

VICTORIA JUNIOR COLLEGE JC 2 PRELIMINARY EXAMINATION 2024 HIGHER 2

NAME:

CT CLASS:

BIOLOGY

Paper 4 Practical

9744 / 04

29/08/2024

2 hours 30 minutes

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

READ THESE INSTRUCTIONS FIRST

Write your name and CT in all the work in hand in.

Give details of the practical shift and laboratory, where appropriate, in the boxes provided. Write in dark blue or black pen.

You may use an HB pencil for any diagrams or graphs. Do not use staples, paper clips, glue or correction fluid.

Answer **all** questions in the spaces provided on the Question Paper.

The use of an approved scientific calculator is expected, where appropriate.

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

At the end of the examination, fasten all your work securely together.

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



Question	Marks
1	
2	
3	
Total	

This document consists of 18 printed pages.

Answer all questions.

Question 1

Researchers are exploring the development of plant-based, biodegradable plastics as an alternative to petroleum-based plastics.

Switchgrass is an example of a crop that synthesises polymers naturally and is used for biodegradable plastic production.

Switchgrass which are genetically modified with genes from another organism can result in the plants synthesising more polymers. These plants are termed as transgenic plants.

The ability to synthesise polymers is highly dependent on the growth rate of the plants, which in turn depends on the rate of photosynthesis.

(a) (i) For the same concentration of chloroplasts, predict how the rate of photosynthesis in the commercially-available switchgrass chloroplast extract would compare to that in the transgenic plant.

To test for the rate of photosynthesis, the blue dye, DCPIP, which acts as a substitute for NADP⁺ can be used.

oxidised DCPIP photosynthesising tissues reduced DCPIP (colourless)

You are required to:

- investigate the rate of photosynthesis in different concentrations of commercially available switchgrass chloroplast extract
- obtain a chloroplast extract from the transgenic plant
- estimate the concentration of chloroplasts in the transgenic plant.

You are provided with:

- 15 cm³ commercially available 10% switchgrass chloroplast extract, in a small vial labelled SE1
- A leaf from transgenic plant labelled T
- cold buffer solution, in a large vial labelled B
- distilled water, in a large vial labelled **W**
- a block of agar stained with DCPIP.

The DCPIP agar may stain your skin. You are advised to wear gloves when handling the agar.

The agar must be covered using aluminium foil when not in use to prevent decolourisation.

Assume that the temperature in the lab remains relatively constant. It is not necessary to use a water bath maintained at room temperature.

Proceed as follows.

1 You are required to carry out a serial dilution of **SE1** to reduce the concentration of the chloroplast extract by **a factor of ten** between each of three successive dilutions, **SE2**, **SE3** and **SE4**.

You will need to make up 8.0 cm³ of each solution. Some of this will be used to make the next solution.

(ii) Describe how you would carry out serial dilution to obtain SE2, SE3 and SE4.

[3]

(iii) Suggest an advantage of carrying out serial dilution.

.....

.....[1]

- 2 Label three small vials, SE2, SE3 and SE4. Use them to prepare the different concentrations of SE2, SE3 and SE4, as described in (a)(ii).
- **3** Place the block of DCPIP stained agar onto a white tile. Using a ruler and a knife, cut the agar block into identical cubes of about 1.5 cm × 1.5 cm × 1.5 cm, as shown in Fig. 1.1.

It may be difficult to cut them to exactly the same size. You will use only **five** agar cubes for this part of the experiment. Choose five that are the most identical to one another. Set aside the rest of the agar cubes for backup.

Place all the agar cubes onto the Petri dish provided and cover the Petri dish with aluminium foil.



3

Fig. 1.1

- **4** Using a white tile and knife, chop up 3.0 cm by 3.0 cm of the fresh leaf **T** into smaller pieces and put them into the mortar.
- **5** Slowly add approximately 15.0 cm³ of cold buffer solution **B** to the mortar. Grind the leaf pieces to obtain a chloroplast extract of **T**.

(iv) Outline the importance of using cold buffer for chloroplast extraction.

.....[1]

- 6 Place a sieve on top of the opening of a large plastic vial. Filter the contents of the mortar to remove the plant debris, keeping filtrate **TE**.
- 7 Use a piece of aluminium foil to cover the vial containing TE to protect it from light.
- 8 Label a small vial **TE** and put 7.0 cm³ of **TE** into the vial.
- **9** You will only need 7.0 cm³ of **SE1**. Use a syringe to discard any excess **SE1** from the small vial into the sink. Put a piece of agar cube into the small vial containing **SE1** and immediately start the stopwatch. Incubate for 10 minutes.
- 10 Repeat step 9 for SE2, SE3, SE4 and TE. You may stagger the start times for incubation of each vial.
- 11 Gently swirl the contents of the vials at regular intervals. While you are incubating the agar cubes, continue to read through this Question Paper up to step 15 and complete (a)(vi).
- **12** Fill a large vial with tap water. This will be used to rinse the agar cubes in step **13** to **stop** the reaction.
- **13** After 10 minutes, use a pair of forceps to remove the agar cubes from each solution and rinse the cubes in the vial.
- **14** Put each agar cube on a white tile and cut it into half.
 - (v) In the square below, draw the decolourised region and DCPIP stained region of the cut end of the agar cube that has been immersed in **SE1**.

Add a ruled line to label the "decolourised region" and a second ruled line to label "DCPIP stained region".



15 Measure the length of the DCPIP stained region for **all** the agar cubes, to the nearest mm. Record these measurements in the table prepared in **(a)(vi)**.

5

Ensure that these measurements are done **immediately** as the agar cubes will continue to decolourise when exposed to light.

(vi) Use this space to record your results for step 15 in a suitable format.

[3]

(vii)	Using the data obtained in (a)(vi), estimate the concentration of chloroplasts in TE.
	estimated chloroplast concentration of TE :[1]
(viii)	Identify one significant source of error in the procedure and describe how you would make an improvement to reduce this source of error.
	error
	Improvement
	[2]

(ix) The percentage decolourisation of an agar cube can be taken as a measure of photosynthetic efficiency. Suppose an agar cube in (a)(vi) had 10 mm of its length stained with DCPIP, calculate its percentage decolourisation. Show all your working clearly.

percentage decolourisation:[3]

Using fresh DCPIP stained agar cubes and **TE**, another experiment was conducted to investigate the effect of different light intensities on the rate of photosynthesis of the transgenic plant.

(b) Table 1.1 shows the percentage decolourisation of each agar cube at four different intensities of light.

Distance between light source and agar cube / cm	Decolourisation of agar cube / %
10	78.0
20	76.0
35	54.0
40	50.0

	Та	b	le	1	.1
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Use the grid provided to draw a line of best-fit for the results shown in Table 1.1.



[4]

[Total: 20]

Question 2

Hydrogencarbonate indicator is a solution that changes colour depending on the concentration of carbon dioxide in the solution. These colours are related to different pH values, as shown in Table 2.1.

colour of hydrogencarbonate indicator solution	рН	concentration of carbon dioxide in the solution
yellow	7.6	•
yellow-orange	7.8	increasing carbon dioxide
orange	8.0	
orange-red	8.2	
red	8.4	atmospheric concentration
red-magenta	8.6	
magenta	8.8	decreasing carbon dioxide concentration
magenta-purple	9.0	
purple	9.2	▼

Chlorella vulgaris is a protoctist that is single-celled, aquatic and photosynthetic. It can be immobilised in alginate beads.

Alginate beads with immobilised *C. vulgaris* can be used to measure the rate of photosynthesis. Sodium hydrogen carbonate solution (NaHCO₃) can be used as a source of carbon dioxide.

A student used alginate beads with immobilised *C. vulgaris* in hydrogencarbonate indicator solution and 0.1M NaHCO₃ to investigate the rate of photosynthesis in different light intensities.

Fig. 2.1 shows some of the apparatus and reagents the student used.



Fig. 2.1

(a) The student noticed that the hydrogencarbonate indicator solution changed from red to magenta, when the alginate beads with immobilised *C. vulgaris* in Fig. 2.1 was exposed to light.

Explain why the colour of the indicator started off as red and later changed to magenta.

- (b) The student wanted to reuse the setup in Fig. 2.1 and colour chart in Table 2.1 to investigate the rate of photosynthesis in different concentrations of carbon dioxide. He has run out of 0.1M NaHCO₃. Instead, he has access to the following materials and apparatus:
 - 0.5M NaHCO₃
 - hydrogencarbonate indicator solution
 - alginate beads with immobilised *C. vulgaris*
 - distilled water
 - table lamp
 - standard lab apparatus
 - access to hot and cold water

Describe a procedure the student could use to carry out this investigation.

Your plan should:

- have a clear and helpful structure such that the method you use is able to be repeated by anyone reading it
- identify the dependent variable and the independent variable
- identify the variables you will need to control
- include a suitable control
- a safety precaution
- use the correct technical and scientific terms
- indicate how the results will be recorded and analysed
- include a prediction and the justification for the prediction.

You do **not** need to include a diagram of the experimental setup.

.....

(c) The student set up a large test-tube containing alginate beads with immobilised *C. vulgaris* in hydrogencarbonate indicator solution at **pH 8.4** (red).

The student kept this set-up in the dark for 12 hours.

Predict and explain the results that will be observed after 12 hours in the dark.

(d) Some scientists wanted to culture cells of *C. vulgaris* on a large scale for use as a biofuel.

To determine the optimal growing conditions for *C. vulgaris*, the scientists needed to determine the number of cells per cm^3 of suspension to monitor the population growth.

They tried two methods to determine the number of cells per cm³ of suspension. The first method used a Secchi stick, as shown in Fig. 2.2.



Fig. 2.2

The Secchi stick is lowered into the suspension of cells until the black and white circle is not able to be seen from above. The depth in cm is recorded from the ruler, as shown in Fig 2.3.





The log_{10} of the number of cells is determined from a graph of log_{10} of cells counted per cm³ suspension against Secchi depth (cm), as shown in Fig 2.4.



Fig. 2.4

(i) When the scientists inserted the Secchi stick into a sample from their cell suspension, the circle (on the Secchi stick) was not able to be seen at a depth of 1.9 cm.

Using the graph in Fig. 2.4, state the log_{10} number of cells per cm³ of suspension. Show how you derive the value on the graph and record your answer below.

The second method used a counting chamber to determine the number of cells per cm³ of suspension. Fig. 2.5 shows a section of a counting chamber with cells present, as viewed using the high power of a light microscope.

The depth of the 1 mm × 1 mm counting chamber is **0.1 mm**.

The scientists counted the number of cells in several sections of a counting chamber.



Fig. 2.5

(ii) Count the number of cells in the 1 mm × 1 mm section of the counting chamber shown in Fig. 2.5.

Use your answer to calculate the number of cells per cm³ of the suspension. Show all your working.

number of cells per cm ³ of suspension[3	3]
Suggest one reason why using the Sechhi stick may be less accurate then using the counting chamber.	g
[Total: 1	[1] 8]

Question 3

Photosynthesis occurs in the leaves of plants. **M1** is a slide of a stained transverse section through a plant leaf.

(a) (i) Draw a large plan diagram of the region of the leaf on **M1** indicated by the shaded region in Fig. 3.1. Use a sharp pencil.



Fig. 3.1

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

No cells should be drawn.

Labels are **not** required.

(ii) Observe the **unshaded** region on slide **M1**. The bottom-most layer of cells is called the lower epidermis and is one cell thick.

Select a group of four touching cells comprising two cells from the lower epidermis and two cells from the layer above the lower epidermis.

Each cell must touch at least two other cells in the group.

Make a large drawing of this group of four cells. Include any internal structures that you can see.

Labels are **not** required.

(iii) A student hypothesised that the ratio of the number of cells in the lower epidermis to the number cells in the layer above is 1:3. He took many sample counts and wanted to determine if the ratio he observed followed the expected. Name the statistical test that he should use and explain the conclusion he should make if the p value is found to be 0.49.

Statistical test:	
Conclusion:	
	[3]

(b) Fig. 3.2 shows a photomicrograph of a stage micrometer scale that is being used to calibrate an eyepiece graticule.

The length of one division on this stage micrometer is 1 mm.





(i) Use Fig. 3.2 to calculate the actual length of one eyepiece graticule unit.

Show your working and include appropriate units.

actual length =[3]

Fig. 3.3 shows a photomicrograph of a transverse section through a different leaf. This was taken with the same microscope and lens used to take the photomicrograph in Fig. 3.2.

The eyepiece graticule has been placed across the leaf section to measure its thickness.



Fig. 3.3

(ii) Use the calibration of the eyepiece graticule from **3(b)(i)** to calculate the actual thickness of the leaf section in Fig. 3.3.

Show your working and write your answer to two decimal places.

actual thickness =[2]

(iii) Identify **three** observable differences, other than colour and the presence of air spaces, between the leaf section on **M1** and the leaf section in Fig. 3.3.

Record these observable differences in Table 3.1.

feature	M1	Fig. 3.3
		[3]

Table 3.1

[Total: 17]