from the layer below it.

Make a large drawing of this group of four cells.

Labels are not required. [4]



1 clear continuous lines + cell wall shown as double lines + correct size

2 correct arrangement

3 correct shape

4 correct proportion

(b)(i) Use the ruler to measure the length of each half of the leaf, along the lines **P** and **Q**. [1]

length of leaf along **P** = 3 mm

length of leaf along **Q** = 3 mm

makes measurement of length in whole numbers in mm

(ii) State which objective lens you have decided to use and give a reason for your choice. [1]

1 low-power + all stomata for each half of the leaf blade are in the field of view
2 high-power + guard cells / stomata can be identified for accurate count

(iii) Using the objective lens selected in **(b)(ii)**, determine the number of stomata on each half of the leaf blade.

Count every stoma for which the **pair of guard cells** surrounding it is visible. Record your results in Table 2.1. [1]

Table 2.1

part of blade	number of stomata
P	17
Q	15

makes count in whole numbers

(iv) Calculate the stomatal density of the leaf on slide **K1**. The number of stomata per unit length of the leaf blade can be calculated. Show your working. [3]

1 summation of number of stomata + summation of length of leaf blade
2 shows division of total number of stomata by total length of leaf blade
3 shows answer expressed to whole number + correct units

(c)(i) Use Fig. 2.3 to estimate the total number of stomata on the leaf.
Show your working. [3]

1 shows conversion of cm^2 to mm^2
2 shows division of leaf area by 0.04
3 shows multiplication by 4 + correct answer

(ii) One way to improve the accuracy of the estimate of the total number of stomata on a leaf is to use a photomicrograph with a larger area.

State **one other** way to improve the accuracy of the estimate of the total number of stomata on a leaf. [1]

use more fields of view / more micrographs

(iii) Identify the observable differences between the leaf surface shown in Fig. 2.3 and the leaf surface shown in Fig. 2.4.

Record the observable differences in Table 2.2. [4]

Table 2.2

feature	Fig. 2.3	Fig. 2.4
1 nucleus in guard cells	nucleus not visible	nucleus visible
2 chloroplasts in guard cells	chloroplasts visible	chloroplasts not visible
3 nucleus in surrounding epidermal cells	nucleus not visible	nucleus visible
4 stomatal opening	stomata closed	stomata opened

(iv) Using the information provided, suggest how a stoma opens during daytime to facilitate gaseous exchange. [3]

1 ref. to accumulation of K^+ makes water potential of guard cells more negative than surrounding epidermal cells

2 ref. to endosmosis / movement of water from surrounding epidermal cells into guard cells

3 ref. to guard cell becomes turgid + ventral wall bends less / dorsal wall bends more

[Total: 25]