Limiting factors in photosynthesis

The Biology team in SSERC has recently been reviewing and updating the range of resources offered through our website [1] to support the new Higher programmes in Biology and Human Biology.

The *CfE* Higher in Biology [2], which will be first taught in the 2014-2015 academic session, has three mandatory units:

1) DNA and the Genome;
2) Metabolism and Survival;
3) Sustainability and Interdependence.

Photosynthesis is covered in the 3rd of the above units and amongst the possible learning activities (in '*The science of food production*' section) is the suggestion that learners might '*Carry out experimental investigations on limiting factors in photosynthesis*'. There are a range of practical activities which one might use to cover this statement and the purpose of this article is to explore some of these in more detail.

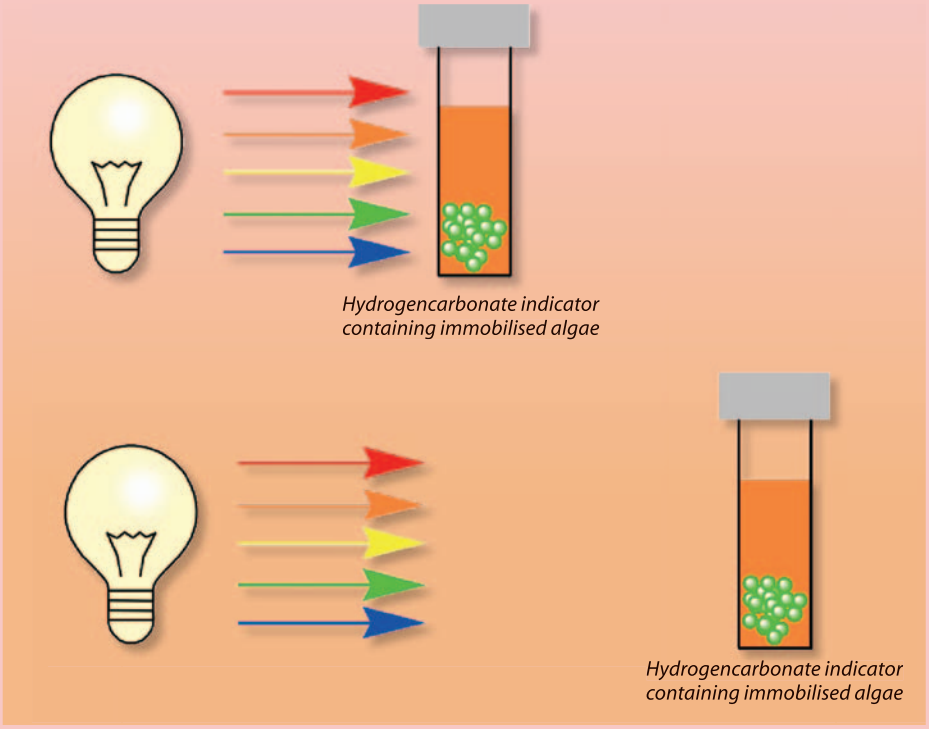

Figure 2 *- Reducing the light intensity in photosynthesis experiments.*

Figure 1 *- Photosynthesis in action.*

Immobilisation of algae

For ease of convenience, many schools opt to use the well-established technique involving immobilisation of algae for which a kit and supporting documentation is available from the National Centre for Biotechnology Education (NCBE, [3]). Within SSERC we have made a number of investigations using immobilised algae and further details can be found in past issues of this Bulletin [4, 5]. In terms of investigating limiting factors, the most common experiment which is undertaken is the measurement of the effect of light intensity on the rate of photosynthesis. Using immobilised algae this is a relatively straightforward experiment to set up. Briefly, you place immobilised algae (a detailed protocol for their production is available [1]) in a suitable vessel containing hydrogencarbonate indicator and place the samples in front of a suitable light source. As carbon dioxide is removed from solution (and the solution becomes less acidic) the indicator will change colour and the rate of colour change can be conveniently measured using a colorimeter. Reducing the light intensity (as shown schematically in Figure 2) can be readily achieved simply by moving samples away from the light source.

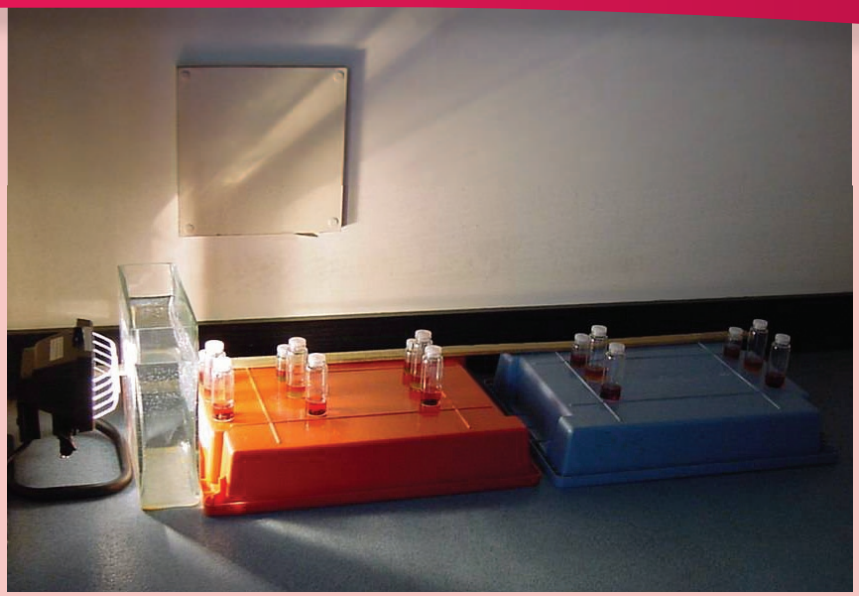

Figure 3 *- An experimental set-up for measuring the effects of light intensity on the rate of photosynthesis.*

There are some drawbacks with this method which are worth highlighting here:

- Light intensity does not reduce linearly as you move your samples away from the lamp. The inverse square law applies and this can be a challenging concept for learners to take into account when planning experiments.
- It is important to minimise the effects of stray room light on your sample especially those samples which are distant from the lamp source.
- Inadvertent shading of samples can occur unless samples are placed correctly.
- Since temperature can also affect the rate of photosynthesis any heating effects from the light source must be minimised.

The set-up shown in Figure 3 demonstrates some of the pit-falls described above. For example the tubes containing the algae are spaced at regular intervals from the lamp and when plots are made the data points, because of the inverse square law, will tend to be bunched at one end of the axis. Additionally the tubes at the front are shading the tubes further away from the lamp. That said, heat effects from the lamp and the extraneous effect of room lighting appear to have been minimised by the insertion of a 'heat sink' and the switching off of room lights.

An alternative way of varying light intensity is to use so-called neutral density filters (shown schematically in Figure 4). These grey filters are available from a number of suppliers and in SSERC we usually source them via Lee Filters (http://www.leefilters.com/) who have a number of distributors throughout the UK. It is possible that your colleagues in the Drama department will have a range of suitable filters since the neutral density filters are marketed as photographic stop filters and often used in lighting rigs for stage productions. Essentially a neutral density filter is designed to reduce light intensity by a given amount across all wavelengths. Lee Filters sell a number of neutral density filters. The product numbers (for which there does not appear to be a logical sequence) and the percentage of light which they transmit is given in the Table below. At the time of writing (August 2013) sheets (measuring 1.2 m x 0.25 m) of each filter were available from Black Light in Edinburgh (www.blacklight.com) for a cost of £2.50 each (+postage).

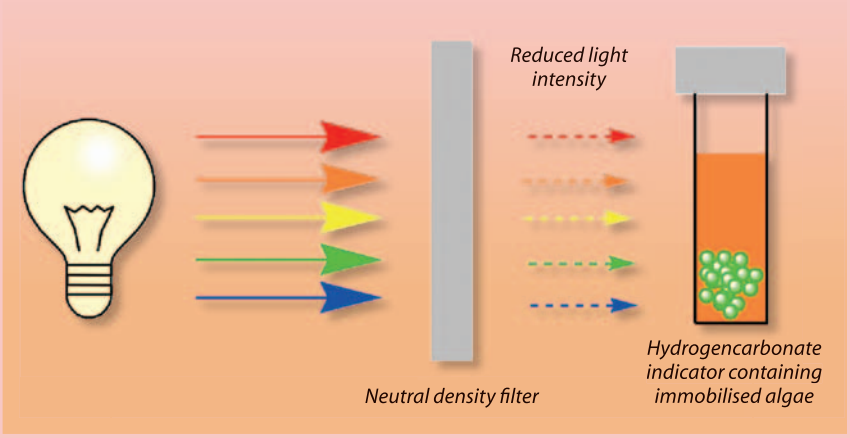

Figure 4 *- Using a neutral density filter to reduce light intensity in photosynthesis experiments.*

Filter number	% transmission
298	71
209	50
210	25
211	12.5
299	6.25

We have made measurements of the transmission properties of the full range of neutral density filters and these are all available as Excel files on the SSERC website [1]. The data for Lee Filter 209 (notionally 50% transmission) is shown in Figure 5. The measured transmission for the neutral density filter is close to the manufacturer's data (the horizontal line is at 50% transmission) but it should be noted that the filter starts to transmit increased light levels towards the far red end of the spectrum - for most purposes this will not be problematic.

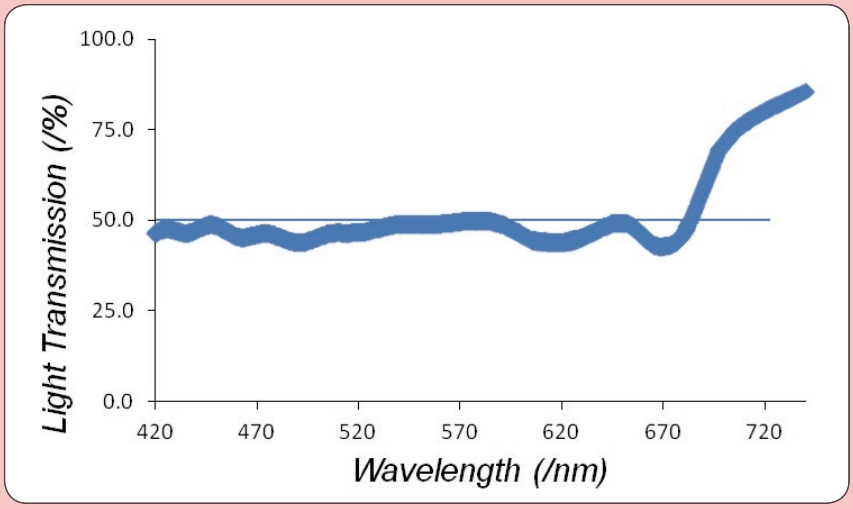

Figure 5 *- Light transmission of a Neutral Density 209 filter from Lee Filters. Data is displayed as percentage of light transmitted as a function of wavelength at 1 nm intervals. The horizontal line represents 'pure' 50% transmission.*

There is no need to buy a complete set of filters. A single layer of filter 298 will notionally allow 71% of light to pass through. A single layer of filter 298 combined with a single layer of filter 210 would allow (0.71 x 25%) = 17.8% of the light to pass through. Two layers of filter 209 is equivalent to 1 layer of 210 etc. The experimental set-up shown in Figure 6 shows how the effects of light intensity might be measured and the advantages using of neutral density filters are apparent. All samples are equidistant from the lamp, any heating effects from the lamp will affect all samples equally, room lighting complications are reduced, and the effects of shading are removed.

Judicious use of filters allows estimates of the compensation point to be determined and this has been the subject of a previous article in this Bulletin [5] and so these will not be discussed further here.

Cabomba

Within the Biology Team at SSERC we have what can only be described as a long-term love affair with the aquatic plant *Cabomba* and we have previously published on its usefulness [6-8] in a number of experiments. The ability to measure rates of oxygen production [6] and the increasing availability from a number of commercial sources makes *Cabomba* 'our pond weed of choice'.

Figure 7 *- Illumination of Cabomba.*

Qualitative and quantitative measurements of the effect of light intensity on the rate of oxygen production using *Cabomba* are relatively straightforward. The basic experimental set-up is shown in Figure 7. A 'sprig' of *Cabomba* is placed in a boiling tube containing 1% sodium hydrogencarbonate and a fresh cut applied to the stem of the *Cabomba*. Bubbles of

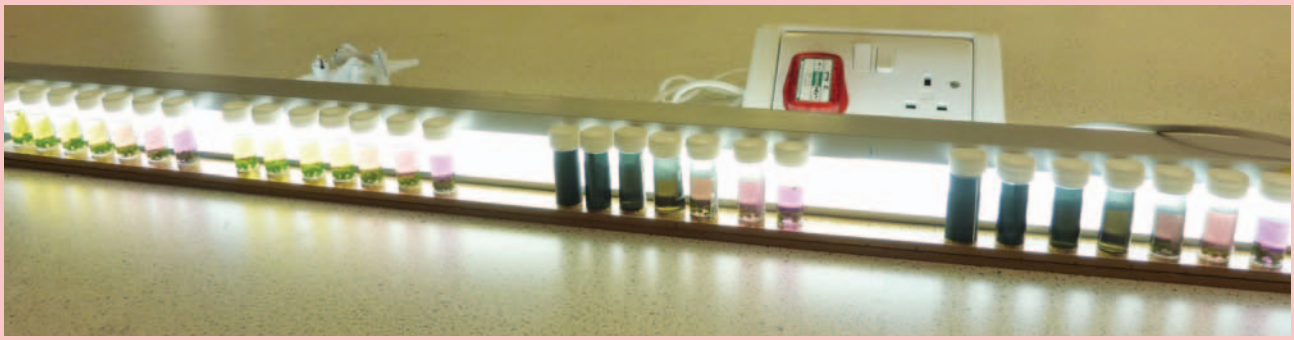

Figure 6 *- An experimental set-up allowing for measurement of the effects of light intensity on the rate of photosynthesis. Bijou bottles containing immobilised algae are exposed to light from a fluorescent tube; light intensity is altered by the addition of a suitable filter.*

gas which emerge from the stem can be counted or arranged to displace a known volume of sodium hydrogencarbonate from a disposable pipette which has been placed over the stem. The rate of gas production can be varied by wrapping the boiling tube with neutral density filters.

Carbon dioxide probes

There are a number of advantages in using carbon dioxide probes to measure rates of photosynthesis and we have reviewed those previously [9]. Results from a typical experiment are shown in Figure 8. In this case leaves from a basil plant (approximately 2.5 g of material [ca. 12 leaves]) were placed in a sealed vessel and exposed to the light from a small desk lamp. The lower data set shows the leaves received the full beam and the upper data set was produced when the intensity of the beam was reduced by 50% using a 209 neutral density filter. Data was collected at 4 second intervals for about 13 minutes in both cases. The best line fits to the data yields slopes with a ratio of 1.8 - close to the value of 2.0 that one might expect. In common with the immobilised algae experiments, issues around changing distance from the light source are overcome.

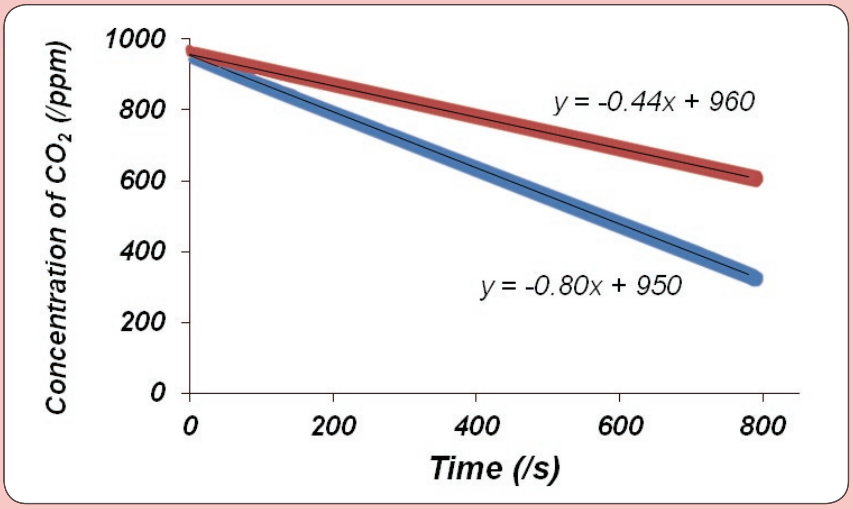

Figure 8 *- The rate of photosynthesis in basil leaves as a function of light intensity. Data were obtained using a Vernier VR105512 probe. A 50% neutral density filter was either present (●) or absent (●).*

Carbon dioxide probes offer additional advantages over the immobilised algae system including:

- a wide range of different plant materials can be investigated
- photosynthesis rates can be investigated in 'real situations' e.g. in the field;
- the readings of carbon dioxide concentration are direct and available in 'real time'.

In principle it would, using carbon dioxide probes, be possible to measure rates of photosynthesis at fixed light intensities with varying carbon dioxide concentrations present. We will endeavour to undertake such experiments and report on our success, or otherwise, in a future issue of the Bulletin. ◀

References

[1] The SSERC website is available at www.sserc.org.uk. Please note that to access all resources on the website you will need to register and be provided with a log-on ID and password.
[2] SQA (2012) Higher Biology Course Support Notes - can be downloaded at http://www.sqa.org.uk/files_ccc/CfE_CourseUnitSupportNotes_Higher_Sciences_Biology.pdf (accessed August 6th 2013).
[3] NCBE (2013), NCBE-SAPS Photosynthesis kit. Available at http://www.ncbe.reading.ac.uk/NCBE/MATERIALS/METABOLISM/photosynthesis.html (accessed August 6th 2013).
[4] Crawford, K. (2006) SAPS Photosynthesis Kit: the use of algal balls to investigate photosynthesis. SSERC Bulletin **219**, 2-5.
[5] McAndrew, R. (2008) Investigating the compensation point of algae. SSERC Bulletin **225**, 6-7.
[6] Crawford, K. (2005), *Cabomba* - a reliable alternative to Elodea? SSERC Bulletin **215**, 10-12.
[7] Adams, A., Moore, G., Crawford, K. and Beaumont, P. C. (2011) *Cabomba* Tricolour. SSERC Bulletin **238**, 2-4.
[8] Adams, A., Moore, G., Rutherford, A., Stewart, F., Crawford, K. and Beaumont, P. C. (2012) *Cabomba* - an exocharmic plant! School Science Review **93**, 9-12.
[9] Beaumont, P. C. (2012) Measuring gaseous carbon dioxide. SSERC Bulletin **238**, 5-7.