Investigation into the effect of light on the rate of photosynthesis

Specification reference:

A level Component: 1.2

Photosynthesis uses light energy to synthesise organic molecules

Introduction

Photosynthesis can be summarised as

$$\text{carbon dioxide} + \text{water} \xrightarrow{\text{light}} \text{glucose} + \text{oxygen}$$

To measure its rate, in principle, the mass of carbon dioxide or water used, the light energy absorbed or the mass of sugar or oxygen produced could be assessed. But in practice, an easier method involves assessing the colour change in a pH indicator. pH increases when carbon dioxide is absorbed from solution in the photosynthesis of aquatic organisms, i.e. becomes less acidic.

When carbon dioxide dissolves in water the carbonic acid produced dissociates releasing hydrogen ions, which lower the pH of water:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

As photosynthesis removes carbon dioxide from the solution, the concentration of hydrogen ions decreases and so the pH increases. This can be visualised by noting the colour change of hydrogen carbonate indicator:

$$\underset{\text{yellow}}{\text{acid}} \rightleftharpoons \underset{\text{red}}{\text{neutral}} \rightleftharpoons \underset{\text{purple}}{\text{alkali}}$$

Scenedesmus quadricauda, a photosynthetic protoctistan, immobilised in alginate beads, is a suitable experimental material. If it turns the indicator purple, its rate of photosynthesis exceeds its rate of respiration; if it turns yellow its respiration exceeds its photosynthesis; if it remains red, photosynthesis and respiration are occurring at equal rates and the algae are at the compensation point.

Use the method below to carry out an investigation into the effect of light on the rate of photosythesis

Apparatus

For making algal balls

5 cm^3 *Scenedesmus quadricauda* culture
3 cm^3 sodium alginate solution (3%)
10 cm^3 syringe without needle
200 cm^3 calcium chloride (2 g /100 cm^3)
Glass rod
Beakers
Distilled water
Tea strainer

For running the experiment

Algal balls
Glass vial + stopper 10 cm^3
Hydrogen carbonate indicator
Colour chart for indicator (*School Science Review* **85** (312) 37–45) or colorimeter with 550 nm filter
Metre ruler
Fluorescent lamp
Timer

Method

Making algal balls

1. Stir a mixture of 5 cm^3 *Scenedesmus* culture and 3 cm^3 3 % sodium alginate solution gently with the glass rod until they are well mixed.
2. Draw the mixture into a 10 cm^3 syringe barrel.
3. With constant pressure on the plunger, drop the mixture, one drop at a time, into 200 cm^3 calcium chloride solution.
4. Leave the balls for 20 minutes.
5. Strain the balls through the tea strainer.
6. Return the balls to the beaker swirl them in distilled water.
7. Repeat steps 5 and 6 twice more.
8. Use immediately or store at 4 ^oC, but bring to room temperature for approximately 20 minutes before use.

Running the experiment

1. Place 20 algal balls in a vial.
2. Add 10cm^3 hydrogen carbonate indicator.
3. Place the vials at a distance from a light source
4. After a given time assess the pH of the indicator in the vial using the colour chart or read its absorbance at 550 nm in a colorimeter.

Risk assessment

Hazard	Risk	Control measure
Solid calcium chloride is an irritant to skin and eyes and if inhaled	Making calcium chloride solution	Solid to be weighed in fume cupboard; Students to be given solution.
Gas accumulation in culture vessel could cause the glass to break	During period of algal culture	Ensure cotton wool stopper allows ventilation; Stand culture vessel in deep tray
Excess heat from lamp may cause burns	When decanting from culture vessel	Ensure no contact with skin

Teacher/ Technician notes

Growing your alga: Prepare a culture of green alga such as unicellular *Scenedesmus quadricauda*. Make up a solution of algal enrichment medium, and subculture the alga into this. Aerate gently and keep at temperatures between 18–22 °C. Constant illumination ensures faster growth of the alga. After 3–4 weeks, the culture should have a green 'pea soup' colour. Subculture the alga again to maintain a healthy culture. You could use other algae, but *Scenedesmus* should produce 2 to 3 litres of dark green 'soup' in about 4 weeks from 50 cm^3 of original culture. (Details from SAPS Sheet 23).

Preparing solutions to make alginate beads (Refer to Recipe card 2):

- Dissolve 3 g of sodium alginate in 100 cm^3 of cold, pure water. Stir with a spatula every half hour or so. Leave overnight and stir in the morning.
- Dissolve 4 g of calcium chloride-6-water in 200 cm^3 of pure water in a 250 cm^3 beaker.

Hydrogencarbonate indicator: Refer to Recipe card 34 and Hazcard 32. Low hazard once made; must be made fresh by qualified staff using fume cupboard. The indicator is very sensitive to changes in pH, so rinse all apparatus with the indicator before use. Avoid exhaling over open containers of the indicator. Make up a 'standard colour scale' of reaction bottles containing buffers from pH 7.6- pH 9.2 with hydrogencarbonate indicator if students will not have access to a colorimeter.

Lamps: You need a brighter light than a standard 40 W or 60 W bench light. Low energy bulbs produce too limited a spectrum of light for full activity. 150 W tungsten or halogen lamps are best. 150 W portable halogen lamps have a stand and handle separate from the body of the lamp which makes them safer to handle. But they do produce heat, so you will need a heat filter for the investigation.

Heat filter: Use a large flat-sided glass vase or a medical 'flat' filled with water. With a high power lamp, the small volume in a medical 'flat' may get too hot for comfort.

Making alginate beads:

- When making up the alginate or diluting the algal culture it is essential to use pure water; otherwise calcium ions in the water will cause the alginate to 'set' prematurely.

Students can run all distances from the lamp at the same time if they 'fan' the vials out in front of the light source.

It takes at least an hour for colour changes to happen – so students will need to return to the lab at break or after lessons to 'read' the results.

More details are available on the link below:

http://www.nuffieldfoundation.org/practical-biology/investigating-photosynthesis-using-immobilised-algae

Sample results

Distance of vial from lamp / cm	Colour of indicator after 1 hour	Absorbance after 1 hour / a.u.	pH after 1 hour
10	purple	0.92	9.0
30	magenta	0.81	8.8
50	red	0.56	8.4
70	orange	0.39	8.2
90	yellow	0.25	8.0

Plot the pH or the absorbance against the distance from the lamp source.
As the light intensity falls in proportion to $\frac{1}{d^2}$, the pH or absorbance could be plotted against $\frac{1}{d^2}$.

Further work

- More than one vial at each distance should be used so that a mean can be calculated from replicate readings.
- Instead of placing vials at different distances, light intensity may be varied by covering the vials with neutral density filters and maintaining the same distance from the lamp. In this way, any potential heating effect has a consistent effect.
- Vials may be covered with coloured filters to expose the algae to different wavelengths. In order that the algae receive the same light intensity, readings must be taken with a light meter with the filters over the probe, to find distance for each wavelength which has the same light intensity, where the vials should be placed.

Practical techniques

- use appropriate apparatus to record a range of quantitative measurements (to include mass, time, volume, temperature, length and pH)
- use appropriate instrumentation to record quantitative measurements, such as a colorimeter or potometer