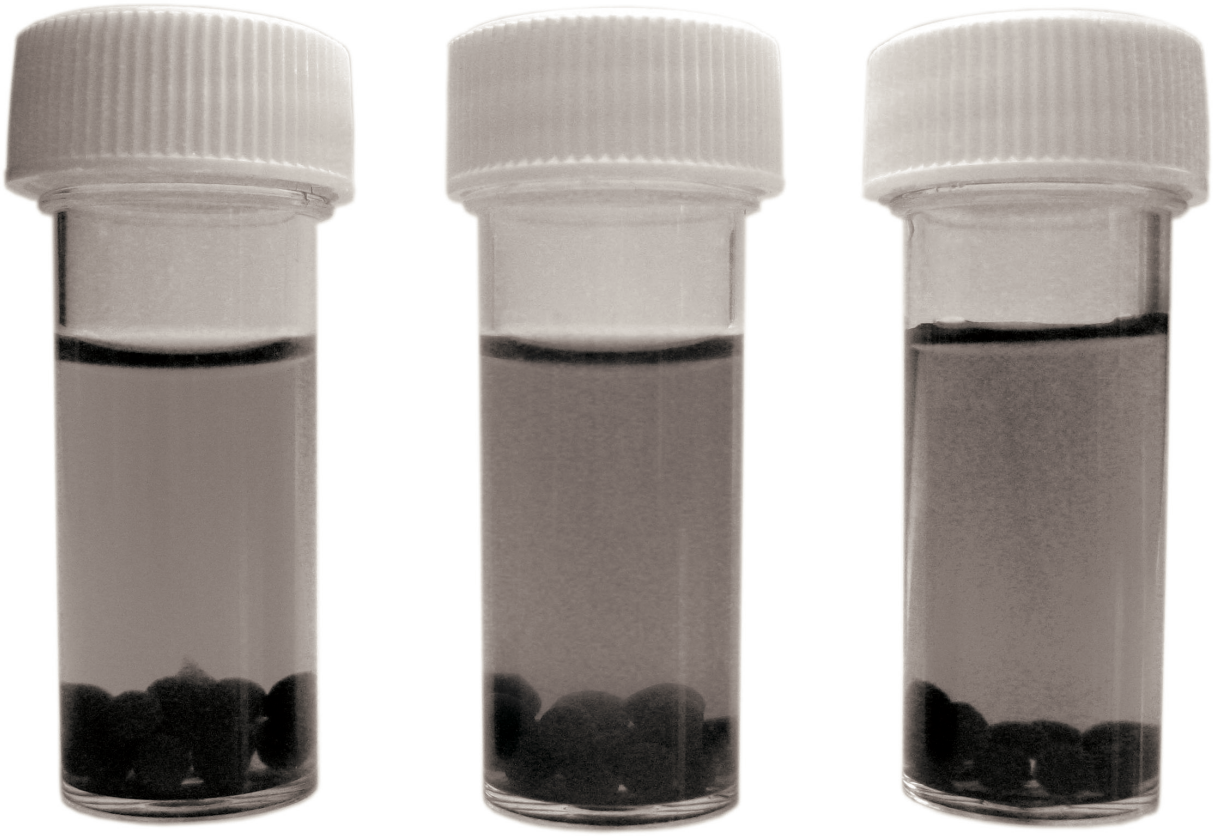
National Centre for Biotechnology Education
and Science and Plants for Schools

University of Reading



Investigating Photosynthesis

Teacher's notes

1.4

Equipment and materials

Equipment and materials

Required by each student or working group

Equipment included in the kit

- Student's guide
- 10 mL syringe
- 7 mL Bijou bottles with caps, ~ 8

In the kit, but needing preparation

- Hydrogencarbonate indicator, diluted from the concentrate in the kit, ~ 60 mL
- Sodium alginate solution, 3% (w/v), ~ 3 mL
- *Scenedesmus quadricauda* culture, concentrated, 3 mL (prepared from 50 mL of the original culture)

OPTIONAL

- Coloured or Neutral density filters (Red, green, blue, ND 0.15, ND 0.3 and ND 0.6) cut to 70 x 40 mm strips (to wrap round the bijou bottles)

Equipment and materials not included in the kit

- Lamp (small fluorescent strip lights or 18 W low-energy — 100 W equivalent — lamps give good results).
- 100 mL beakers or plastic cups, 2
- Tea strainer
- Glass or plastic stirring rod
- Permanent marker pen
- Ruler (*e.g.*, 1 metre rule)
- Graph paper or suitable graphing software
- Calcium chloride solution, 1.5% (w/v) if using anhydrous $CaCl_2$; 2% (w/v) if using the dihydrate, $CaCl_2.2H_2O$, ~100 mL
- Distilled or deionised water, ~100 mL
- Colorimeter and cuvettes
  or
  Class set(s) of standard solutions, prepared as described on page 3 of the Teacher's notes (opposite).

OPTIONAL

- Transparent adhesive tape, *e.g., Sellotape*
- Scissors

Preparation and timing

Cultivating the algae

The algae should be grown for 3–4 weeks then allowed to sediment out before the lesson by leaving the culture to stand overnight.

Sodium alginate solution

The sodium alginate takes some time to dissolve, so the solution is best prepared the day before the lesson. Ideally, leave the alginate overnight to dissolve, preferably on a magnetic stirrer. Large volumes can be prepared in a blender. Note that excessive heating can reduce the strength of the alginate by depolymerising it. If you wish to store sodium alginate solution for more than a few days, it is advisable to autoclave it. To prevent excessive depolymerisation of the alginate, however, you should increase the pH of the solution to 7–8 before autoclaving.

The practical protocol

Immobilised algae can be prepared by students in 15–20 minutes. It takes a further 20–30 minutes to set up the experiment, then 1–2 hours for the indicator to change colour.

Suppliers

Colorimeters

A colorimeter suitable for school use is the CO7500 from *Biochrom Ltd*, 22 Cambridge Science Park, Milton Road, Cambridge CB4 0FJ, UK. **www.biochrom.co.uk** This colorimeter has a digital readout, it is simple to use, reliable, robust and the results are repeatable.

An inexpensive alternative is the Mystrica Colorimeter, available from *Mystrica Ltd*, 39 Charterhall Road, Edinburgh, EH9 3HS, UK. **www.mystrica.com**

Both colorimeters are supplied with software and can be linked to computers.

Filters

Coloured and neutral density filters for studying the effects of different wavelengths or light intensity may be obtained from *Lee Filters*, Central Way, Walworth Industrial Estate, Andover SP10 5AN, UK. **www.leefilters.com**

Algae

Scenedesmus quadricauda and other algae are available from *Sciento*, 61 Bury Old Road, Whitefield, Manchester M45 6TB, UK. This firm also sells the enrichment medium needed for cultivating algae. **www.sciento.co.uk**

Growing the algae

- 2 L, clear, colourless PET lemonade bottle
- Aquarium air pump
- Aquarium airline tubing and airstone or glass sparger
- Cotton wool or foam, ideally non-absorbent, for closing the top of the bottle
- Low-temperature lighting, *e.g.*, 18 W low-energy bulbs (equivalent to 100 W), ideally double-walled, 2
- Algal enrichment medium, 1 L.

a. Add 1.5 g of the enrichment medium to 1 L of distilled water in a 2 L lemonade bottle and shake to dissolve. *Some residual powder will settle out; this is normal and it will be utilised by the algae as the soluble nutrients are depleted.*
b. Inoculate the bottle with the algae.
c. Insert an airline to aerate the culture with an air pump. *This will provide extra dissolved carbon dioxide and keep the algae and nutrients circulating.*
d. Stopper the bottle loosely with cotton wool or a foam bung. Continuously illuminate the culture with a bright light while it is growing. *Small fluorescent strip lights or 18 W low-energy lamps give good results. The best results are obtained when extraneous light in the laboratory is minimised.*

WARNING

Do not place hot lamps near to the culture. Please refer to the safety guidelines overleaf also.

Hydrogencarbonate indicator

To make hydrogencarbonate indicator

Makes one litre of 10x stock solution

- Cresol red, 0.1 g
- Thymol blue, 0.2 g
- Sodium hydrogencarbonate (sodium bicarbonate, $NaHCO_3$), 0.85 g
- Ethanol, 20 mL
- Freshly-boiled distilled water, ~1 L

Note

Hydrogencarbonate indicator is very sensitive to changes in pH and it is therefore important that all glassware, *etc* is rinsed with a little of the indicator before use.

a. Dissolve 0.1 g of cresol red and 0.20 g of thymol blue in 20 mL of ethanol.
b. Dissolve 0.85 g of sodium hydrogencarbonate in ~200 mL of freshly-boiled (and therefore CO_2-free) distilled water.
c. Add the ethanolic solution of cresol red and thymol blue and dilute to 1 L with freshly-boiled distilled water.

For use, dilute this stock solution with nine volumes of freshly-boiled distilled water and adjust the pH to ~7.4. *Ideally, this solution should be fully aerated before use so that it becomes a bright red colour.*

To prepare a set of standard solutions

If a colorimeter is not available, the colour change of the indicator can be semi-quantified by comparing it to a series of coloured buffered solutions. Solutions ranging from pH 7.6 to pH 9.2 can be made using boric acid/borax buffer.

- 7 mL bijou bottles with lids, 9
- Boric acid, 12.4 g
- Sodium tetraborate decahydrate (borax) $Na_2B_4O_7.10H_2O$, 19.5 g
- 4.5 mL stock (*i.e.*, 10x) hydrogencarbonate indicator

a. Dissolve the boric acid in 1 L of distilled or deionised water.
b. Dissolve the borax in another litre of distilled or deionised water.
c. To 25 mL of the boric acid solution, add the volume of borax indicated in the table below and make up to 100 mL with distilled or deionised water.
d. Place ~4.5 mL of each of the prepared solutions into each of a series of vials.
e. Immediately before the lesson, add 0.5 mL of the stock (that is, concentrated) hydrogencarbonate indicator solution to each bottle. Compare your results with the colours in this range of 'standard' bottles.

Borax solution, mL	1.00	1.55	2.45	3.60	5.70	8.70	15.00	29.50	57.50
pH	7.6	7.8	8.0	8.2	8.4	8.6	8.8	9.0	9.2

Safety guidelines

Cultivating the algae

A build-up of gas within the culture vessel, especially one made of glass, could be dangerous. Consequently you should ensure that the container used to cultivate the algae is adequately vented.

Care should also be taken to ensure that lights used to illuminate the culture and the aquarium pump cannot come into contact with liquid should it spill or leak from the culture vessel. It is a wise precaution to place the culture vessel in a deep tray with sufficient capacity to hold all the liquid in the event of a leak. Any electrical equipment should then be placed *outside* this tray.

Chemicals

None of the chemicals used in this protocol are considered to be harmful when handled as directed. Solid calcium chloride is an irritant: avoid contact with the skin or eyes, or breathing in dust from the dry powder.

Lamps

Care should be taken to ensure that lamps are not used in such a manner that there is a risk of electrical shock or overheating that may cause burns or fire. Low-energy or fluorescent lamps should be disposed of appropriately.

Additional investigations

The basic procedure described in the Student's guide can be extended in several ways. Examples of open-ended investigations include:

Varying light intensity
This can be achieved in two ways: either vary the distance from the lamp to the bijou bottles or cover the bottles with one or more neutral density filters.

Wavelength of light
Wrap a coloured filter, secured with tape, round each bijou bottle. Note that different light sources produce light of different wavelengths and that different wavelengths of light are transmitted by each of the filters. Details can be found on the *Lee Filters* web site: **www.leefilters.com**

Temperature
Set up water baths under a bank of lights and float the sealed bijou bottles in the water with light shining from above.

Number of algal cells
Make several sets of algal beads with different numbers of algae in them. Do this by diluting the concentrated algal culture before making a the beads. A hæmocytometer could be used to estimate the number of cells in each bead.

Should you wish to recover the algae from the alginate beads, 50 mM sodium citrate or phosphate buffer at pH 7.0 can be used to dissolve the calcium alginate gel.

Further information
Science and Plants for Schools (SAPS) has numerous articles, support materials and additional practical protocols for studying photosynthesis, including the original version of this immobilised algae protocol together with sample data and other useful information: **www.saps.org.uk**

Debbie Eldridge's *School Science Review* paper, describing this work, can be downloaded from the NCBE's web site, as can *Keynote* and *PowerPoint* presentations featuring the illustrations from the Student's guide.

Acknowledgements

Science and Plants for Schools

The original work which led to the development of the Photosynthesis kit was undertaken by Debbie Eldridge who at that time was Head of Science at King Ecgbert School in Sheffield. The work was published: Eldridge, D. (2004) A novel approach to photosynthesis practicals. *School Science Review* **85** (312) 37–45.

Funding for Debbie's work was provided through a *Science and Plants for Schools* (SAPS)/Robinson College Schoolteacher Fellowship which enabled her to spend several months working at SAPS in Cambridge. SAPS is funded by the Gatsby Charitable Foundation.

NCBE, University of Reading

These Teacher's notes and the accompanying Student's guide were written, typeset and illustrated by Dean Madden at the *National Centre for Biotechnology Education*, University of Reading.

National Centre for Biotechnology Education, University of Reading
Tel: + 44 (0) 118 9873743. Fax: + 44 (0) 118 9750140. eMail: NCBE@reading.ac.uk Web: www.ncbe.reading.ac.uk