Measurement of limiting factors in photosynthesis

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ABSTRACT The new Higher biology curriculum in Scotland encourages students to carry out experimental investigations on limiting factors in photosynthesis. This article describes experimental systems for measuring the effect of light intensity and carbon dioxide concentration on the rate of photosynthesis that will allow such work to be carried out.

The teaching of post-16 biology in Scotland is undergoing a radical transformation with the recent introduction of new Curriculum for Excellence (CfE) Higher programmes in biology and in human biology. The CfE Higher in biology (Scottish Qualifications Authority, 2014) has three mandatory units:

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

Among the topics covered in *Sustainability and Interdependence* are studies of photosynthesis and among the possible learning activities is the suggestion that learners might '*Carry out experimental investigations on limiting factors in photosynthesis*'. There are a range of practical activities that one might use to cover this statement and the purpose of this article is to explore some of these in more detail.

A number of factors are known to affect photosynthesis rates in plants. Classically, the variation of factors is as shown in Figure 1 (Hall and Rao, 1999).

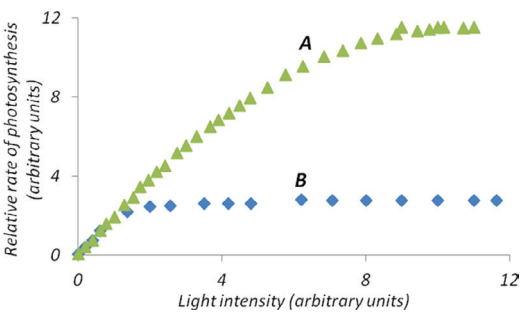

Figure 1 Effect of external factors on the rate of photosynthesis in chlorella: (A) effect of light intensity at 25 °C and 0.04% CO_2, (B) effect of light intensity at 25 °C and 0.01% CO_2; adapted from Hall and Rao (1999)

The interpretation (Hall and Rao, 1999) of the plots can be summarised as:

- at low light intensities, the rate of photosynthesis increases linearly as a function of light intensity;
- at higher light intensities, the rate of photosynthesis is limited by the available CO_2 concentration (curve B).

Effect of light intensity

For convenience, many schools opt to use the well-established technique involving immobilisation of algae (Eldridge, 2004), for which a kit and supporting documentation are available from the National Centre for Biotechnology Education (see *Websites*). In terms of investigating limiting factors, the most common experiment undertaken is probably 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 in a suitable vessel containing hydrogencarbonate indicator and place the samples in front of a suitable light source. As CO_2 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.

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

- Light intensity does not fall linearly as the samples are moved away from the lamp. The

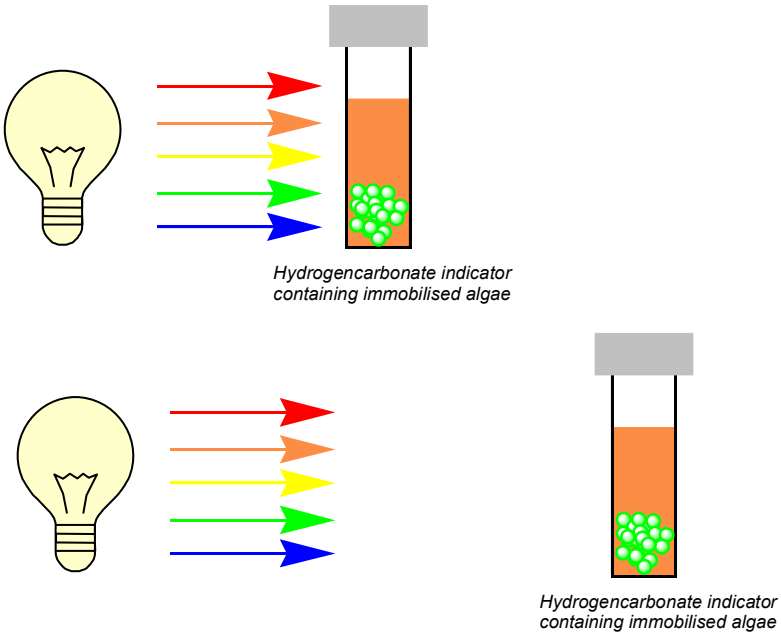

Figure 2 Reducing the light intensity in photosynthesis experiments

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 lights on your samples, especially those that 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.

An alternative way of reducing light intensity is to use neutral density filters (shown schematically in Figure 3).

Neutral density filters are available from a number of suppliers and we usually source them from Lee Filters (see *Websites*) 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 neutral density filters are often used in photography and in lighting rigs for stage productions. Essentially, a neutral density filter is designed to reduce light intensity by a given amount across all wavelengths (filters available include 71%, 50%, 25%, 12.5% and 6.25% transmission). The data for a Lee 209 filter (notionally 50% transmission) are shown in Figure 4. The measured transmission 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. We have made measurements of the transmission properties of the full range of neutral density filters and these are available as a set of *Microsoft Excel* files from the authors.

There is no need to buy a complete set of filters. A single layer of a Lee 298 filter will notionally allow 71% of light to pass through and, if combined with a single layer of a Lee 209 filter would allow 71% × 50% = 36% of the light to be transmitted; other filter combinations can be made.

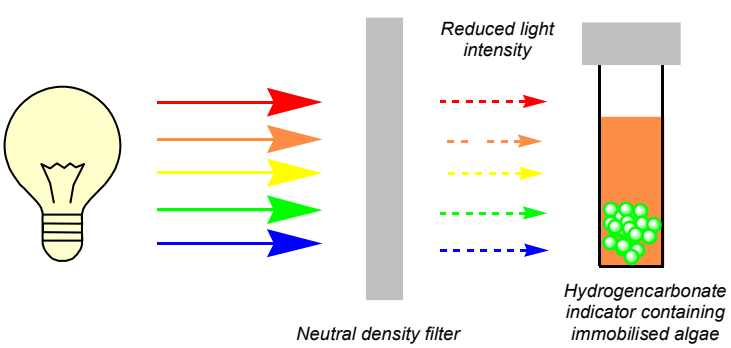

Figure 3 Using a neutral density filter to reduce light intensity in photosynthesis experiments

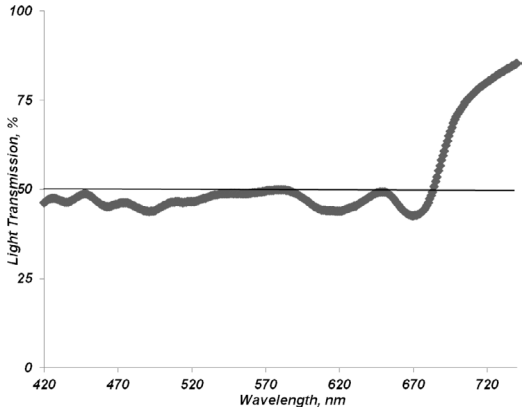

Figure 4 Light transmission of a neutral density 209 filter from Lee Filters. Data are displayed as the percentage of light transmitted as a function of wavelength at 1 nm intervals. The horizontal line represents 'pure' 50% transmission.

The experimental set-up in Figure 5 shows how the effects of light intensity might be measured and the advantages of using 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.

Effect of carbon dioxide concentration

Carbon dioxide probes are convenient devices that can be used experimentally in the classroom (Delpech, 2006). Such probes have a number of advantages, including the following:

- a wide range of different plant materials can be investigated;
- photosynthesis rates can be investigated in 'real situations', such as in the field;
- the readings of CO_2 concentration are direct and available in 'real time'.

The experimental set-up using CO_2 probes has been described previously (Redding and Masterman, 2007) and is shown in Figure 6.

Figure 6 Experimental set-up for measuring respiration and photosynthesis rates in plants. Basil leaves are placed into a 'reaction chamber' together with a CO_2 gas sensor from Instruments Direct Services Ltd (product code VR105512). The sensor is linked to a computer via a Vernier Go!Link interface.

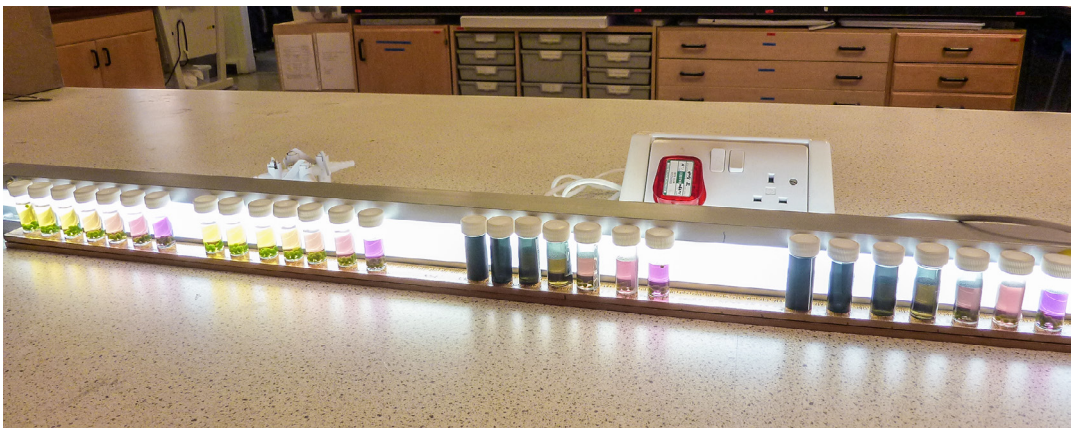

Figure 5 An experimental set-up allowing for measurement of the effects of light intensity on the rate of photosynthesis. Bijou bottles containing immobilised algae are exposed to light from a fluorescent tube; light intensity is altered by the addition of a suitable filter.

The light source used here was a small desk lamp although a range of lamps could be used. A tissue culture flask filled with water (to act as a heat sink) was placed in front of the experimental chamber. Leaves from a basil plant (approximately 2.5 g of material, about 12 leaves) were placed in a chamber that was wrapped in aluminium foil to exclude light. Under these conditions, CO_2 levels rise as respiration takes place in the leaves. Data were collected for 11 minutes (Figure 7). During the first 5.5 minutes, the concentration of CO_2 present increased from its initial level of about 335 ppm to approximately 560 ppm. During this period, the dominant process was respiration leading to an increase in CO_2 concentration. The aluminium foil was then removed and the lamp switched on. Over the next 2 minutes, CO_2 levels continued to rise as the system equilibrated. Once photosynthesis became predominant, CO_2 levels started to fall. By the end of the collection period, the concentration of CO_2 had fallen to 520 ppm.

To calculate the rate of fall of the CO_2 concentration, we measured it over a limited timescale (thus, for the data in Figure 7, it was measured in the region between initial and final CO_2 concentrations of 600 and 520 ppm respectively). During the period when photosynthesis was predominant, there was a linear fall in CO_2 concentration, the rate of fall being 0.8 ppm s^{-1}. We have opted to include a line of best fit to the data in Figure 7 although we recognise that, while a reasonable fit is obtained (the R^2 value for the data in Figure 7 is 0.99), a linear plot is somewhat misleading since, as is implied in Figure 1, the rate of photosynthesis is related to the CO_2 concentration present and as this is reduced the rate will fall. Given sufficient time of observation, such plots will appear curved. However, measuring the initial rates allows comparisons between different data sets to be made.

It is possible to repeat the experiment with different initial CO_2 concentrations. To achieve this, we filled a syringe (5 cm^3) with pure CO_2 (this was taken from a cylinder that we had available but there is no reason why it could not be generated chemically, for example by using marble chips and dilute acid). Taking care not to disturb the experimental set-up shown in Figure 6, we added CO_2 from the syringe to the chamber containing the basil leaves. We used a separate larger syringe (30 cm^3) to ensure thorough mixing of the contents of the chamber and allowed a period of equilibration (1–2 minutes) with the lamp switched off. The lamp was then switched on, the system allowed to further equilibrate (approximately 5 minutes), and data on the CO_2 concentration recorded. We generated similar plots to that shown in Figure 7 for a further five initial concentrations of CO_2. The data from these experiments are shown in Figure 8, where we have plotted the rate of fall of CO_2 concentration as a function of initial CO_2 concentration.

The data in Figure 8 clearly support the proposition that the rate of photosynthesis is enhanced by an increase in CO_2 concentration. Such a conclusion is not new but, in our judgement, experimental systems that allow students to confirm this within the confines of school timetables are not readily available. We thus contend that results such as those shown in Figure 8 could form the basis of a number of student investigations and support those areas of CfE Higher biology where learners are invited to '*Carry out experimental investigations on limiting factors in photosynthesis*'.

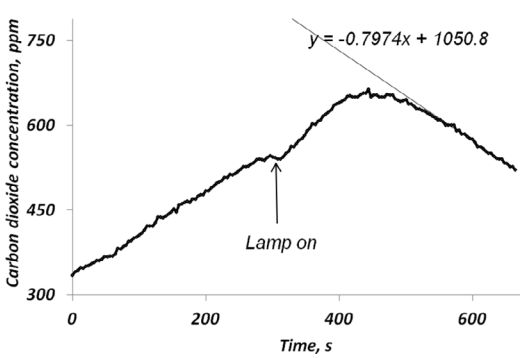

Figure 7 The CO_2 concentration in the chamber as the experiment progressed

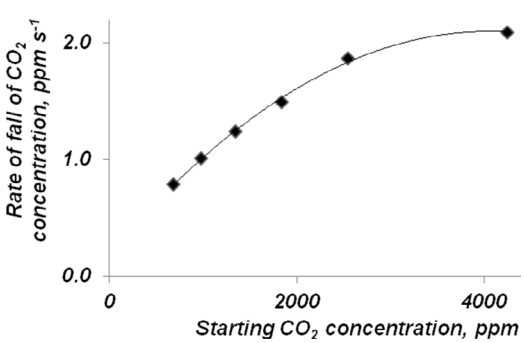

Figure 8 The rate of fall of CO_2 concentration in the chamber as a function of initial CO_2 concentration

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References

Delpech, R. (2006) Making the invisible visible: monitoring levels of gaseous carbon dioxide in the field and classroom. *School Science Review*, **87**(320), 41–51.

Eldridge, D. (2004) A novel approach to photosynthesis practicals. *School Science Review*, **85**(312), 37–45.

Hall, D. O. and Rao, K. K. (1999) *Photosynthesis*. 6th edn. pp. 24–26. Cambridge: Cambridge University Press.

Redding, K. and Masterman, D. (2007) *Biology with Vernier*. 97005-2886. Beaverton, OR: Vernier Software & Technology. Parts of this publication are available at: www.vernier.com/cmat/bwv.html.

Scottish Qualifications Authority (SQA) (2014) *Higher Biology Course Support Notes*. Available at: www.sqa.org.uk/files_ccc/CfE_CourseUnitSupportNotes_Higher_Sciences_Biology.pdf.

Websites

Lee Filters: www.leefilters.com.

National Centre for Biotechnology Education – NCBE-SAPS Photosynthesis kit: www.ncbe.reading.ac.uk/NCBE/MATERIALS/METABOLISM/photosynthesis.html.

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