A novel approach to photosynthesis practicals

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Traditional practical work on photosynthesis is rarely stimulating. The use of immobilised algae in hydrogencarbonate indicator is suggested as an accessible and enjoyable alternative at key stage 4.

Traditional school science experiments on photosynthesis focus on starch tests, usually in *Pelargonium*. Classic practicals largely take the form of demonstrations. The presence of starch is considered proof of photosynthetic activity, and a series of controlled experiments can be set up where one variable is excluded to demonstrate the essential requirements for this process. Barker and Carr (1989) showed that many students did not understand the starch test. Students are distracted by the complex mechanics involved in starch testing and do not link starch production with the photosynthetic process. As an example, one student thought that when iodine and chlorophyll were mixed together they produced starch. Kinchin (2000) also criticises the idea of starch production as proof of photosynthesis because of the many examples that seem to conflict with the idea that starch production is dependent on light. He makes the point that students know that a potato contains starch but also that a potato is produced in the dark and without the need for chlorophyll.

ABSTRACT
The topic of photosynthesis is rarely greeted with enthusiasm by students. This complex process requires a mature understanding of many abstract scientific ideas and it is not easy to illustrate in a practical way. In order to try to change students' attitudes to photosynthesis, a new practical procedure is proposed for key stage 4. It involves the immobilisation of algae and measurement of the colour changes that take place as the algae use up carbon dioxide from hydrogencarbonate indicator. All students involved in school trials were able to obtain reliable data. Overall impressions of student motivation towards this activity were encouraging.

Some other problems of these practical activities arise because students do not understand the concept of a controlled experiment. The removal of carbon dioxide from the air surrounding a plant cannot be seen and therefore does little to promote the idea of it being an essential requirement for photosynthesis. Eisen and Stavy (1993) have suggested that there is insufficient emphasis on the basic chemistry of the process. Students cannot make the link between the carbon from carbon dioxide in the air and the carbon present in carbohydrates. To date there is little practical work that can be done to reinforce this.

Other experimental work may focus on the production of oxygen as a product of photosynthesis. Oxygen evolution by *Elodea* and other aquatic plants can be used as proof of photosynthesis, and if the oxygen is collected the rate can be determined under different conditions. Such experiments, often used for investigations at key stage 4, can produce good data but they are notoriously difficult to complete on a regular basis. Although they may serve a function in terms of assessment, they do little to improve student understanding of the whole process.

Plant nutrition has always been an area of student misconception (Driver *et al*., 1994). Students come to science with some deeply entrenched ideas, and the need for students to restructure their 'working' knowledge of plant nutrition as they develop higher level concepts is widely accepted. Much of the recent

work involved in trying to help students overcome their difficulties has been based on challenging them to construct theoretical models or become involved in confrontational exchanges with others to help clarify their ideas (Lumpe and Staver, 1995; Kinchin, 2000).

While this is very important, there is also a need to motivate students in this area of biology. Photosynthesis is such a central scientific idea that it appears throughout the National Curriculum. If teachers are not careful, students may feel that they are repeating the same topic several times. Experience shows that by the time that students get to key stage 4 they may be very 'turned off' plants, and there is a need to introduce something stimulating to challenge them. One way of keeping their interest is to provide them with a novel practical procedure.

This article describes such a procedure which, while demanding, allows students to develop new skills and use apparatus that they may not have met before. It suggests the use of immobilised green algae as photosynthetic organisms which are easy to manipulate. Students may then determine the rate of carbon dioxide absorption by the algae using hydrogencarbonate indicator and a colorimeter or colour scale. In addition, the importance of carbon dioxide as a requirement for photosynthesis is emphasised in an implicit way, as students are in effect taking measurements of how much carbon dioxide is taken up by the algae, which causes the change in the colour of the indicator. A major aim of this work was to make the topic of photosynthesis more interesting from a practical viewpoint.

Growing a healthy culture of algae

A healthy culture of *Scenedesmus quadricauda* (or other fast-growing green algae) is needed in order to carry out the practical. The starter culture used here was obtained from Sciento (see *Apparatus*) and the culture initially grown on for four weeks in order to supply enough material for several classes of pupils. The culture was maintained using an enrichment medium (see *Apparatus*) in a 2 litre clear plastic bottle. In order to allow circulation and aeration with carbon dioxide, a lift pump was used. A 15 mm diameter plastic pipe, just shorter than the total height of the bottle, was inserted into the neck of the bottle. The pipe had small holes at the bottom and one just above the water surface. An air lead without a diffuser was

Figure 1a Algal generator.

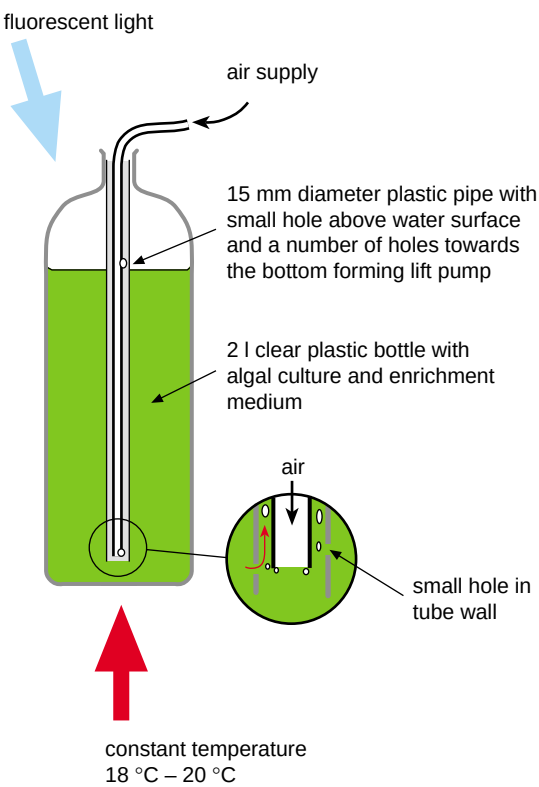

Figure 1b A simple algal generator showing the operation of a lift pump.

pushed down to the base of the pipe and the lift pump flow adjusted to give a small amount of splashing at the top surface. The pump works by decreasing the density of the column of water in the pipe, causing it to rise, which then draws water in at the base resulting in a gentle circulation – see Figure 1a and b. The bottle was illuminated under a fluorescent light bank and the temperature was kept at between 18 and 20 °C. After about four weeks a dark green 'pea soup' colour was obtained. From this it was very easy to maintain the culture by subculturing into fresh medium. It is not vital that the culture remains pure, as any unicellular green algae are suitable for these investigations. However, it is important to subculture while the algae are in the exponential growth phase.

Immobilisation of the algae

The algae need to be immobilised; in effect, this means trapping large numbers of algal cells in 'jelly like' balls so that they can be kept in one place. The sodium alginate is not harmful and the algae can photosynthesise and reproduce within the balls for several weeks. This means that students can make the algal balls in one lesson and then use them immediately or store them for use in future lessons.

Method

- A 3% solution of sodium alginate is prepared. This needs be dissolved slowly using a warm hotplate, and it takes several hours to mix fully. Once prepared it lasts for several days in a fridge as long as it is covered to prevent contamination. It was found experimentally that different brands of sodium alginate have different consistencies when made up. Sodium alginate from Philip Harris and BDH are recommended.
- A concentrated solution of algae is obtained – the best way of doing this is to pour out approximately 100 cm^3 of dark green algal 'soup' into a measuring cylinder and leave it for 30 minutes settle out. The supernatant is poured off and the last 5 cm^3 collected. This is added to approximately 5 cm^3 of the alginate. The resulting mixture should be dark green. If time is short or the algae remain in suspension and don't settle out, as with smaller algae such as *Chlorella*, a dark green pellet can be obtained by centrifuging the culture gently and decanting off the supernatant (5 minutes at 4000 rpm is sufficient).
- A 2 cm^3 syringe, with the plunger removed, is secured in a clamp stand at a height of approximately 10 cm above a beaker containing 2% calcium chloride. The mixture is poured into the syringe so that the gel containing algae drops through slowly into the calcium chloride and forms small spheres. The calcium chloride is swirled gently as the balls form. After 10 minutes the algal balls are washed thoroughly in distilled water. This makes about 100 algal balls.

The algal balls are now ready for experimentation and will be active for several weeks, as long as they are kept in a light room and not allowed to dry out. The advantage of using algal balls is that it is very easy to standardise the amount of photosynthetic tissue in any experiment. The balls are uniform in size and chlorophyll concentration as long as they are from the same batch. This means that accurate replication of any experiment is possible.

Using hydrogencarbonate indicator to measure photosynthetic rates

Hydrogencarbonate indicator is commonly used to detect changes in pH. It is extremely sensitive and can therefore be used in a precise way to determine the level of carbon dioxide in a solution. It is non-toxic, readily available for purchase (see *Apparatus*) or can be made easily in the laboratory. This indicator can be used to help measure the rate of photosynthesis. When the indicator is equilibrated with atmospheric air it is orange/red. With more carbon dioxide it becomes yellow, and as carbon dioxide is removed it becomes more red and finally purple.

If a standard number of algal balls are placed in a standard volume of hydrogencarbonate indicator and placed near to a light source, they will take up the carbon dioxide as the algae photosynthesise. The indicator will change from orange through red to purple. The rate at which the algae remove the carbon dioxide obviously depends on a number of factors but noticeable colour changes can be as fast as 15 minutes in a sealed vial using 20 dark green algal balls in approximately 7 cm^3 indicator, under bright light. (150 W halogen lamps which are freestanding can be purchased from hardware stores for less that £10. These were found to be most effective in these experiments.)

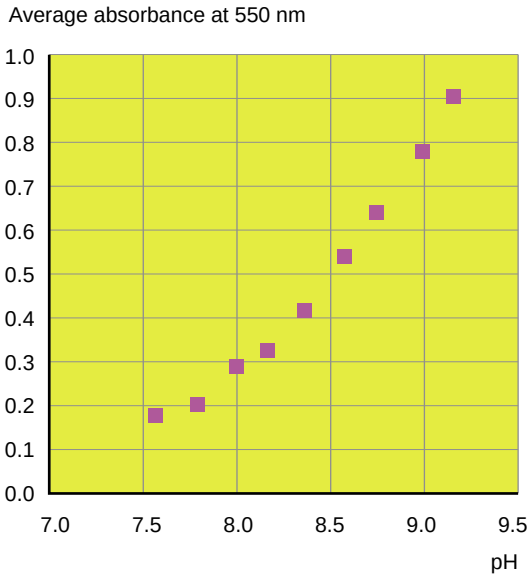

Figure 2 Graph to show the absorbance at 550 nm of pH buffers ranging from 7.6 to 9.2 with added hydrogencarbonate indicator.

Changes in the colour of the indicator are clearly visible and can be quantified using a colorimeter. A green filter (550 nm) is used to measure the absorbance of the indicator because the range of colours produced all absorb green light. The absorbance of the indicator increases as the pH increases. Simple confirmation of this was established by preparing a range of buffers of pH 7.6–9.2 to which the indicator was added. The absorbance of each was measured and plotted against pH, showing a linear response over the range of interest (Figure 2).

A really excellent colorimeter, which is very easy for key stage 4 students to use, is the CO7500 from Biochrom (see *Apparatus*). This has a digital read-out, and is very robust and reliable.

Sample investigations

1 Is there a relationship between light intensity and rate of photosynthesis?

Into each of twelve small, clean, glass vials with plastic lids were placed 15 algal balls, along with

Table 1 Absorbance values, taken at 550 nm, of hydrogencarbonate indicator, each containing 15 algal balls at five distances from a 150 W light source, and in the dark, at various time intervals.

Distance D from light source/cm	*Relative light intensity $1/D^2$(x 10^{-5})*	*Time interval /min*	*Absorbance at 550 nm*		
			Replicate 1	*Replicate 2*	*Average*
250	1.6	60	0.71	0.75	0.73
		120	0.83	0.86	0.85
		180	0.93	0.95	0.94
350	0.81	60	0.66	0.68	0.67
		120	0.75	0.8	0.78
		180	0.8	0.89	0.85
500	0.4	60	0.57	0.57	0.57
		120	0.6	0.63	0.62
		180	0.62	0.66	0.64
780	0.16	60	0.48	0.51	0.50
		120	0.52	0.56	0.54
		180	0.53	0.57	0.55
1250	0.06	60	0.42	0.44	0.43
		120	0.43	0.45	0.44
		180	0.44	0.46	0.45
dark	0	60	0.33	0.33	0.33
		120	0.34	0.34	0.34
		180	0.29	0.29	0.29

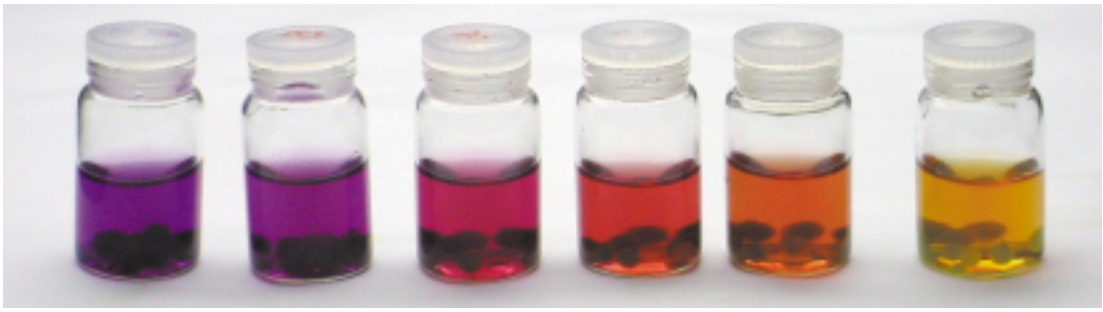

Figure 3 The colours of replicate 1 after 180 minutes. The vials are arranged in the sequence, from left to right, of greatest to least illumination.

7 cm^3 of hydrogencarbonate indicator which had been equilibrated with the atmosphere. Ten of the sealed glass vials were placed at five different distances from a strong tungsten lamp (150 W light source), two at each position, with a flattened glass tank filled with water between the lamp and the vials to act as a thermal insulator. Two vials were placed in the dark. A visible change was first noticeable after 30 minutes.

After 60 minutes the indicator was removed from the closest vial and poured into a clean cuvette. The absorbance was measured with a colorimeter using a bright green filter (550 nm). The indicator was placed back into the vial, the lid put back and it was repositioned in the experiment. This was repeated with the other vials and measurements taken again after 120 minutes and 180 minutes.

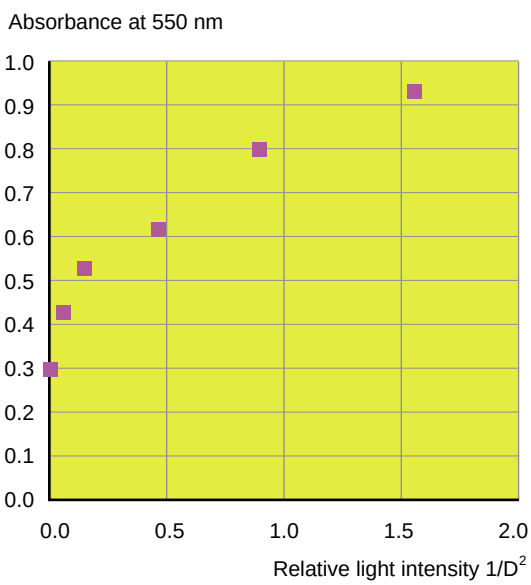

Figure 4 Graph to show the absorbance (at 550 nm) of indicator from six vials over a range of light intensities. Light intensity was a relative measurement calculated as 1/(distance)^2.

Results

See Table 1 and Figure 3. If a graph is plotted with relative light intensity (estimated as 1/(distance)^2) against absorbance, then a classic photosynthetic curve is obtained (see Figure 4).

Analysis

The graph shows that as light intensity increases, the rate of photosynthesis increases. There is a proportional relationship at low light intensities, but the graph levels off at higher light intensities suggesting that there is another limiting factor.

In this instance the work prompted another investigation to find out what the limiting factor might be. It was decided that carbon dioxide was unlikely to be limiting since the hydrogencarbonate ions present in the indicator were at a concentration far in excess of what is normally available. It was decided to investigate the effect of increasing the number of algal cells in each batch of algal balls.

2 Investigation into effects of light intensity on rate of photosynthesis in batches of algal balls with different numbers of algal cells

A large container of algal culture was shaken thoroughly to mix it. Into three measuring cylinders, 500 cm^3, 250 cm^3 and 100 cm^3 of algal solution were poured. The solution was allowed to settle for the same period of time and then all but the final 5 cm^3 were decanted off. The final 5 cm^3 from each cylinder was added to the equal volumes (5 cm^3) of alginate. Thus the remaining solutions contained decreasing amounts of algae. The resulting algal balls are shown in Figure 5.

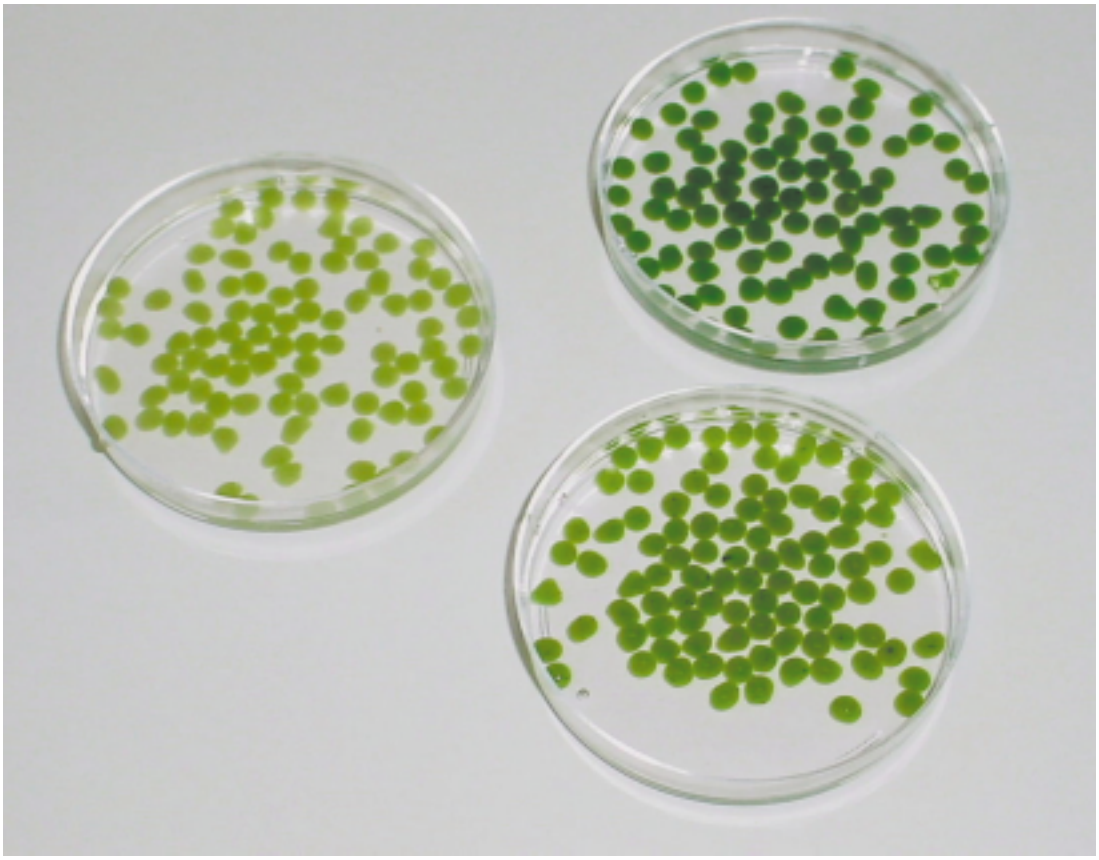

Figure 5 Algal balls made from three different volumes of algal suspension.

Fifteen vials were then each rinsed with hydrogencarbonate indicator and 7 cm^3 fresh, equilibrated indicator added to each. To each of five of the vials, 15 algal balls with a high concentration of algae were added. This was repeated with the medium and low concentrations of algae. Three vials (containing balls of each algal concentration) were placed at different distances from the light source. The results are shown in Table 2 and Figure 6.

Table 2 Absorbance values at 550 nm for algal balls made with three different concentrations of algae, at different distances from a light source.

	Volume of culture used to make algal balls/cm^3	*500 cm^3*	*250 cm^3*	*100 cm^3*
Distance from source/cm	*Relative light intensity $1/D^2$ (x 10^{-5})*	*Absorbance of indicator at 550 nm*		
250	1.6	0.85	0.81	0.75
350	0.81	0.76	0.74	0.67
500	0.4	0.68	0.56	0.47
780	0.16	0.49	0.39	0.37
1250	0.06	0.26	0.25	0.25

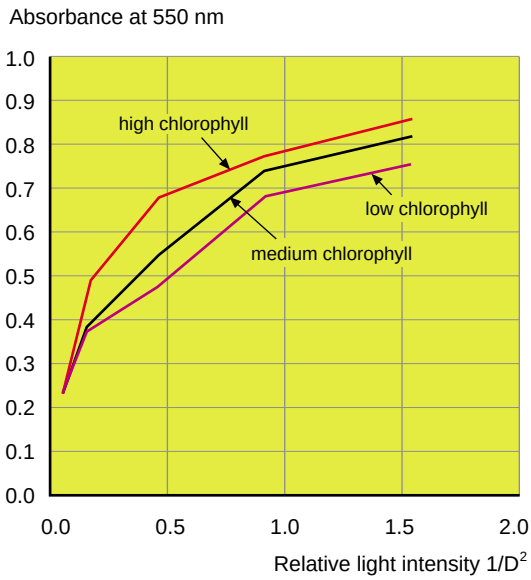

Figure 6 Graph to show the absorbance (at 550 nm) in relation to light intensity of balls made of three different concentrations of algal cells.

Analysis

As before, the general relationship between light intensity and rate of photosynthesis can be observed. There is a steep increase initially as light intensity is increased and a flattening of the curve when saturation occurs. Additionally, however, these data show that the saturation point might be raised by increasing other factors.

In this case, with an increasing number of algal cells there is a greater availability of chlorophyll and an increasing concentration of photosynthetic enzymes. These graphs should be familiar enough for students to recognise the classic shape that illustrates the principle of limiting factors. While they should see that the rate of photosynthesis is higher with more algae, they should also be able to see that it is not in proportion to the increase in number of algae – therefore something else must be limiting the rate.

Notes

- If access to a colorimeter is not possible, then the colour change can be semi-quantified by comparing the indicator to a series of coloured buffered solutions. Solutions ranging from pH 7.6 to 9.2 can be made up using borax/boric acid buffers. If 9 cm^3 of each buffer solution is placed into a vial of the same dimensions as that used in the experiment and 1 cm^3 of *concentrated* hydrogencarbonate indicator is added to this just prior to the lesson, a lovely spread of colours is obtained with which students can then compare their results (Figure 7).
- Standard 40 W or 60 W bench lamps that are used in schools are not bright enough for these investigations – they do not create a large enough gradient in light intensity, particularly when there is often extraneous illumination from the lab/outside, etc. 150 W tungsten or 150 W halogen lamps were found to be effective. Low energy bulbs are not useful because they are very selective in the wavelengths of light produced. Portable halogen lamps can be bought from hardware stores for a very reasonable price, but they do get hot so heat filters are essential.

Safety

There are no hazards associated with any of the chemicals used in these practicals. If teachers purchase 150 W halogen lamps they would be advised to obtain the portable lamps, as these have a stand and handle which is separate from the body of the lamp. Students should not look directly at the lamps and not pick them up or touch them immediately after use. The lamps should not be left near combustible material.

Figure 7 Buffer solutions ranging from pH 7.6 to 9.2 (at intervals of 0.2). 9 cm^3 of each buffer solution was mixed with 1 cm^3 of stock hydrogencarbonate indicator.

Notes on other investigations which have been trialled

- **Is there a relationship between light intensity and rate of photosynthesis?** This can be investigated by varying the distance from a lamp as described above or by using neutral stop filters from a photographic shop. It is best to work in a darkened room.
- **Is the rate of photosynthesis influenced by the wavelength of light?** Coloured filters can be obtained from photographic shops, which can be wrapped around the vials. Be aware that different light sources produce different wavelengths of light anyway. The filters can be purchased with details of their transmission properties and students can use these to interpret their results.
- **Does the size of algal ball affect the rate of photosynthesis?** It is possible to make algal balls of different sizes – so students can try keeping the mass the same but varying the number. Does the ratio of surface area to volume have an effect? Tiny balls can be made with a micropipette, and larger ones by running the alginate through wide-bore plastic tubing.
- **Does temperature influence rate of photosynthesis?** It is difficult to keep the indicator and balls at a specific temperature, but one possibility is to set up water baths under fluorescent light banks. Condensation builds up in the vials as they warm up and this may reduce the available light.
- **Does the number of algal cells influence the rate of photosynthesis?** At a simple level the more algal balls you use, the greater the rate of photosynthesis. This is a simple investigation but very straightforward for less able students. Alternatively students can make several batches of balls with different concentrations of algae as in the example above. Some students in the trials actually quantified the concentration of cells with the colorimeter before they started.

Discussion

The preliminary trials of these investigations, completed in schools during summer 2002, have been very encouraging. As long as the algae are healthy and reasonably concentrated the results are reliable and repeatable. (If the algae start to clump together and settle out despite aeration it suggests contamination. Although they still photosynthesise it is more difficult to get an even distribution of the algae in the balls if they are in this state. It is advisable to subculture the algae at four-week intervals to maintain a healthy culture.) All students were able to generate lots of data yet there was enough variation in the results to give them scope to discuss and evaluate. The trials indicate that students enjoy making the algal balls and indeed often want to take them home with them. Beware, they may just want to keep making more and more, leaving less time for the investigatory work!

Students do need to be aware of the sensitivity of the indicator, because if vials or cuvettes are used that are not clean, this can influence the colour of the solution. It is always best to rinse out containers with a little of the indicator beforehand.

The colour changes are attractive and the use of the simple colorimeter makes the results easily quantifiable – this is essential for key stage 4 coursework. It also introduces students to a different piece of apparatus, which they have to learn to use and understand. The best students will soon become aware of the precautions that are needed for reliable data, giving scope for assessment by the teacher.

Conclusion

Initial evidence from the schools trials suggests that the visual representation of carbon dioxide uptake by the algae helped to reinforce that carbon dioxide is a raw material for photosynthesis. The students who wanted to take the algal balls home with them added a small amount of indicator to the sealed container and kept it on a windowsill. Students were aware of the algae living and causing a colour change over a 24-hour period. They could relate this to the presence of light, carbon dioxide (which they had come to associate strongly with the indicator) and water. The trials were completed with a group of 17 year 9 students with SAT scores ranging from level 6 to level 8. The intention is to track these students over the next two years, during which time they will complete an open-ended investigation based on photosynthesis in algae. The accuracy of their responses together with an assessment of their motivation towards the topic of photosynthesis will be compared to a control group of the same ability who have not experienced this activity.

No one involved in science education can be unaware of the importance of photosynthesis. As a

process it requires an understanding of many concepts which are more often associated with physical science topics at key stage 3. Students need to understand the concepts of energy, conservation of matter and the cycling of materials – to name but a few! These conceptual difficulties may be resolved by getting students to discuss opposing views and construct concept maps but one has to assume that there is the motivation to do so. Demanding practical work, which is relevant and accessible, can go a long way towards increasing the enjoyment and sense of achievement of students, and perhaps give them new incentive to get to grips with the finer detail.

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Apparatus

- Sciento, 61 Bury Old Road, Whitefield, Manchester, M45 6TB. 0161 773 6338. Algal enrichment medium was purchased from Sciento.
- The best hydrogencarbonate indicator is obtained from Beecroft , Northfield Road, Rotherham, S. Yorkshire S60 1RR.
- Biochrom Ltd, 22 Cambridge Science Park, Milton Road, Cambridge CB4 0FJ. Telephone: 01223 423723.

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Debbie is happy to answer e-mails on the practicalities of running this activity.