SAPS Photosynthesis Kit : the use of algal balls to investigate photosynthesis

Introduction

The use of immobilised algae and hydrogencarbonate indicator provides an engaging and interesting approach to practical work that supports the learning and teaching of photosynthesis in the school laboratory. The technique was developed by Debbie Eldridge[1] from King Ecgbert School, Sheffield while working on a SAPS/Robinson College Schoolteacher Fellowship. SAPS has now produced a *Photosynthesis Practical Kit* based upon Debbie's work. This article describes briefly the kit and some of its possible uses in the classroom.

A new technique

Traditional practical work in photosynthesis focusses on testing the products of photosynthesis, either by monitoring the evolution of bubbles containing oxygen in *Elodea* or using iodine to test for starch in geranium leaves that have been decolorised. Standardisation of the quantity of photosynthetic material under test is difficult and quantification of the starch is impossible using standard classroom procedures. Using these methods, it has been very difficult to carry out investigative work on the rates of photosynthesis and students rarely find the work stimulating. The *Cabomba* plant techniques shown in *Bulletin 215*, also developed by Debbie Eldridge, can give more reliable results than with the traditional *Elodea* :-

http://www.sserc.org.uk/members/SafetyNet/bulls/215/Biology.htm

Algae can be considered as single-celled plants. They can be grown easily, concentrated, and then, using immobilisation techniques, divided into standard quantities in the form of so-called algal balls. These algal balls can be easily manipulated and, in association with hydrogencarbonate indicator, used in practical investigations of photosynthesis. The hydrogencarbonate indicator will change colour according to the concentration of dissolved carbon dioxide (CO_2). The concentration of dissolved CO_2 will be governed by the balance of photosynthesis and respiration. The colour changes in the hydrogencarbonate indicator can be quantified either by measuring the absorbance of the indicator at 550 nm with a colorimeter or by comparing the colours with a set of standard buffer solutions :-

http://www-saps.plantsci.cam.ac.uk/worksheets/ssheets/ssheet23.htm

1 Eldridge, Debbie (2004). A novel approach to photosynthesis practicals. *School Science Review*, 85 (312), 37-45

Figure 2 - SAPS Photosynthesis Kit

The SAPS Photosynthesis Kit (Fig. 2) contains algae (*Scenedesmus quadricauda*); enrichment medium for growing up the algae; sodium alginate for immobilising the algae; syringes for creating appropriately- and evenly-sized drops that can form jelly balls; hydrogencarbonate indicator in a concentrated form; Bijou bottles (Figure 1) for carrying out the reactions; and coloured filters that can be used in investigations of wavelength of light on photosynthesis. The school has to supply a vessel for growing the algae, such as a large lemonade bottle; a simple air pump such as that used in a fish tank to aerate the growing culture; illumination for growing the culture (a lightbank is useful, here); clamps and stands to hold the syringes; plastic cups and spoons for mixing the algae with alginate and for holding calcium chloride solution; 2% calcium chloride solution; tea strainers; lamps for experimental work. The kit contains full instructions for growing the algae, for producing algal balls and suggestions for experimental work.

Growing the algae

The kit contains a small bottle of the alga, *Scenedesmus quadricauda* and concentrated enrichment medium. The algae need to be grown in diluted enrichment medium for 3 – 4 weeks in order to produce enough algae for a 'class set'. Full instructions for inoculation and growth of the algae are contained within the kit.

Making the algal balls

To make the algal balls, algae are mixed with sodium alginate solution and this mixture is then allowed to drip gently into calcium chloride solution. This forms jelly balls of calcium alginate with algae trapped inside i.e. this process immobilises the algae.

Figure 1 - Immobilised algae in hydrogencarbonate indicator

The powdered sodium alginate provided with the kit needs to be dissolved in water to make the 2-3% solution required. Sodium alginate can take some time to dissolve and so it is recommended that it be made up at least 24 hours before the class practical work is due to take place. It is important not to heat the sodium alginate as this would be likely to affect the consistency of the algal balls. A 2% calcium chloride solution is also required.

The algal culture that has been grown needs to be dispensed (about 50 cm^3 per student or group) and concentrated. Concentration of the algae is achieved either by leaving them to stand (Figure 3) or by centrifuging.

Figure 3 - Concentrating the algae

On discarding the supernatant, a much-reduced volume of concentrated algae remains. Approximately 3 cm^3 of this concentrated algae is mixed (Figure 4) with an equal volume of sodium alginate solution in a clean vessel such as a universal container. This mixture is added to the syringe and then allowed to drip gently into the calcium chloride solution (Figure 5). The syringes supplied with the kit have long, narrow nozzles that are particularly well suited to producing

fairly small, uniformly-shaped and sized algal balls. The calcium chloride solution should be swirled gently as the algae drop into it. The result is the formation of balls of calcium alginate of uniform size and containing approximately equal quantities of algae.

The algal balls should be left for approximately five minutes to harden, washed in tap water (a tea strainer is useful to hold the algal balls (Figure 6)) and then given a final rinse with distilled water. These rinsing steps are required to ensure that all the calcium chloride is completely removed. At this stage, the balls can be kept refrigerated under distilled water for up to six months. When ready to carry out experimental work, the balls can then be counted out (Figure 7) and added to hydrogencarbonate indicator.

Figure 4 - Mixing algae and alginate

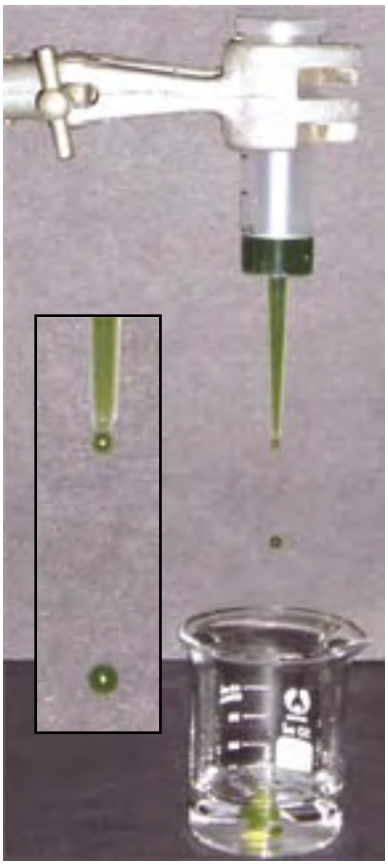

Figure 5 - Forming the algal balls

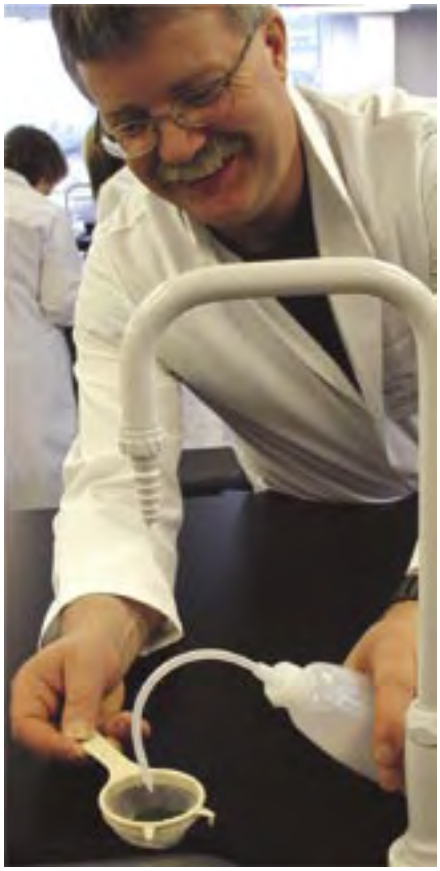

Figure 6 - Washing algal balls in tea strainer

Figure 7 - Counting algal balls

Hydrogencarbonate indicator

Hydrogencarbonate indicator is very sensitive to changes in pH and hence to dissolved carbon dioxide level. The indicator is orange/red when the dissolved carbon dioxide concentration is in equilibrium with air. It changes through orange to yellow as the pH falls, i.e. as carbon dioxide concentration increases (for example when the rate of respiration exceeds the rate of photosynthesis). When the carbon dioxide level falls, the hydrogencarbonate indicator changes through red to a deep purple (for example, when the rate of photosynthesis exceeds the rate of respiration). The colour of the hydrogencarbonate indicator can thus be used to monitor both respiration and photosynthesis. The colour change can be measured either by using a colorimeter to measure absorbance at 550 nm or by comparing with a set of standard buffer solutions (Figure 8).

250 cm^3 hydrogencarbonate indicator is provided with the kit. It needs to be diluted 10x for use. Because of its sensitivity to changes in pH, it is essential that all glassware etc. is rinsed out with a little indicator before use. Due to variations in commercially prepared hydrogencarbonate indicator, SAPS recommends that any additional solution required is prepared according to the instructions provided with the kit.

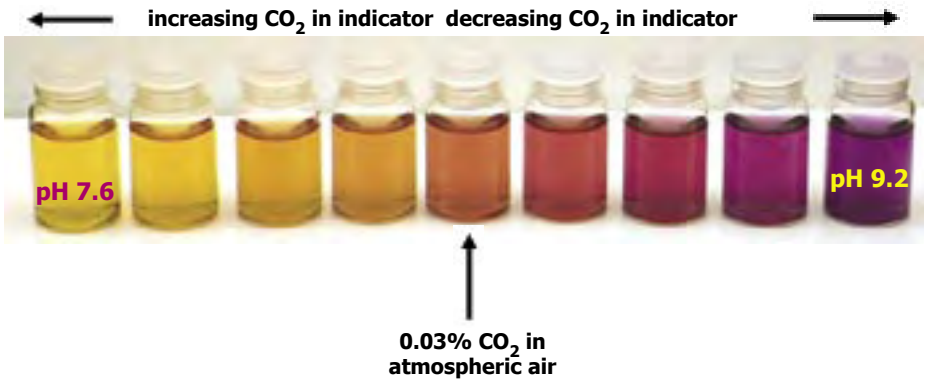

Figure 8 - Colour chart for hydrogencarbonate indicator in standard buffer solutions from pH 7.6 – pH 9.2 at 0.2 increments

Light sources

The light source needs to be stronger than a standard 40 W bench lamp. SAPS recommends two types of light source:

Figure 9 - 150 W lamp

1. Halogen lamps such as the one shown in Fig. 9 are typically rated at 150 W. They, therefore, give out a lot of heat and so a heat filter should be employed when using them as a light source for experiments on photosynthesis. A good heat filter might simply be a medical flat bottle filled with water placed between the lamp and the algal balls.

2. Fluorescent tubes do not give out as much heat and therefore do not require a heat filter. As it is easier to measure the distance between sample and lamp (Figure 10), more samples can be used with a single fluorescent tube, therefore reducing the effects of variability in the light source.

We have found fluorescent tubes more convenient to use and that they provide very satisfactory results.

Experiments using the algal balls

Whilst the instruction set for making the algal balls is quite prescriptive, the use of the algal balls themselves allows a range of open-ended investigations and provides students with an opportunity to plan and carry out their own investigations. Algal balls and a standard volume of hydrogencarbonate indicator should be added to the Bijou bottles that are provided with the kit. As the algae absorb CO_2 from or release CO_2 into the hydrogencarbonate indicator, it changes colour. This set-up can be used to investigate a number of variable factors that affect the rate of photosynthesis. In the next section we look at how to examine the effects of changing light intensity and wavelength as well as how the quantity and type of algae can affect the rate of photosynthesis.

Light intensity

The effect of light intensity on the rate of photosynthesis in algal balls can be approached in two ways:

1. Bijou bottles containing a standard quantity of algal balls and hydrogencarbonate indicator can be placed at different distances from the lamp and the rate of change of colour monitored and recorded. The hydrogencarbonate indicator

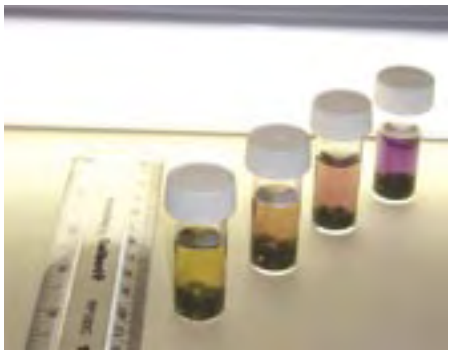

Figure 10 - Algal balls at varying distances from the light source (in this case, a fluorescent tube)

changes colour from orange through red to purple most rapidly in the Bijou bottle closest to the light source. The algal balls contained therein are carrying out photosynthesis at a faster rate than those in the Bijou bottles at a greater distance from the light source, the concentration of the CO_2 reduces more rapidly and a colour change is observed. Care must be taken that the bottles closer to the light source do not obscure the bottles further away.

2. The Bijou bottles can be covered with different neutral density filters. Neutral density filters are 'grey' filters and five different such filters are provided with the kit. An ideal neutral density filter would reduce the intensity of light of all wavelengths equally. In practice, neutral density filters do not achieve this. Each of the filters provided in the SAPS Photosynthesis Kit does reduce the intensity of light reasonably evenly across the range 400–660 nm. The names and the notional amount of light transmitted in the spectral range 400-660 nm by each of the neutral density filters is summarised in the Table 1 below.

Using these neutral density filters as an alternative to varying the distance of the Bijou bottles from the light source reduces possible sources of error that might arise from:

- changes in light intensity which arise from a change in angle between the lamp and sample
- difficulties in interpretation and application of the inverse square law which governs the relationship between light intensity and distance from the light source
- any heating effects caused by varying distances from the lamp.

Wavelength of light

The effect of wavelength of light on the rate of photosynthesis in algal balls can be investigated using filters of different colour (Figure 11). In addition to the five neutral density filters, the kit provides nine coloured filters. These can be wrapped around the Bijou bottles and provide a straightforward way of altering the wavelength of light reaching the algal balls within. The rate of photosynthesis in each of the bottles can be compared by observing changes in the colour of the hydrogencarbonate indicator.

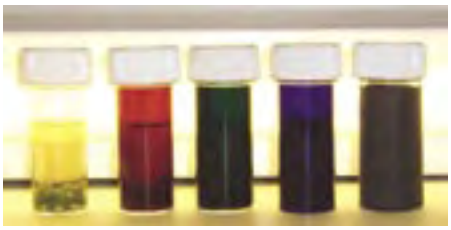

Figure 11 - Algal balls and hydrogencarbonate indicator in Bijou bottles covered with coloured filters

More information on the filters and their uses in these practicals is provided on the SAPS website:

http://www-saps.plantsci.cam.ac.uk/articles/broad_light.htm

Quantity of algae

The effect of numbers of algae on the rate of photosynthesis can be achieved in two ways:

1. The number of algal balls used with a standard volume of hydrogencarbonate indicator can be varied.

2. The concentrated algae produced from settling or centrifuging can be diluted and the different dilutions used to make algal balls that contain different numbers of algae. In this case, equal numbers of balls should be added to the standard volume of hydrogencarbonate indicator.

Type of algae

The rates of photosynthesis carried out by different algae can be investigated if different algae are grown up and immobilised. The concentration of algae can be quantified using a haemocytometer and so the rates of photosynthesis by similar concentrations of algae under given conditions can be compared. Guidelines for using a haemocytometer can be found on the SSERC website in the Microbiological Techniques section of SafetyNet :-

www.sserc.org.uk/members/SafetyNet/Microbio2/EM/countcells.htm

Filter name	298 (0.15 ND)	209 (0.30 ND)	210 (0.6 ND)	211 (0.9 ND)	299 (1.2 ND)
Light transmitted (%)	71	50	25	12.5	6.3

Table 1 - Names and the notional amount of light transmitted in the spectral range 400-660 nm by each of the neutral density filters.

Equivalent concentrations of algae, however, do not necessarily contain equal concentrations of chlorophyll and it is would be difficult to ensure that the quantity of photosynthetic pigment for each organism is standard. It would, however, be possible to investigate, qualitatively, whether blue-greens photosynthesise optimally at a different wavelength of light from standard algae. At SSERC, we have successfully grown *Scenedesmus quadricauda*, *Chlorella sp.* (both algae) and *Cynechoccus sp* (Cyanobacteria or blue-greens).

Appraisal of the kit

Using the SAPS Photosynthesis Kit makes the implementation of practical work with algal balls in the classroom easy. Any materials not supplied in the kit are readily available in schools. Students enjoy making and working with algal balls (Figure 12). They can plan practical work for use with algal balls and investigate a range of factors on the rate of photosynthesis, by observing colour changes caused by differences in levels of CO_2.

Figure 12 - Cheers! Success at making algal balls.

The kit is provided in a plastic box and brings together some equipment that is not normally available in school laboratories and apparatus whose design has been found to be particularly useful for investigations with algal balls. It also makes available, in quantities practicable for school use, materials that are available for bulk purchase (for example the filters and the Bijou bottles). At £37.50, including postage and packing (£30.00 without P & P), the cost of the kit is significantly less than the sum that would have to be expended on similar quantities of materials and has the advantage that the principal resources are to hand. The syringes, Bijou bottles and filters are reusable. Details of where to purchase replacement consumables for the practical work are included with the kit. Additional materials required for the practical work are commonplace in schools. The kit provides hardware sufficient for fifteen students or groups to carry out work at one time. However, there is sufficient medium, alginate, hydrogencarbonate indicator and algae when cultured to allow 45 sets of experimental work. Hence the materials within a single Photosynthesis Kit could support 3 x 15 sets of practical work over a short time period. The hardware would, of course, be available for subsequent occasions with only the consumables requiring to be purchased. We consider that the SAPS Photosynthesis Kit represents very good value and is an excellent resource.

Further information and purchases can be obtained from:

SAPS, Homerton College, Hills Road, Cambridge CB2 8PH, Tel: 01223 507168 ; email saps@homerton.cam.ac.uk

Revisiting useful friends - now even cheaper

An opportunity has arisen which may benefit microbiology work carried out in schools. This has been made possible through collaboration with Scientific & Chemical Supplies Ltd. and Prestige Medical.

Here we announce an exclusive offer in Scotland where you can purchase an autoclave which we have found to be ideal for use in schools. At SSERC we evaluate equipment received from suppliers to ensure safety and suitability for school use. The Classic 2100 met all criteria regarding pressurised systems (BS3970: Part 4). The results of our tests are available in full detail on the Members part of the SSERC web site (SafetyNet-What's New).

The Model 2100 Classic, which has the Standard Body, can operate at 126 ^{o}C for 11 mins. The Extended Body version operates at 121 ^{o}C for 15 mins and is specially designed for media. It has temperature and pressure gauges and a thermal jacket. Both models come with instrument tray, basket, lifter and 'V' support. Both standard and extended body versions can be purchased through Scientific & Chemical by quoting the catalogue numbers shown in Table 1 and offer savings of £200 and £300 respectively over the normal catalogue prices.

Contact Scientific & Chemical Supplies Ltd, 39 Back Sneddon Street, Paisley, Renfrewshire PA3 2DE

Telephone: 0141 1887 3531 E Mail: paisley@scichem.com

Figure 1 - Standard Body autoclave at new price of £465

Catalogue No.	Description	Old Price	New Price
AUT 010 015	Standard Body	£678.57	**£465**
AUT 010 055	Extended Body 'Plus' Media	£959.18	**£640**

Table 1 - Standard Body autoclave at new price of £465

"These models provide effective steam sterilisation for educational microbiological work. Prestige Medical autoclaves are easy and convenient to use for both sterilising most media and disposal of microbiological waste. The taller model with the extended body is particularly useful."

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