

Photosynthesis . . . using algae wrapped in jelly balls

Algae can be considered as one-celled plants, and they usually live in water. You are going to use algae to look at the rate of photosynthesis. The algae are tiny and are difficult to work with directly in the water so the first part of the practical involves 'immobilising' the algae. This effectively traps large numbers of algal cells in 'jelly like' balls so that we can keep them in one place and not lose them. We use sodium alginate to help make the jelly. Sodium alginate is not harmful to the algae.

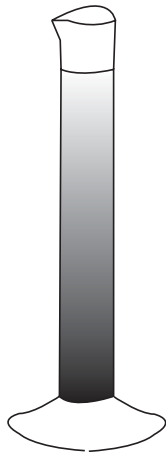
When these algae are 'wrapped up' in the jelly balls they are excellent to use in experiments on photosynthesis. These algal balls are:

- cheap to grow and easy to make – you will be able to make hundreds in a very short time
- easy to get a standard quantity of plant material because each of the balls is approximately the same volume
- easy to keep alive for several weeks so you can keep them for future experiments

Making the algal balls

1. First you need to obtain a concentrated suspension of algae. Do this by removing some of the liquid medium in which they are growing in one of two ways.

- *either*
Leave 50 cm³ of dark green algal suspension to sediment out and gently pour off the supernatant to leave approximately 5 cm³ at the bottom.
- *or*
Place 50 cm³ of dark green algal suspension in a centrifuge and spin gently for 5 minutes. Pour off the supernatant leaving approximately 5 cm³.



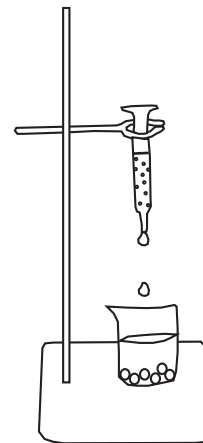
2. Now you have millions of algal cells in a small volume of liquid. It's time to mix them into your 'jelly' . . .

- Pour about 2.5 cm³ of jelly (sodium alginate solution) into a very small beaker.
- Add approximately 5 cm³ of concentrated algal cells.
- Stir the mixture with a clean cocktail stick until you have an even distribution of algae in your jelly.



3. Finally we're going to make the balls . . .

- Pour the green mixture through an open-ended syringe into a 2% solution of calcium chloride.
- Swirl the calcium chloride gently as the drops fall through the syringe to form small balls of algae.
- Leave for 10-15 minutes in the calcium chloride and then wash the balls with distilled water. (A plastic tea strainer is useful to separate the algal balls from the solution.)



Your algal balls are now ready to use in experiments. They will remain alive for several weeks as long as they are kept in the light and not allowed to dry out.

When you have made your algal balls you can use them to determine the rate of carbon dioxide absorption - which indicates how fast photosynthesis is taking place. You can detect carbon dioxide absorption using hydrogencarbonate indicator.

Hydrogencarbonate indicator is very sensitive to changes in carbon dioxide level. The indicator is orange/red in colour when equilibrated with atmospheric air. It changes to yellow when more carbon dioxide is added and changes through red to a deep purple colour when carbon dioxide is removed. The diagram below shows these colours.



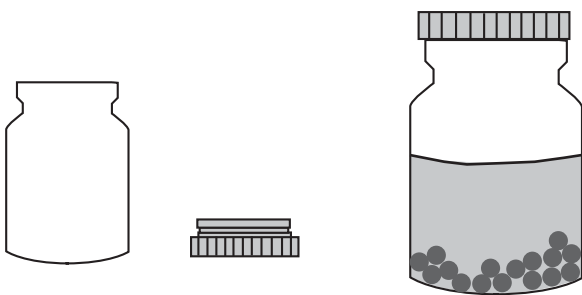
Doing investigations with algal balls

When you place the algal balls in hydrogencarbonate indicator solution, the colour of the indicator changes from orange / red to purple. This is because the algae are taking carbon dioxide out of the indicator thereby lowering the concentration in the indicator as they use carbon dioxide in photosynthesis.

Here is an outline of how you could investigate the effect of light intensity on the rate of photosynthesis. You will need to decide on details of quantities and how to vary the light intensity.

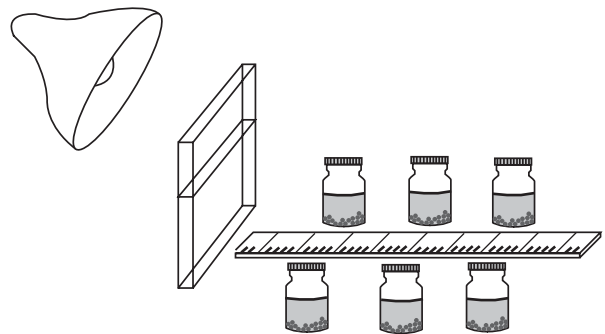
4.

- Take several small glass containers with lids and rinse all of them with a small volume of hydrogencarbonate indicator.
- Add equal numbers of algal balls to each container.
- Add a standard volume of indicator to each container.
- Replace the lid.



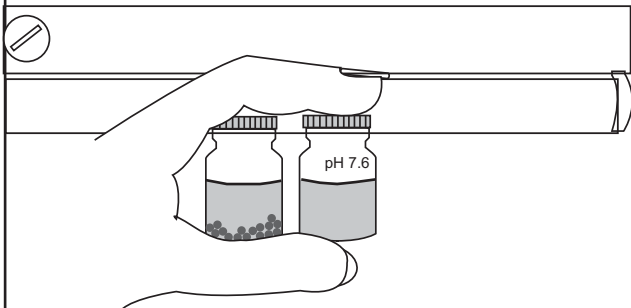
5.

Place the containers at different light intensities. Leave them until there is a visible colour change in some of the containers. (This may take 1-2 hours.)



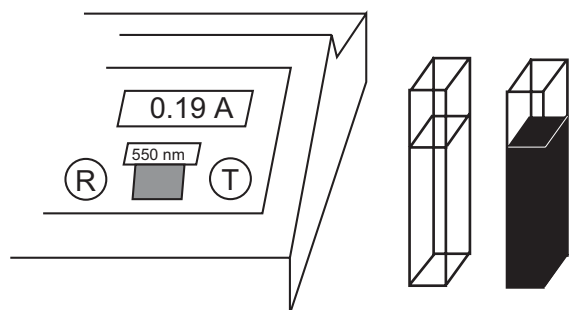
either 6a

Compare your colour changes with the standard buffer solutions. Hold each container to the light and match it to the buffer nearest in colour to your sample.



or 6b

- Use a colorimeter to measure the absorbance of your solution.
- Fill a cuvette $\frac{3}{4}$ full with distilled water and place in the colorimeter. Press the zero or reset button.
- Fill a second cuvette $\frac{3}{4}$ full with the indicator from one of our test solutions. Place in the colorimeter. Press the test button and take the reading.
- Repeat with each of your test solutions.



A colorimeter can be used to measure the amount of light absorbed by the coloured solution. Different colours absorb different wavelengths of light to a different extent, so we shine light of a single wavelength through the mixture. It is best to use green light (wavelength 550 nm), because the colours of the indicator (purples, reds and yellows) absorb green light quite well.

Notes for teachers *(see Technicians' notes on page 2)*

Photosynthesis is such a vital biological process that it inevitably appears, in a variety of forms, throughout the National Curriculum. By the time it crops up at Key Stage 4, students may be very 'turned off' by the word photosynthesis and cries of 'not again' can be heard in classrooms throughout the land. Many teachers consider the traditional experimental work to be slow, laborious and unexciting. Additionally there is some evidence to suggest that the emphasis on starch testing does little to improve students' understanding of the topic.

The experiments described here have several aims:

- To introduce students to algae as organisms with a fast rate of photosynthesis and which can be cultured easily in large quantities
- To focus on carbon dioxide as a raw material which is vital for photosynthesis
- To provide students with techniques which are reliable enough for them to get lots of data for a full investigation
- To stimulate and interest students

The experiments make use of the technique of immobilisation so that the algae can be 'contained' within balls of sodium alginate. The advantage of using algal balls is that it is very easy to standardise the amount of photosynthetic tissue present. In each batch the balls are remarkably uniform in size and chlorophyll concentration as long as they have been produced with the same syringe.

The experiments can be carried out with any green algae but one of the best is the unicellular alga *Scenedesmus quadricauda* *. It is easy to culture and photosynthesises well. Starting with a 50 cm³ culture, you can get 2 to 3 litres of dark 'green soup' in about 4 weeks. This would be enough for several groups of students.

Students can determine the rate at which the algae absorb carbon dioxide, using hydrogencarbonate indicator. The colour changes 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 made up beforehand.

Possible investigations

These instructions to students for making the algal balls are quite prescriptive but the way that the pupil takes this forward into investigations of photosynthesis has been presented in a deliberately 'open ended' way. This is so that students have the opportunity to plan and carry out their own investigation. There are a number of variables that they can consider and different ways that the method can be adapted to investigate each variable. Here are some suggestions:

1. Light intensity

- either vary the distance from the lamp. (Students can achieve high marks for planning if they have researched the relationship between light intensity and distance.)
- or cover the containers with neutral stop filters (available from photographic shops) in various combinations

2. Wavelength of light - use coloured filters from photographic shops or gels from theatre lighting companies. (If students are to analyse the results well they will need to be aware of different light sources producing light of different wavelengths and they will need to know what wavelengths of light each of the filters lets through.)

3. Temperature - set up water baths under a bank of lights and float the sealed containers in the water with light shining from above.

4. Number of algal cells - make several sets of algal balls with different numbers of algae in them. This can be achieved by shaking the algal culture initially and then using different volumes of culture to obtain the sediment.

A few more comments

For the best results, the lights used in these experiments need to be stronger than the standard 40 W bench lamp. 150 W is ideal but these lamps give out a lot of heat so it is important to use a heat filter. Medical flats filled with water are useful as heat filters. It is also important to keep the room as dark as possible apart from the experimental light source.

Use of the colorimeter - The best colorimeter I have found for students at Key Stage 4 to use is one by WPA (Walden Precision Apparatus), The Old Station, Linton, Cambridge CB1 6NW. This colorimeter has a digital readout and is simple to use. It is reliable, robust and the results are repeatable. It also remains standardised for 10 minutes at a time. 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.

* Algal enrichment medium can be purchased from Sciento. They are offering 20% discount to schools purchasing a culture of *Scenedesmus* and 25 g of algal enrichment medium at the same time. Sciento are at: 61, Bury Old Road, Whitefield, Manchester M45 6TB
Tel: 0161773 6338

An article, which describes in more detail experiments that were carried out with *Scenedesmus* algal balls, is being prepared for publication.

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Dr Eldridge carried out this work during her SAPS-Robinson College Schoolteacher Fellowship in Plant Science. SAPS is very grateful to King Ecgbert School, Sheffield for releasing her.

Technicians' notes (for photosynthesis . . . using algae wrapped in jelly balls)

3 - 4 weeks before the investigation

Instructions for the culture of the algae

Obtain a culture of *Scenedesmus*. Make up a solution of algal enrichment medium* and sub culture the alga into this medium. Aerate gently and keep at temperatures between 18 to 22 °C and under constant illumination. (Constant illumination is not essential but growth is faster if this is available.) After 3 to 4 weeks the solution should be a dark 'pea soup' colour at which point you should subculture the algae again to maintain a healthy population.

1 day before the investigation

Making up the alginate

Make up a 3% solution of sodium alginate 100 cm³ should be enough for a class of 30 students. This needs to be dissolved slowly on a warm hotplate and takes several hours to mix fully but lasts for several days in the refrigeration once made up. Different brands of sodium alginate have different consistencies when made up but this is not crucial as long as a fairly viscous mixture is obtained to drip steadily through the syringe.

Making up buffer solutions

If access to a colorimeter is not realistic then the colour change can be semi-quantified by comparing it to a series of coloured buffered solutions.

Solutions ranging from pH 7.6 – pH 9.2 can be made up using borax/boric acid buffers. If 9 cm³ of buffer solution is placed in a series of vials and 1 cm³ of concentrated (i.e. off the shelf) hydrogencarbonate indicator is added to this just prior to the lesson it gives a lovely spread of colours and students can then compare their test solution with the buffer. The buffers can be kept for weeks but the colour of the indicator deteriorates so it is best to add the indicator just before the lesson.

1 hour before the practical

Each student will need 50 cm³ of algal culture solution and it is best if this can be poured out and left to stand for at least 30 minutes so that the algae begin to sediment out. This avoids wasting time waiting for this to happen in the lesson.



Photosynthesis - using algae wrapped in jelly balls

Practical information for teachers and technicians

Notes prepared by Dr D Eldridge, King Egbert School, Furniss Avenue, Sheffield S17 3QN.

1. Suppliers of algae

Sciento, 61 Bury Old Road, Whitefield, Manchester M45 6TB
Tel 0161 773 6338 E-mail: sales@sciento.co.uk

Scenedesmus quadricauda (A290) is £5.85 for a starting culture; algal enrichment medium (K10) £5.85 for 25 g

Sciento offer 20% discount if these are bought together – mark it on the order form. You should not need to purchase the enrichment medium each time you buy a new culture. Jumbo cultures (JA290) of *Scenedesmus quadricauda* are also available.

The culture should be prepared about three to four weeks before it is required. Make up the algal enrichment medium as instructed and subculture the algae into this medium. It should then be aerated gently and maintained at 18–22 °C, ideally under constant illumination for fast growth. Depending upon light and temperature, after two to four weeks, the suspension should be a dark 'pea soup' colour. If it is not needed immediately, the algae can be sub-cultured (taking about 100 cm³) to maintain a healthy population. To aid aeration of the algae, it should be prepared in large, round vessels e.g. 5 dm³ round bottomed flasks or 2 dm³ volumetric flasks. 2 dm³ 'pop' bottles have been tested, but were found to be unsuitable since the algae sedimented out in the dimples of the base.

Each student requires 50 cm³ of dark green algal culture solution which should ideally be dispensed about 30 minutes before the lesson for the algae to sediment out. This avoids wasting time in the lesson. (If the culture is not dark green they may need a greater starting volume.)

2. Colorimeter

A suitable colorimeter to purchase for pupils at Key Stage 4 is the CO7500 from Biochrom Ltd., 22 Cambridge Science Park, Milton Road, Cambridge CB4 0FJ. Tel 01223 423723 e-mail enquiries@biochrom.co.uk

The best filter to use is the green (550 nm).

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

Make up solutions of boric acid (12.4 g per dm³ i.e. 0.2 mol dm³) and borax (19.05 g per dm³ i.e. 0.5 mol dm³ (0.2 mol dm³ in terms of sodium borate)). To 25 cm³ of the boric acid add the indicated volume of borax solution and make up to 100 cm³ with deionised water:

Borax solution / cm ³	pH	Borax solution / cm ³	pH
1.00	7.6	8.70	8.6
1.55	7.8	15.0	8.8
2.45	8.0	29.5	9.0
3.60	8.2	57.5	9.2
5.70	8.4		

Place 9 cm³ of the buffer solution in a series of vials and just before the lesson add 1 cm³ of concentrated hydrogen carbonate indicator solution. This should give a good range of colours for the students to assess their results against.

Hydrogen carbonate indicator can be purchased from all the main scientific suppliers but it does vary a great deal. It is bought in concentrated form and has to be diluted x10 and then aerated before use. Some indicator is barely red when bought in a concentrated form and another bottle might contain a deep purple liquid. I am still in discussion with suppliers about this but the key thing is to use your indicator from a single source and not to mix them. If you can find a good source – the deep purple colour seems to give the best depth of colour and most obvious colour changes.

The indicator can also be made quite easily from cresol red and thymol blue: Dissolve 0.10g of cresol red and 0.20 g of thymol blue in 20 cm³ ethanol. Dissolve 0.85 g sodium hydrogencarbonate in about 200 cm³ of freshly boiled distilled water. Add the ethanol solution and dilute to 1000 cm³ with water. For use, dilute the stock solution ten times with freshly boiled distilled water and bubble air through the diluted solution to equilibrate it with atmospheric carbon dioxide.

Hydrogen carbonate indicator is a very sensitive pH indicator so it is important that all the glassware etc. used is rinsed out with a little of the indicator before use.

3. Sodium alginate

This needs to be made up at least one day before the lesson. 100 cm³ is sufficient for a class of 30 students. The solution should not be heated at any stage of preparation since this will compromise the gel strength of the algal balls. The 2-3% w/v solution is made by adding the sodium alginate powder to cold water on a magnetic stirrer – the solution can then be left overnight to fully dissolve. The solution will then keep for a couple of weeks if refrigerated.

4. Lights

Normal bench lamps are not bright enough for experimental work on photosynthesis so you will need to purchase some brighter ones.

Ideas – 150 W Halogen portable lamps are excellent if you can get them (available from Rapid Electronics). If not then 150 W bulbs in holders are quite bright or types of inspection lamps from motor dealers can be used effectively. None of the above is too expensive – many are cheaper than traditional bench lamps.

All these lamps give out a lot of heat and therefore a heat screen is necessary so that light is the only variable. A large flat glass vessel filled with water is sufficient. A chromatography tank is ideal, or large (1 dm³) medical flats. If flat glassware is unavailable, 2 dm³ 'pop' bottles will suffice – anything is better than nothing, since the heat from the lights will kill the algae if placed too close. Pupils should be instructed not to look directly at the light source and not to touch the lamp during the course of the experiment.

The best results are obtained when all extraneous light in the laboratory is minimised. Very successful experiments have been done with the light within a large cupboard or with a light shining through a hole in a large cardboard box and the experiment arranged within it but beware of overheating of the box itself.

Photographic filters can be used to experiment with light wavelength. Lee Filters produce a huge range and details of the transmission spectrum of each filter are supplied (available from Lee Filters, Central Way Walworth Industrial Estate, Andover, Hampshire SP10 5AN. Tel 01264 366245; www.leefilters.com).