

The effect of light colour and intensity on the rate of photosynthesis

1. Introduction

Debbie Eldridge has described in detail how immobilised algae can be used to measure rates of photosynthesis (see [here](#) and School Sci. Rev. (2004), 85, 37-45).

The information that follows is offered in support of post-16 experiments that use the immobilised algal system. It is possible to study a number of variables when measuring the rate of photosynthesis. Such variables include (but are not limited to):

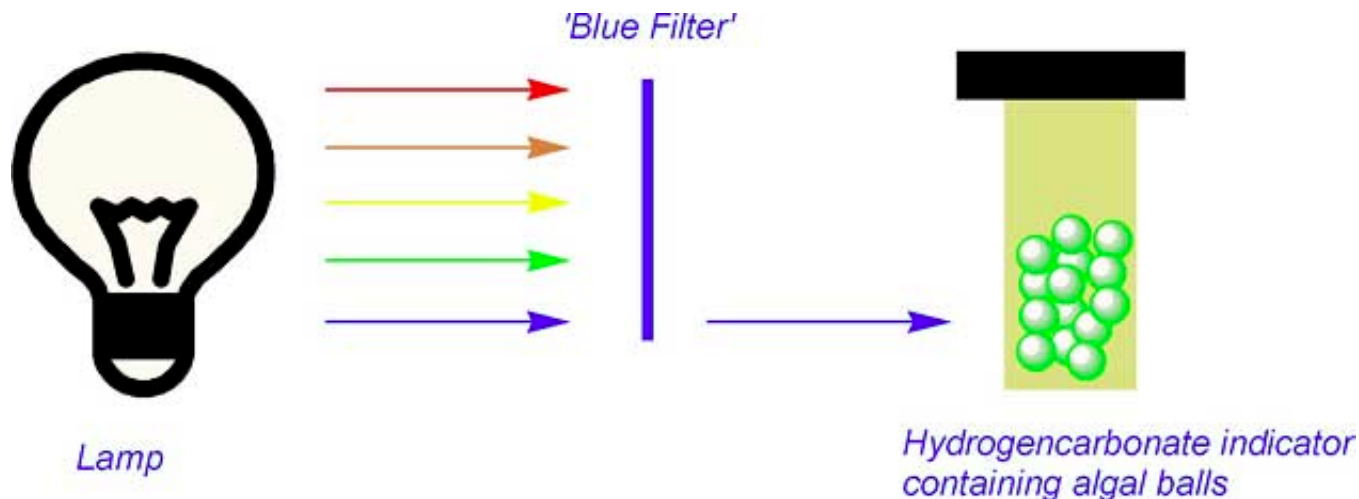
- Colour of light
- Light intensity
- Number of algal balls
- Algal ball size
- Concentration of algae
- Temperature
- Starting concentration of CO₂
- Rate of oxygen production

We describe some resources which may be useful when designing experiments to investigate the first two variables from the above list (Colour of Light, and Light intensity).

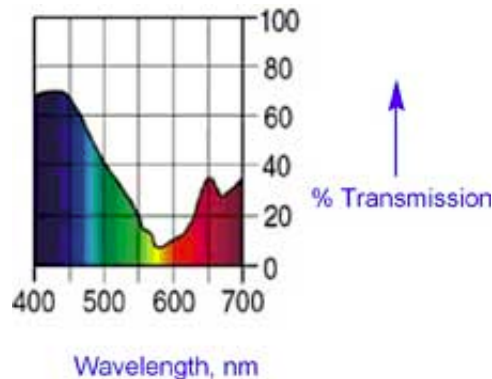
2. Colour of Light

2.1 Choice of filters

A convenient way to investigate the effect of light colour on the rate of photosynthesis is to place a filter between the light source and the sample and an experimental set-up, using a blue filter, is shown schematically below.



Using different filters for experiments is relatively simple to do and offers scope for a large number of potential investigations. The properties of the filter will, of course, influence the colour of the light that is transmitted onto the sample and we have been investigating different sources and types of filters. One company which supplies a suitable range of filters is LEE Filters (Central Way, Walworth Industrial Estate, Andover, Hants SP10 5AN. Tel 01264 366245; www.leefilters.com - see the attached list of local [distributors](#)). Typically a sheet of filter (measuring 1.22 m x 0.55 m) costs £3.65 + VAT. The transmission properties of each of the filters are available from the Lee Filters website. For example, the transmission properties for the filter True Blue (filter number 196) as taken from the Lee Filters website are:



The information available through the Lee Filters website is useful in showing the spectral distribution of transmitted light but is not presented in the most useful format if, for example, you wish to predict the effect of combining more than one filter. We have decided, therefore, to measure the absorption spectrum of a number of filters produced by Lee Filters and make these spectra available as Microsoft Excel® files through the SAPS website. We have selected those filters which we feel will be useful when making studies of light colour on the rate of photosynthesis. The data for each filter is contained in a separate file with the filename corresponding to its Lee Filters number. So, for example, the filter "182 Light Red" has the filename 182light_red.xls.

The filters that we have chosen to use are:

[104 Deep Amber](#)

[106 Primary Red](#)

[119 Dark Blue](#)

[124 Dark Green](#)

[139 Primary Green](#)

[182 Light Red](#)

[344 Violet](#)

[716 Mikkel Blue](#)

[722 Bray Blue](#)

You can access the files by clicking on the links above. Before you do that, however, we advise you to have a quick look through the remainder of this section together with sections 2.2 and 2.3.

[In addition to the above coloured filters, we have made measurements of a number of neutral density filters and these are detailed in [section 3](#) below].

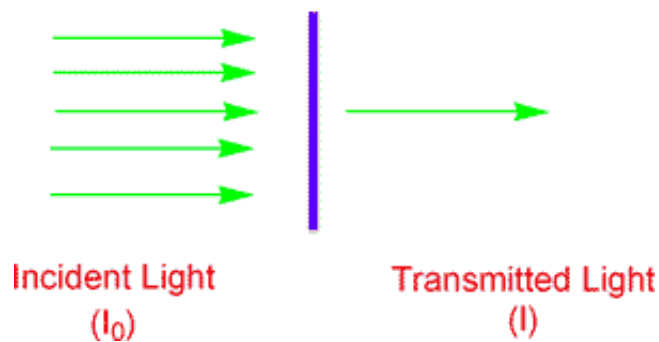
The absorbance of a single thickness of each filter has been measured at 4 nm intervals using a Perkin-Elmer Lambda 9 Spectrometer and the data recorded. Each file contains four columns of data as shown in the following table (taken from file 722(Bray Blue)).

<u>Wavelength (nm)</u>	<u>Absorbance</u>	<u>Transmittance</u>	<u>% Transmittance</u>
402	0.87	0.13	13.5
406	0.79	0.16	16.2
410	0.72	0.19	19.1
etc.	etc.	etc.	etc.
etc.	etc.	etc.	etc.
722	0.53	0.30	29.5
726	0.47	0.34	33.9
730	0.41	0.39	38.9

In the following section we look at how the data have been generated and how transmittance is calculated from a knowledge of the amount of light absorbed.

2.2 Absorbance and transmittance

The terms absorbance and transmittance can be explained by considering the schematic below.



Let's consider a blue filter that is exposed to green light. The amount of light falling on the filter (also called the incident light) is defined as I_0 whereas the amount of light that passes through the filter is defined as I .

As can be seen we would expect the amount of light transmitted in this case to be quite small (remember blue filters will tend to allow blue light to pass through them and absorb other colours of light). The transmittance, T , of the sample is defined as the ratio of transmitted light to incident light:

$$T = \frac{I}{I_0}$$

You should be able to see that as the filter allows more light to pass through the transmittance will be higher and in the case where all the incident light passes through then T will be 1.00. If the filter absorbs all of the light then I will become zero and T will also be zero.

Most devices (these include colorimeters, spectrometers and spectrophotometers) which measure the amount of light absorbed or transmitted by a sample report the results in terms of absorbance (often given the symbol A). The absorbance of a sample is a measure of the amount of light absorbed by the sample. It follows that absorbance and transmittance are related to one another but the exact relationship is not simple. The absorbance of a sample is defined as:

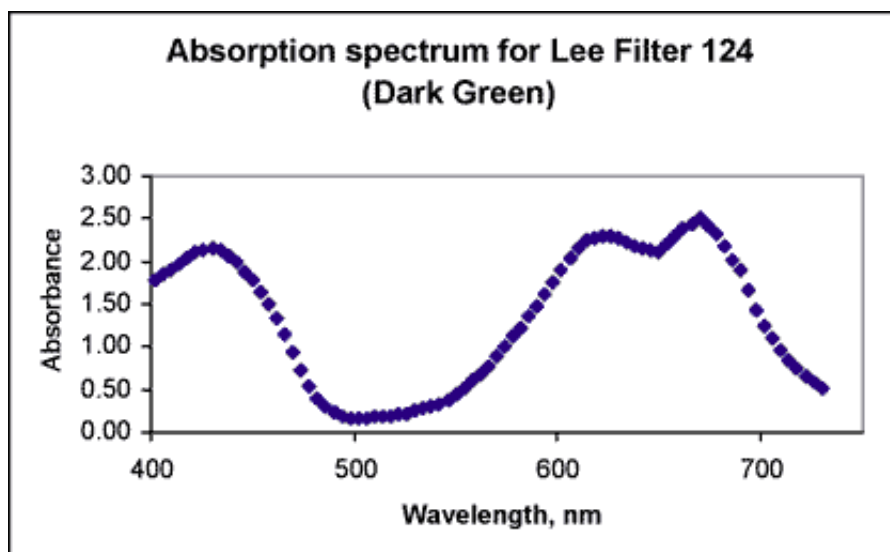
$$A = \log_{10} \left(\frac{I_0}{I} \right)$$

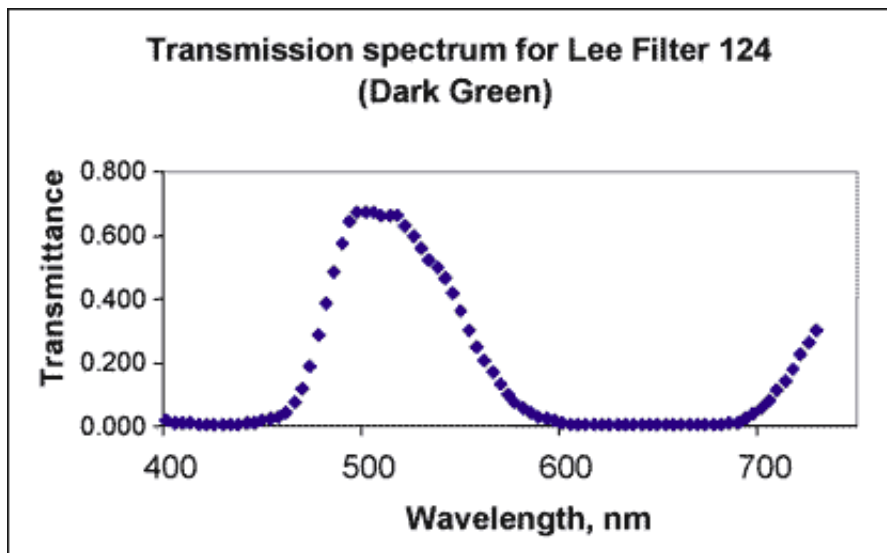
We have measured the absorbance of each filter at 4 nm intervals and by combining the above 2 expressions together we calculate the transmittance from:

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

2.3 Using the files to produce absorption and transmission spectra

You can use the data in the various files in a number of ways. You might simply wish to produce absorption and/or transmission spectra for individual filters. For example suppose you want to produce an absorption or transmission spectrum for Lee filter 124 (Dark Green). To do this you need to open the spreadsheet file ([124dark_green.xls](#)) and plot wavelength against either absorbance or transmittance. You should be able to produce graphs similar to the two that follow:





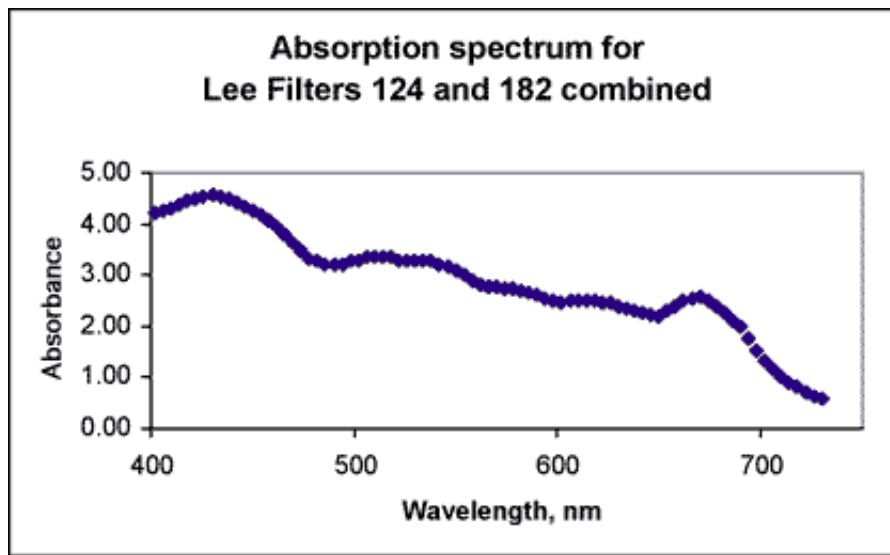
2.4 Using the files to produce absorption and transmission spectra of filter combinations

Suppose you wish to decide to use two filters 124 (Dark Green) and 182 (Light Red) in an experiment. You could use the data in the spreadsheet files to predict the effect of combining these two filters together. You would need to add the absorbance values (at any given wavelength) for the filters together and the resulting sum will be the absorbance of the new filter combination. One way of doing this is to:

- Open file [124\(Dark Green\)](#) and save it as a new file (the name doesn't matter but you might choose "124182combined").
- Delete columns C and D from this new file.
- Open file [182\(Light Red\)](#) and copy the absorbance values in column B.
- Paste the values of absorbance for 182(Light Red) into the vacant column C in file 124182combined.
- Add columns B and C in file 124182combined together and place the answer in column D. This column now contains the sum of the absorbance for the two filters. The first few rows and columns of file 124182combined should look like this:

<u>Wavelength (nm)</u>	<u>Absorbance 124</u>	<u>Absorbance 182</u>	<u>Combined</u>
402	1.79	2.41	4.20
406	1.85	2.39	4.24
410	1.91	2.39	4.30
414	1.98	2.39	4.37
418	2.05	2.39	4.44
422	2.10	2.41	4.50

You can now plot the absorption spectrum for the combined filters and you should be able to produce something that looks like:



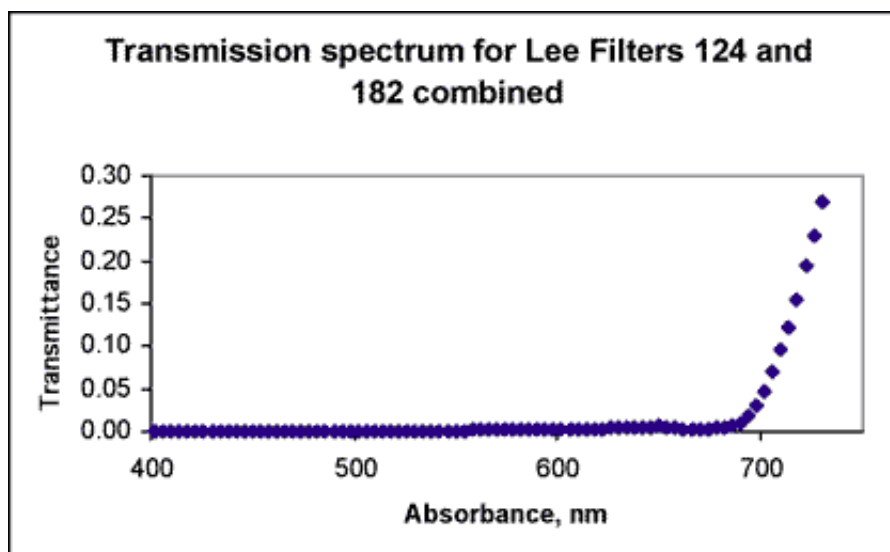
It is sometimes difficult to look at absorption spectra and get a feel for what they mean in terms of light that will be passed through the filter. You can see that the absorbance scale has a maximum of about 4.5 in this particular case - does that mean that a lot of light is absorbed or just some of the light? In fact an absorbance value above 2.0 is very high! A more useful way of presenting the data would be to produce a transmission spectrum. You can use the spreadsheets to calculate the transmittance of the new filter combination. You can convert the combined absorbance value to transmittance using the following instruction in your file 124182combined:

$$E4 = 1/(10^{D4})$$

where D4 is the cell that contains the sum of the two absorbance values. Repeat this exercise for all the values in column D and the first few rows of your spreadsheet should now look like:

<u>Wavelength (nm)</u>	<u>Absorbance 124</u>	<u>Absorbance 182</u>	<u>Combined</u>	<u>Transmittance</u>
402	1.79	2.41	4.20	0.00
406	1.85	2.39	4.24	0.00
410	1.91	2.39	4.30	0.00
414	1.98	2.39	4.37	0.00
418	2.05	2.39	4.44	0.00
422	2.10	2.41	4.50	0.00

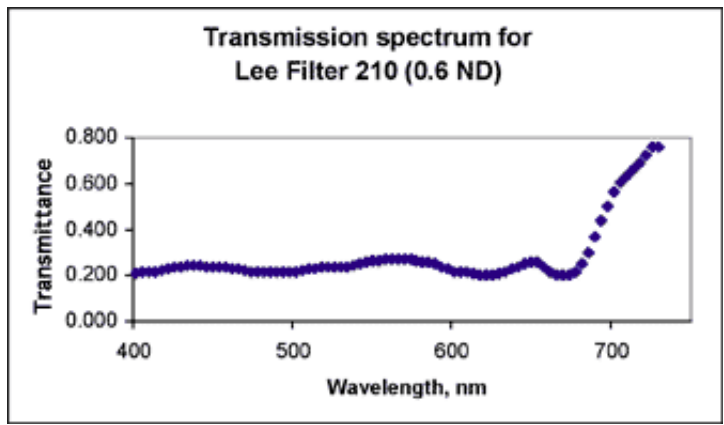
You can now plot the transmission spectrum for the combined filters and you should be able to produce something that looks like:



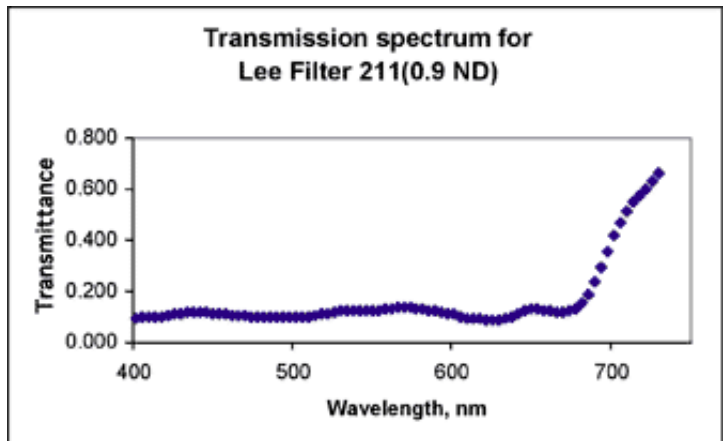
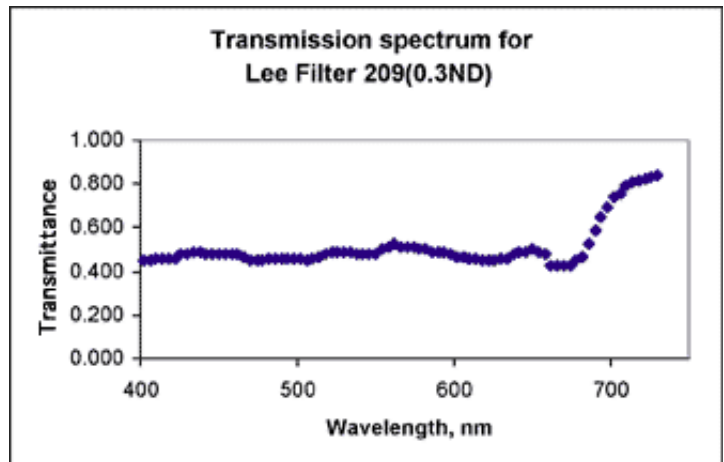
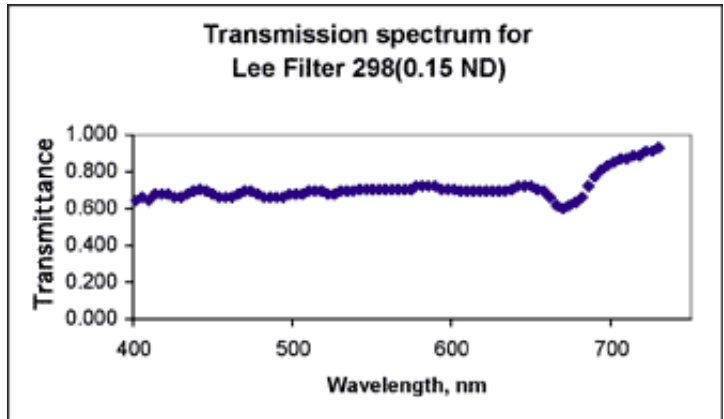
This would not be a very useful filter combination for photosynthesis experiments! Can you see why?

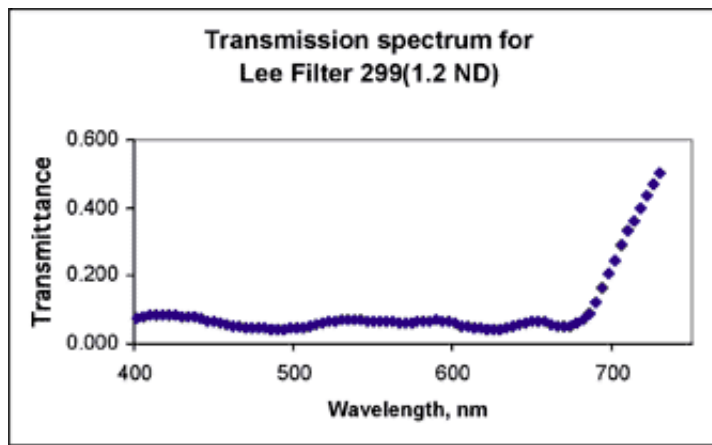
3. Light Intensity

One of the variables commonly investigated in photosynthesis experiments is to measure the effect of light intensity on the rate. The most common



You can see from this plot that the filter will allow approximately 20-22% of all light in the spectral range 400 – 680 nm to pass – i.e. light intensity would be reduced by 78 - 80 %. Other neutral density filters are available and the transmission characteristics are shown in the plots below:





The neutral density filters supplied by Lee Filters offer an alternative way of reducing light intensity across the spectral range. Notionally the amount of light transmitted in the spectral range 400-680 nm by each of the neutral density filters is summarised in the table below:

Filter name	298(0.15 ND)	209(0.30 ND)	210(0.6 ND)	211(0.9 ND)	299(1.2 ND)
Light transmitted (%)	71	50	25	12.5	6.3

The neutral density filters can be used in combination with colour filters or with each other. We have found, for example, that the rate of photosynthesis in the presence of 4 layers of Filter 298 is the same as that found in the presence of one layer of Filter 210. Clearly, other combinations are possible.

We hope that the information presented here is useful in the design of experiments that support, amongst your students, a better understanding of photosynthesis. We would welcome your comments and feedback. Please contact us by e-mail [here](#) or by writing to us at SAPS, Homerton College, Cambridge CB2 2PH.