



# Investigating Photosynthesis

## Student's guide

## Photosynthesis and respiration

Photosynthesis is the *energy-acquiring* process in which light energy is used to produce oxygen, glucose and water from water and carbon dioxide. The reactions can be summarised in an equation:

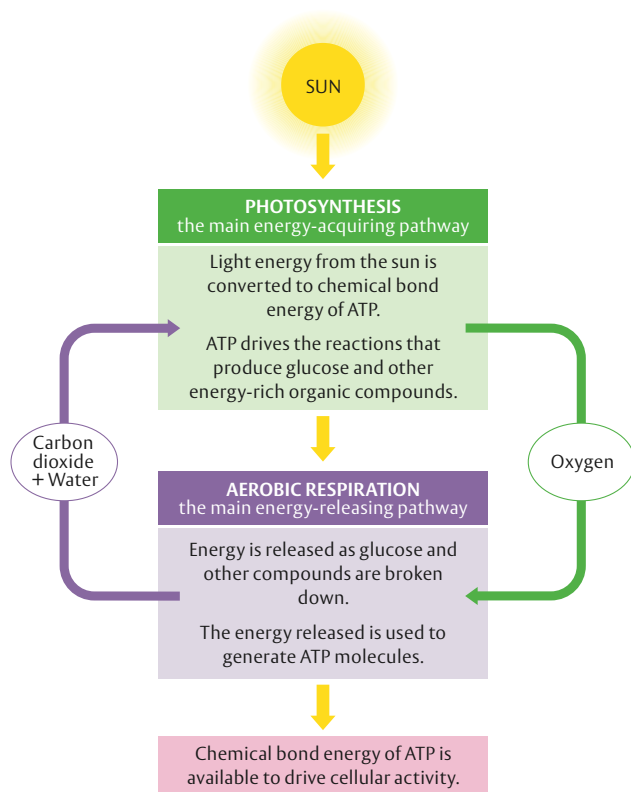


Note that this is a simplified equation, and that the glucose produced by photosynthesis is immediately converted into other carbohydrates, such as starch and sucrose.

Alongside photosynthesis, *respiration* is also taking place. Respiration is the main *energy-releasing* pathway in the cell. In *aerobic respiration*, oxygen is taken up and carbon dioxide and water are released:



Again, this equation is simplified (and remember that *anaerobic respiration* can also take place in the absence of oxygen). The relationship between the two processes can be shown as follows:



## Algae for studying photosynthesis

Green algae (phylum Chlorophyta) can be thought of as single-celled plants. They grow quickly and concentrated suspensions of algal cells are easy to prepare. In the practical procedure described here, green algae are immobilised in beads of calcium alginate. Each bead contains roughly the same number of algal cells, so it provides a standard amount of photosynthetic material. This means that semi-quantitative investigations of photosynthesis are possible.



G. FAHNENSTIEL, U.S. NOAA.

*Scenedesmus quadricauda*. This species forms colonies of four (or two, eight, or 16) cells attached side by side, arranged linearly or in a zigzag. The terminal cells in each cluster have spiny projections. Each cell is 11–18 µm long and 3.5–7 µm wide.

The rate of carbon dioxide uptake by the immobilised algal cells is used to measure the rate of photosynthesis — this can be done simply by observing the colour change of hydrogen carbonate indicator, either by eye or by using a colorimeter. The indicator will change colour according to the concentration of dissolved carbon dioxide. The concentration of carbon dioxide will be governed by the balance of photosynthesis (which takes up carbon dioxide) and respiration (which produces it).

The effect of varying the intensity or wavelength of the light may be studied, as can the effect of temperature or cell density. The species of algae provided in the kit is *Scenedesmus quadricauda*. Different species of algae or photosynthetic cyanobacteria may also be tested and compared using this method.

## Immobilisation in alginate

Immobilisation in alginate is a relatively gentle process; it does not harm the cells and the beads are porous, so that gases and other substances can diffuse into and out of them. The same method can be used to immobilise isolated organelles (such as chloroplasts or mitochondria) or even large organic molecules (such as enzymes).

There are two steps to immobilising algae. First, a concentrated suspension of the algae is prepared and mixed with a solution of sodium alginate. The mixture is then allowed to fall from a syringe, a drop at a time, into a solution of calcium chloride.

The calcium ions in the solution form cross-links between the chains of alginate molecules (see diagram). This causes the alginate to set, making a semi-solid gel, with the algae trapped inside. After several minutes in the calcium chloride, the calcium alginate hardens and the beads become stronger. Alginates from different species of seaweed have different physical properties: the amount of cross-linking (and hence the strength of the beads) will vary.

Once hardened, the beads can be separated from the calcium chloride, washed, and used to study photosynthesis.



LEISLEY/JACQUES, ISTOCKPHOTO.

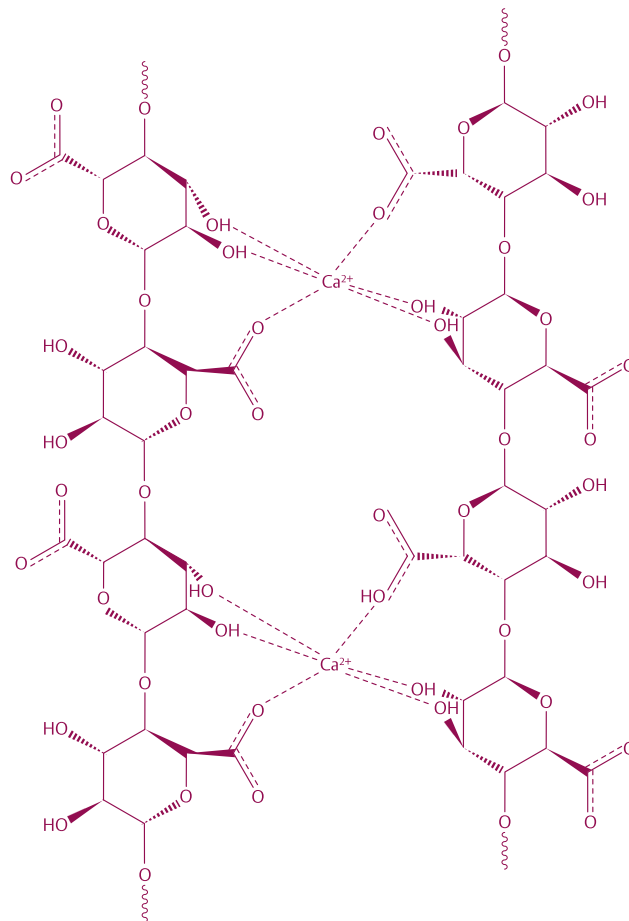
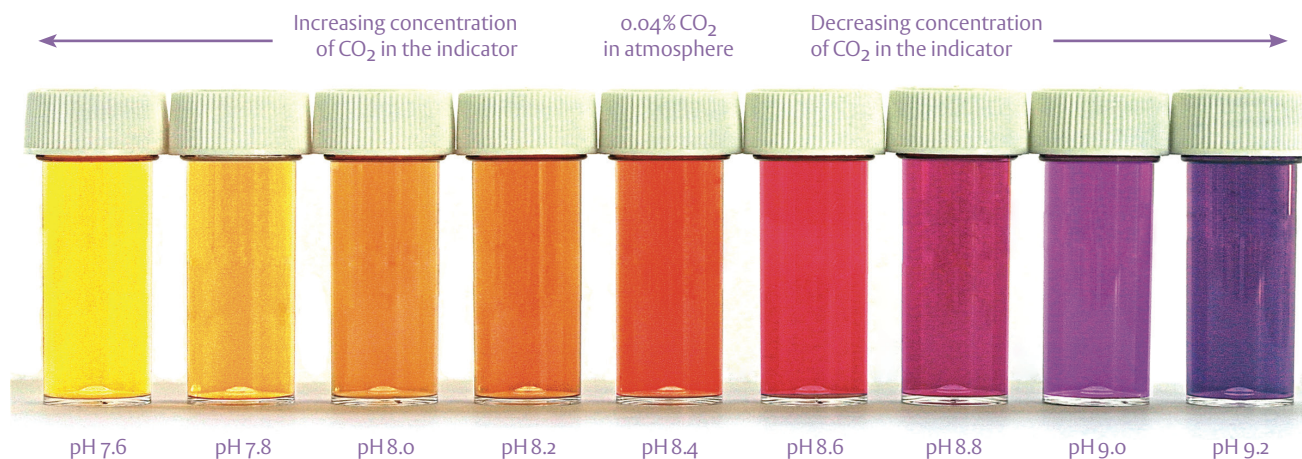
Sodium alginate is the sodium salt of alginic acid. It is obtained from the cell walls of brown algae (seaweeds), including Giant kelp (*Macrocystis pyrifera*), *Laminaria* species and Egg wrack (*Ascophyllum nodosum*, above).

Beads like this may be kept in water for several weeks at room temperature or for several months if stored in a fridge. Surprisingly, the immobilised algae survive quite well when refrigerated, even in the dark.

## Hydrogencarbonate indicator

Hydrogencarbonate indicator (sometimes called bicarbonate indicator) is very sensitive to changes in pH. Even carbon dioxide from the air, dissolved in water to form carbonic acid, is sufficient to make the indicator change colour. The indicator is coloured red when the dissolved carbon dioxide concentration is in equilibrium with the surrounding air. As the dissolved carbon dioxide concentration increases and the pH falls, the colour changes through orange to yellow. This will happen, for example, when the rate of respiration, in which carbon dioxide is produced, exceeds the rate at which carbon dioxide is utilised by photosynthesis.

Standard solutions of hydrogencarbonate indicator can be used to estimate the relative concentration of carbon dioxide in solution.



Two sodium alginate chains, cross-linked with calcium ions to form semi-solid calcium alginate gel.

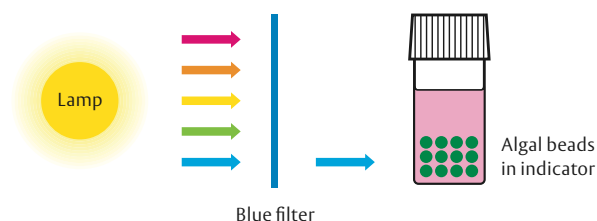
Conversely, when the carbon dioxide concentration falls, the indicator changes to a deep purple (for example, when the rate of photosynthesis exceeds the rate of respiration).

The colour of 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 (that is, using a green filter) or by comparing the colour of the indicator solution with a set of standard buffer solutions (see photograph below, but note that the colours shown in this printed document do not show the exact colours of the indicator).



## Transmittance and absorbance

One way to study the effect of the colour (wavelength) of light on the rate of photosynthesis is to place a coloured filter between the light source and the algae. This is shown in the diagram below:



In this example, the filter is blue, so blue light will tend to pass through it, and all other colours will tend to be absorbed. In the same way, a green filter will let green light pass through it, and a red filter will let red light pass through (in reality, the filters are not 'pure' blue, red and green — so they allow a range of wavelengths to pass through them).

Imagine that green light is shone on a blue filter. Only a small amount of light would pass through (blue filters will allow blue light to pass but they will absorb other colours of light). The *transmittance*,  $T$ , of the filter is defined as the ratio of transmitted light to the amount of light falling on the filter (also known as the *incident light*):

$$\text{transmittance, } T = \frac{\text{transmitted light}}{\text{incident light}}$$

If a filter allows more light to pass through,  $T$  will be greater and if *all* the incident light passes through, then  $T$  will be 1. Conversely, if a filter absorbs *all* of the light and *no* light passes through, then  $T$  will be 0.

Devices (e.g., colorimeters) which measure the amount of light passing through a sample often give the results in terms of *absorbance* ( $A$ ), the amount of light absorbed by the sample. Absorbance and transmittance are related to one another as follows:

$$T = \frac{1}{10^A}$$

## The Inverse Square Law

The *Inverse Square Law* allows you to calculate how much light will fall on bottles of algae placed at different distances from a light source, such as a lamp.

Clearly, as the distance is *increased*, the light intensity will *decrease*: this is what *inverse* means here. The relationship between the distance and the light intensity is not linear, however. The light intensity decreases in proportion to the *square* of the distance, thus:

$$\text{Light intensity} = \frac{1}{\text{Distance}^2}$$

So, for example:

$$2 \times \text{the distance gives } \frac{1}{2^2} = \frac{1}{4} \text{ of the light intensity}$$

$$3 \times \text{the distance gives } \frac{1}{3^2} = \frac{1}{9} \text{ of the light intensity}$$

$$10 \times \text{the distance gives } \frac{1}{10^2} = \frac{1}{100} \text{ of the light intensity}$$

## Neutral density filters

Neutral density filters are grey-coloured. They reduce the amount of light passing through them across the spectrum (in the 400–680 nm range). They can be used singly or combined with one another to investigate the effect of light intensity on the rate of photosynthesis.

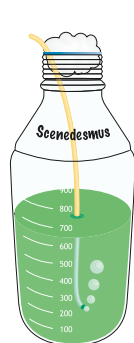
Using filters like this has several advantages over altering the distance between the bottles and the light source (see 'Additional investigations' on the back cover of this booklet).

The name of each Neutral Density filter includes the approximate absorbance of the filter at all wavelengths. For example, Filter 210 (0.6 ND) has an absorbance of approximately 0.6–0.7 across the spectrum. This translates into a transmittance of ~25% — a quarter of the light falling on this filter will pass through it.

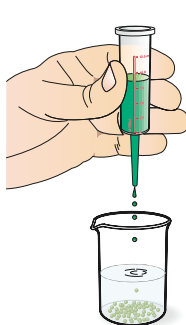
Figures for the absorbance and transmittance of the three types of Neutral Density filter provided in this kit are given on page 6 of this booklet.

## Summary of the procedure

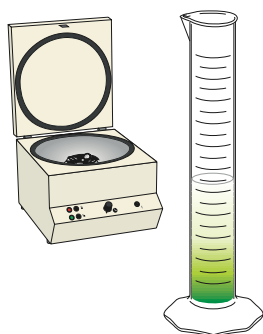
Grow the algae



Immobilise the algae



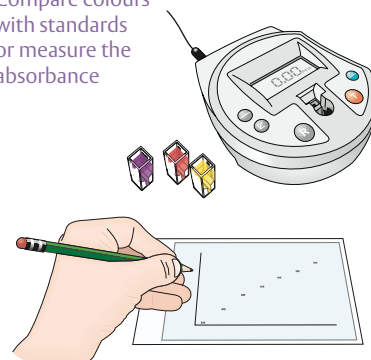
Concentrate the cells



Expose the algae to light



Compare colours with standards or measure the absorbance



Plot a graph of the results

# The procedure

## 1 Preparing the algae

Instructions for cultivating the algae are given in the Teacher's notes. The algae will need to be grown for 3–4 weeks before they can be used for this investigation.

Prepare a concentrated suspension of algae. There are two ways of doing this:

*Either*

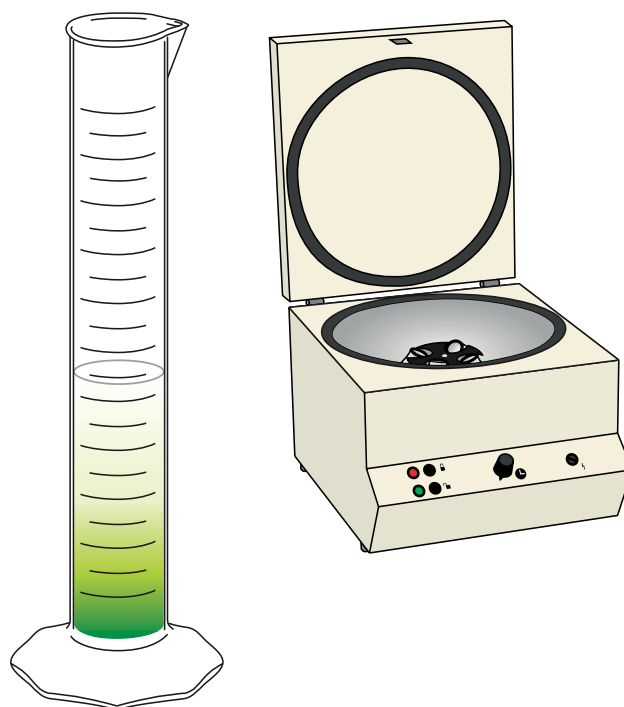
Leave the 50 mL of dark green algal suspension to settle out (ideally, overnight) then carefully pour off the supernatant to leave approximately 3 mL of concentrate.

*Or*

Centrifuge 50 mL of the suspension at low speed for five minutes. Pour off the clear supernatant to leave approximately 3 mL of concentrate.

### Notes

You need a thick, green 'soup' of algae to obtain the best results. If you use a small centrifuge, obviously you'll need to spin the cells down in batches. Ensure that you balance the centrifuge and spin the algal suspension in *sealed* tubes (the latter to prevent the formation of aerosols).



## 2 Immobilising the algae (1)

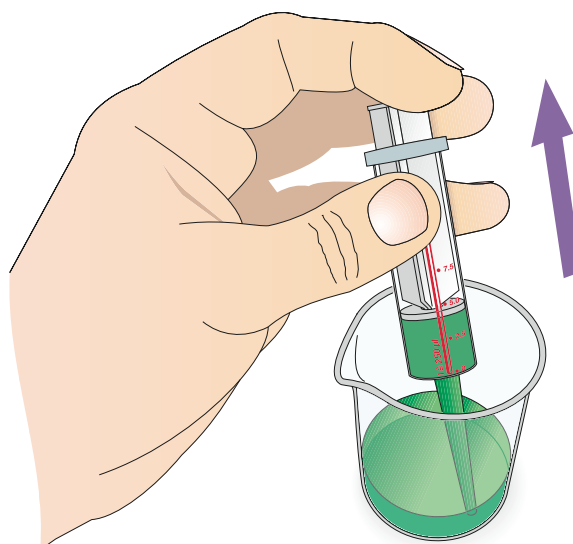
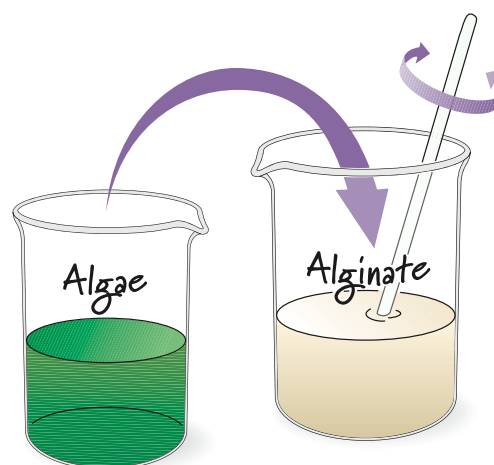
- Pour ~3 mL of the algal suspension into a small beaker and add an equal volume of 3% (w/v) sodium alginate solution. Stir gently until the algae are evenly distributed.
- Draw the algal suspension into a syringe.

### Notes

The sodium alginate solution will gel if it is mixed with a liquid containing calcium ions. Therefore it is *absolutely essential* to use distilled or deionised water, not tap water, when making up the alginate solution.

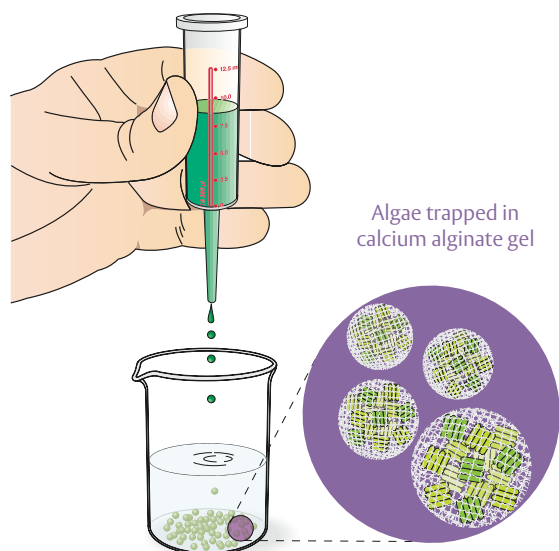
Sodium alginate dissolves only slowly, so it must be prepared in advance. Continuous stirring and warm water helps. The solution should not be heated, however, as this can adversely affect the consistency of the alginate solution. It can also be left overnight in a covered beaker or flask to dissolve.

Different sources of sodium alginate have different gelling properties. If you use sodium alginate other than that provided in the kit, you may have to experiment a little to obtain an alginate concentration that yields satisfactory beads.



### 3 Immobilising the algae (2)

- Place a beaker of 1.5% (w/v) calcium chloride solution under the syringe.
- Withdraw the plunger and allow the algae plus alginate mixture to drip slowly by gravity from the syringe into the liquid below. Swirl the calcium chloride solution gently as this happens (a magnetic stirrer is useful, although not essential, here). Hint: If you find that the beads have 'tails', hold the syringe higher, so that the alginate has time to form spheres as it falls.
- Leave the beads of immobilised algae to harden in the calcium chloride solution for 5–10 minutes. *The alginate molecules will be cross-linked by the calcium ions, trapping the cells in a matrix of calcium alginate.*
- Separate the beads from the calcium chloride solution using a tea strainer and gently wash the beads with cold tap water. Give the beads a final rinse with distilled water.

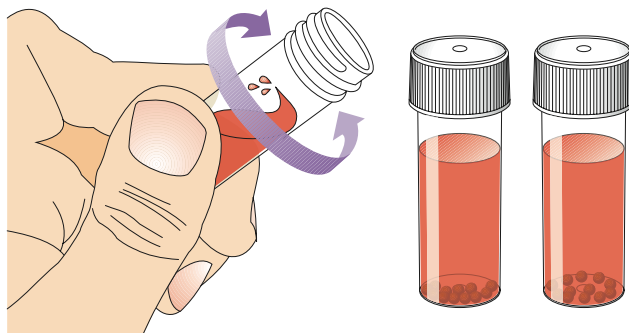


#### OPTIONAL STOPPING POINT

If they are illuminated and not allowed to dry out, the beads may be kept for several weeks. Alternatively, the beads may be stored in distilled water in a fridge for several months. Beads removed from the fridge should be allowed to warm to room temperature for about 30 minutes before use.

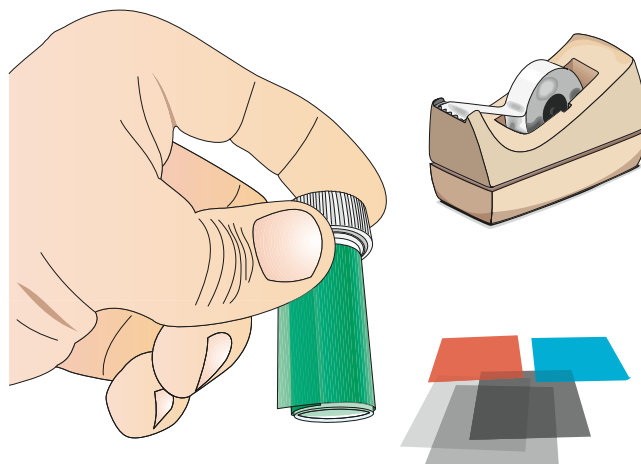
### 4 Preparing the bottles

- Take several bijou bottles and rinse each of them with a small volume of diluted (that is, not the concentrated stock) hydrogencarbonate indicator solution.
- Place equal numbers of algal beads in each bottle, then add 5 mL of diluted hydrogencarbonate indicator solution. Replace the lid of each bottle. *Approximately 12–15 beads will be required in each bottle.*



#### OPTIONAL INVESTIGATIONS

The effect of coloured or grey (neutral density) filters on the rate of photosynthesis can be investigated simply by wrapping a filter round each bottle and securing it with clear adhesive tape. Several neutral density filters can be combined to provide a wider range of light intensities. The properties of each of the filters are described on the Lee Filters website: [www.leefilters.com](http://www.leefilters.com)



Coloured filters <i>Lee filters product codes</i>	Neutral density filters <i>Lee filters codes (%transmittance)</i>
<b>Primary red</b> <i>Product code 106</i>	<b>0.15 ND</b> (71% @ 400–680 nm) <i>Product code 298</i>
<b>Primary green</b> <i>Product code 139</i>	<b>0.3 ND</b> (50% @ 400–680 nm) <i>Product code 209</i>
<b>Bray blue</b> <i>Product code 722</i>	<b>0.6 ND</b> (25% @ 400–680 nm) <i>Product code 210</i>

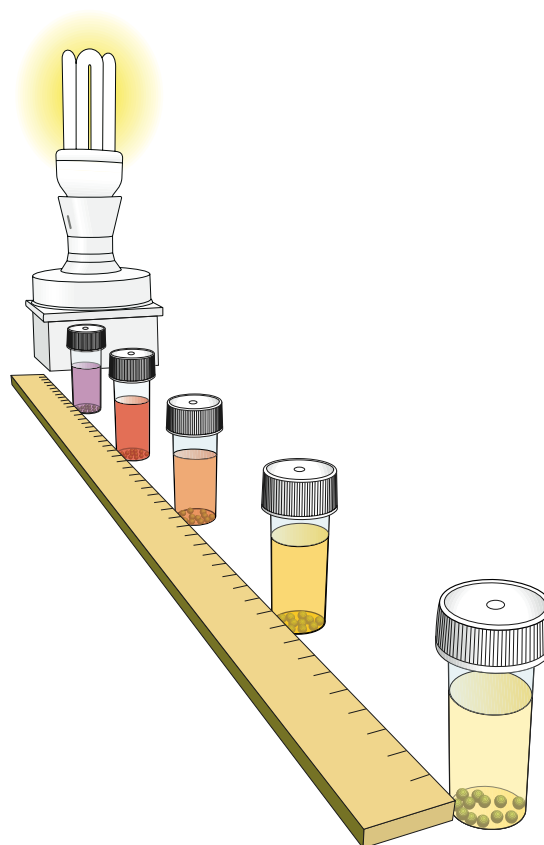
## 5 Photosynthesis

- Place the sealed bijou bottles in different light intensities by standing them at various measured distances from a bright light (for example, over a 500 cm distance). *Ensure that the bottles nearer the lamp do not shade those further away.*
- Leave them for 1–2 hours until the indicator in some of the bottles changes colour.

### Note

If you have wrapped a filter round any of the bottles, ensure that the adhesive tape that secures it faces away from the light source.

Ensure that the tape faces away from the light source



## 6 Measuring the carbon dioxide concentration

- Determine the relative carbon dioxide concentration in each bijou bottle. There are two ways to do this:

### Either

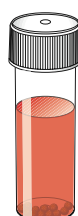
Match the colours of the hydrogencarbonate indicator in the bottles with algal beads with a range of standard solutions (instructions for preparing these standards are given in the Teacher's notes);

### Or

Use a colorimeter to measure the absorbance of the solutions at ~550 nm (that is, using a green filter or light source, depending upon the type of colorimeter available). *Note: It is important to measure absorbance (not transmission) in these experiments since there is a linear response between absorbance and pH of the indicator over the range studied.*



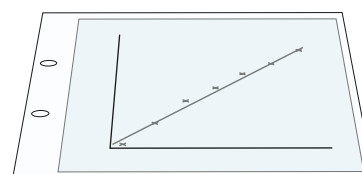
Use standard solutions...



... or a colorimeter

## 7 Plotting the results

- Plot a graph to show the absorbance at 550 nm, or the pH of the hydrogencarbonate indicator against the relative light intensity, that is,  $1/(\text{distance}^2)$ .



# Safety guidelines

Please also refer to the safety data sheets supplied

## 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, and therefore contact with the skin or eyes, or breathing in dust from the dry powder should be avoided. Refer to the Safety Data Sheets provided.

## 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 and/or a fire.

# Additional investigations

The basic method described in this booklet can be extended to provide open-ended investigations. These 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 (grey) filters. The use of neutral density filters instead of varying the distance reduces possible sources of error that might arise from:

- changes in light intensity due to differences in the angle between the lamp and the bottles;
- heating effects due to a bottle's distance from the lamp;
- difficulties in applying or interpreting the inverse square law which governs the relationship between light intensity and distance from the light source.

### Different species

How does photosynthesis by cyanobacteria (e.g., *Cynechoccus* sp.) differ from that of algae?

### Wavelength of light

The effect of wavelength can be investigated by wrapping 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. Information about the properties of the different filters can be found on the *Lee Filters* website: [www.leefilters.com](http://www.leefilters.com). The SAPS website (see below) also has useful background information.

### Temperature

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

### Number of algal cells

Dilute the algal culture and make several sets of beads with different numbers of algae in them. A haemocytometer could be used to estimate the number of cells in the concentrate and hence the number of cells in each bead.

# Further information

## Printed publications

Eldridge, D. (2004) A novel approach to photosynthesis practicals. *School Science Review* **85** (312) 37–45. *This article, upon which this kit is based, describes photosynthesis experiments with immobilised algae in more detail.*

Smidsrød, O. and Skjåk-Bræk, G. (1990) Alginate as an immobilization matrix for cells. *Trends in Biotechnology* **8** (3) 71–78.

## Websites

### Science and Plants for Schools (SAPS)

SAPS has numerous articles, support materials and additional practical protocols for studying photosynthesis.

This includes the original version of this protocol, written and video resources and detailed information on light absorption and filters. These materials are available at: [www.saps.org.uk](http://www.saps.org.uk)