Immobilised algae and respiration

Background

There are a number of well-established practicals which can be used in the classroom to show plant photosynthesis. For studies at Biology National 4 and 5 it is likely that experimental systems involving the use of *Elodea* and/or *Cabomba* will be recommended. The Biology Team in SSERC has previously published, in this Bulletin and elsewhere, details of methods involving the aquatic plant *Cabomba* [1 - 3]. Immobilised algae have increasingly been used since details of their preparation and descriptions of appropriate experimental conditions for classroom practicals were first published [4-6]. A comprehensive kit for the production of immobilised algae, together with supporting documentation, is available from the National Centre for Biotechnology Education [7].

A common misconception amongst students is encapsulated in the statement 'Plants respire at night but breathe in carbon dioxide during the day' [8]. Whilst plants do indeed respire at night they also respire during the day; under 'appropriate' conditions of light intensity plants are net producers of oxygen and when light conditions fall below a certain level plants are net consumers of oxygen as respiration dominates. Early indications from the draft arrangements documents relating to Biology National 4 and 5 indicate that experimental systems which allow students to investigate the process of respiration will be recommended. Whilst there are a number of possible experiments which one might use to cover this aspect we feel that the use of plants, specifically immobilised algae, is a particularly powerful system since such studies allow the misconception outlined above to be explored and addressed.

Experimental System

For the experiments described here 100 immobilised beads of algae are required for each temperature run. The standard protocol which we use in SSERC for the production of immobilised algae is available on the Science3-18 website [5].

Briefly suspensions of algae are combined with a solution of sodium alginate and the resulting mixture allowed to flow into a solution of calcium chloride. The immobilised beads thus formed (Figure 1) are rinsed with cold tap water and distilled water to remove excess calcium chloride.

A stock of hydrogencarbonate indicator is prepared as follows:

1 Cresol red (0.10 g) [**Caution HARMFUL** if ingested in quantity or inhaled as dust] and thymol blue (0.20 g) [**Caution HARMFUL** if ingested in quantity or inhaled as dust] is dissolved in ethanol (20 cm^3) [**Caution** – ethanol is **HIGHLY FLAMMABLE** and an **IRRITANT**]

Figure 1 - *Immobilised Scenedesmus quadricauda.*

2 Sodium hydrogencarbonate (0.85 g) is dissolved in freshly boiled, cooled distilled water (approximately 200 cm^3) and combined with the ethanolic solution of cresol red/thymol blue and made up to 1.0 dm^3 with distilled water.

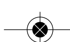
For routine use 100 cm^3 of the stock solution is diluted to 1.0 dm^3 with freshly boiled, cooled distilled water and the pH of this solution adjusted by the addition of small volumes of dilute sodium hydroxide or sulfuric acid solutions.

Into each of 5 Bijou bottles are placed 20 immobilised algal beads together with exactly 4.0 cm^3 of hydrogencarbonate indicator at pH 9.2. At this starting pH the solutions are purple in colour and have a relatively high absorbance when measured at 580 nm. The 5 Bijou bottles are then placed in a water bath at the desired temperature (or in the refrigerator for measurements at 4°C) and the absorbance measured at regular intervals. It is important that all solutions are kept in the dark in order to ensure that no photosynthesis takes place - so put a lid on your water bath. Immediately prior to measuring their absorbance, solutions are gently shaken to ensure a uniform distribution of colour.

Results

In the absence of light photosynthesis does not take place whereas respiration does occur and produces carbon dioxide and a consequent lowering of pH which is reflected in a change of colour from purple to red and eventually through orange to yellow.

This effect can be seen in the sample shown in Figure 2; it should be noted that we have placed the immobilised algae in a Universal bottle - we did this merely to allow for greater ease of photography. Immobilised algal beads were added to hydrogencarbonate indicator and left in the dark for 3 hours at room temperature (20°C).



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An interesting observation about the image in Figure 2 is that whilst a purple colour remains at the top of the solution the algae are clearly respiring since the layer of solution immediately surrounding them is a yellow/orange in colour indicating an increased localised carbon dioxide concentration.

Figure 2 - Respiring immobilised algae in hydrogencarbonate indicator.

The data in Figure 3 show the change in absorbance of the hydrogencarbonate indicator as a function of temperature. As can be seen the rate of respiration increases with temperature. We have continued measurements beyond the 2 hours shown in Figure 3; so, for example, the absorbance at 4°C is only reduced to 1.07 (from a starting value of 1.45) after a period of 22 hours in the dark.

The experimental system described lends itself to investigative work for students in the senior phase. One could, for example, envisage using the basic technique of monitoring rate of carbon dioxide production and varying other factors which might affect respiration rate. In an accompanying article we describe how gaseous carbon dioxide levels can be measured [9] and the method described here complements that publication.

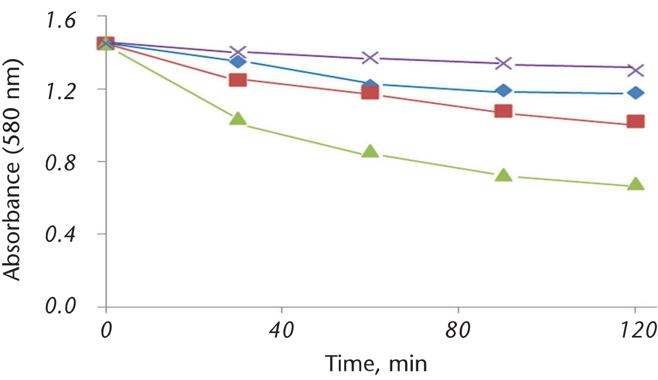

***Figure 3** - Effect of temperature on the rate of respiration in immobilised Scenedesmus quadricauda. Absorbance was measured at 580 nm in 1 cm cuvette using distilled water as the reference. The starting pH was 9.2 and the fall in absorbance reflects a drop in pH as a function of time.*

Key: X = 4°C; ◆ = 20°C; ■ = 30°C; ▲ = 40°C.

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