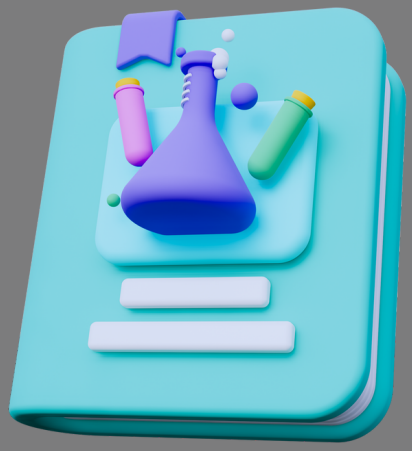
PROTOCOL
INVESTIGATING ACTION SPECTRA OF PHOTOSYNTHESIS

Immobilise algae and investigate photosynthesis using bicarbonate indicator

Page



Background

Photosynthesis is the process of converting light energy into the energy of chemical bonds in organic substances. For photosynthesis, pigments in plant tissues, including the chlorophylls and carotenoids, absorb energy of photons of specific wavelengths to initiate the chain of chemical reactions of photosynthesis. Various researchers, including T.E. Kuleschova, have investigated the influence of lighting of defined spectral characteristics on the growth of plants. This has wider implications for the global food chain, upon which there are significant demands as the human population continues to increase.

Chlorophyll a and b (Figure 1) are the primary pigments, capable of absorbing light and converting it to chemical energy. The carotenoids extend the absorption range.

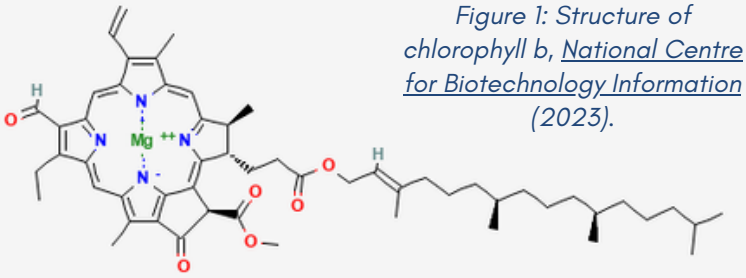


Figure 1: Structure of chlorophyll b, National Centre for Biotechnology Information (2023).

As outlined on the BBC Bitesize website, when light is exposed to a leaf, approximately 12% of light is reflected, 5% of light is transmitted, and 83% of the light is absorbed. Out of this 83%, approximately 4% is used for photosynthesis (Figure 2).

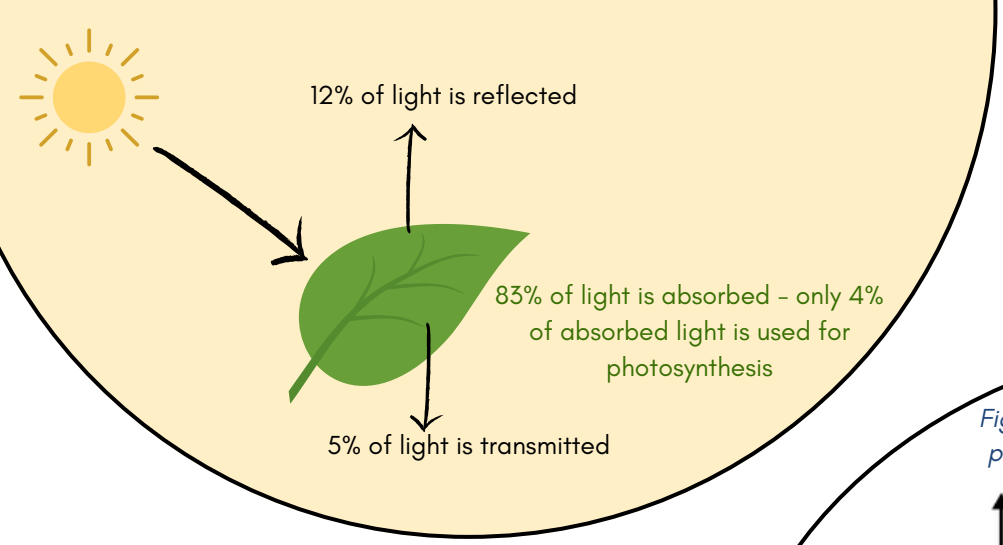


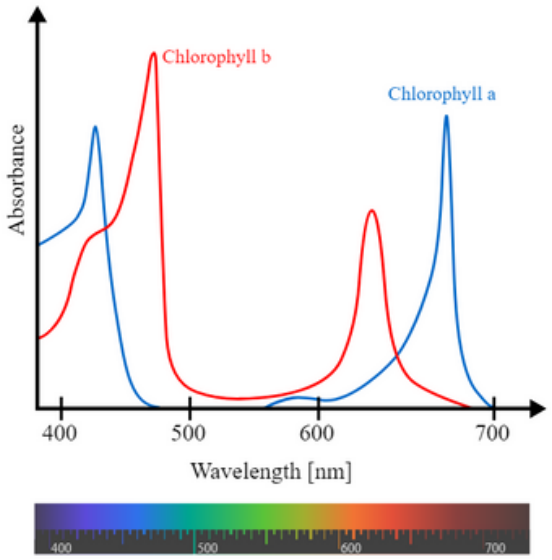
Figure 2: Fate of light as it strikes a leaf. Which wavelength of light might be reflected and transmitted? Which wavelength of light are absorbed and ultimately required for photosynthesis to take place?

This image was adapted from BBC Bitesize

This protocol allows learners to investigate Figure 3 (opposite). This shows a typical published "absorption spectra", where light of specific wavelengths are absorbed by a leaf which, presumably, contribute to higher photosynthetic activity.

In this protocol, this image is used to form the basis of the experiment. Coloured filtered that allow specific wavelengths of light to pass through will be used to investigate the impact of photosynthetic activity of algae.

Figure 3: Absorption spectra for photosynthesis in plants. Image courtesy of Wikimedia Commons.



AIM

To investigate the effect of wavelength of light on photosynthesis activity in immobilised algae

This protocol can be adapted to investigate:

- effect of temperature on photosynthesis activity
- effect of light intensity on photosynthesis activity
- effect of plant type on photosynthesis activity.

In addition, this protocol could be adapted to use alternative plant sources, including:

- *Egeria najas*
- *Fucus vesiculosus* (Bladderwrack seaweed)

However, care must be taken to consider confounding variables – without immobilised the plant species, it is more challenging to be confident that plant mass / surface area is constant in each experiment.

RISK ASSESSMENT

A risk assessment for this activity can be downloaded from the SSERC website. Click here. This should be adapted for your centre, where appropriate.

There are no significant hazards presented to learners or teachers. However, technicians should take care when preparing calcium chloride solutions. Further, the disposal of *Scenedesmus quadricauda* requires 1% Virkon (w/v) and must not be released into the natural water system.

MATERIALS REQUIRED PER PAIR

Part 1 - Preparation of the algae beads

- 3 cm^3 concentrated *Scenedesmus quadricauda* (prepared by technician)
- 3 cm^3 2% sodium alginate
- 2% calcium chloride in beaker / cup
- retort stand
- 10 cm^3 syringe barrel
- tea strainer
- distilled water

Part 2 - Photosynthesis Experiment

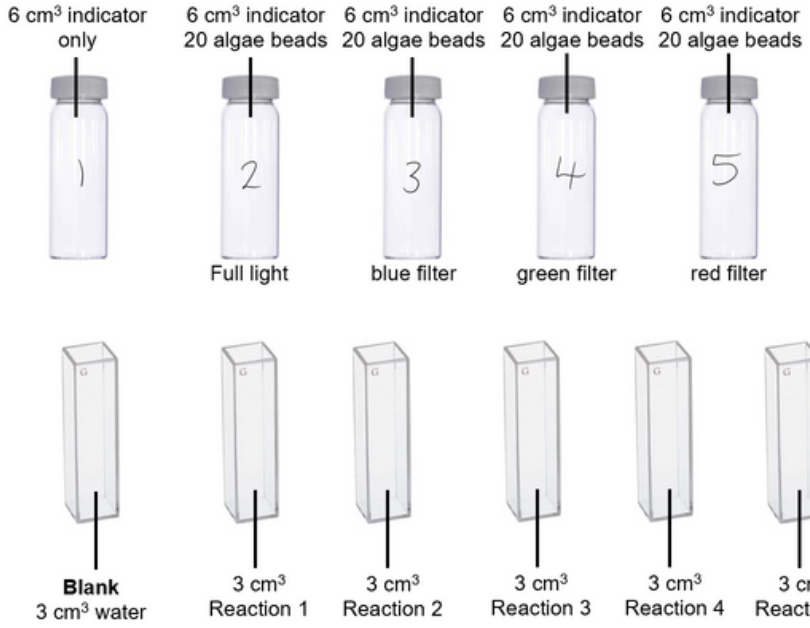
- 80x immobilised beads of *Scenedesmus quadricauda*
- 5x bijou bottles
- 36 cm^3 bicarbonate indicator
- spoon
- paper towels
- 3 cm^3 distilled water
- wash bottle of distilled water
- tea strainer
- accessto fluorescent tube light
- 3x coloured filters
- colorimeter
- 6x cuvettes
- 3 cm^3 plastic pipettes
- marker pen
- stopwatch

Bicarbonate indicator

Bicarbonate indicator is sensitive to carbon dioxide levels. When CO_2 levels are higher, the indicator is yellow; as CO_2 levels decrease, as a result of photosynthesis, the indicator becomes increasingly purple, as indicated in the diagram below.

$$6CO_2 + 6H_2O \xrightarrow[\text{Sunlight}]{\text{Chlorophyll}} C_6H_{12}O_6 + 6O_2$$

OVERVIEW OF METHOD



Immobilised algae, immersed in bicarbonate indicator, will be exposed to light through various coloured filters, which limit the wavelengths of light that pass through.

After 30 minutes, the absorbance of the indicator will be measured. The higher the absorbance, the less carbon dioxide is present, indicating a higher level of photosynthetic activity.

COLOURED FILTERS

The coloured filters used in this experiment were purchased from Black Light (manufactured by Lee Filters). The filters used were:

- 119 - Dark blue
- 139 - Primary green
- 182 - Light red

Daubeny (1836) stated that "photosynthesis proceeds with unequal speed in certain ranges of the spectrum. The filters were chosen based on their unique spectral properties, allowing a defined range of wavelength of visible light to transmit through to the algae sample. In the protocol, the algae in bijou 2 accesses the full range of visible light (approximately 400 - 700 nm).

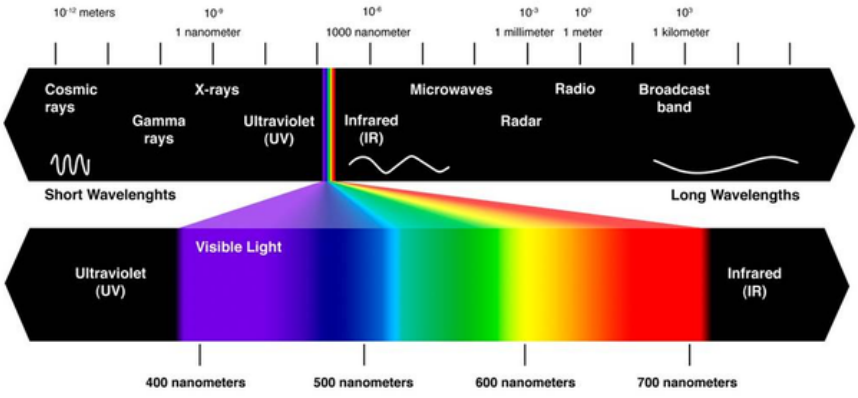


Image courtesy of Wikimedia Commons.

The next page details the spectral characteristics for each of the coloured filters used in the experiment. These details were obtained from Lee Filters.

Filter 119 allows 3.1% of all light to transmit through the filter to the sample. The algae has access to a narrow range of light wavelengths, centered on **455 nm**.

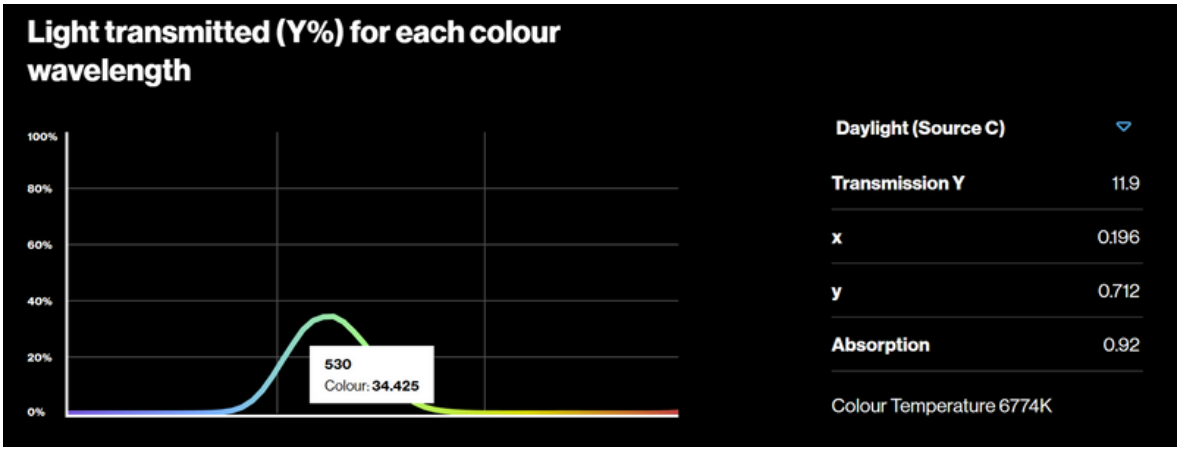
Compare colours Add to my list

119 Dark Blue

Filter 139 allows 11.9% of all light to transmit through the filter to the sample. The algae has access to a narrow range of light wavelengths, centered on **530 nm**.

Compare colours Add to my list

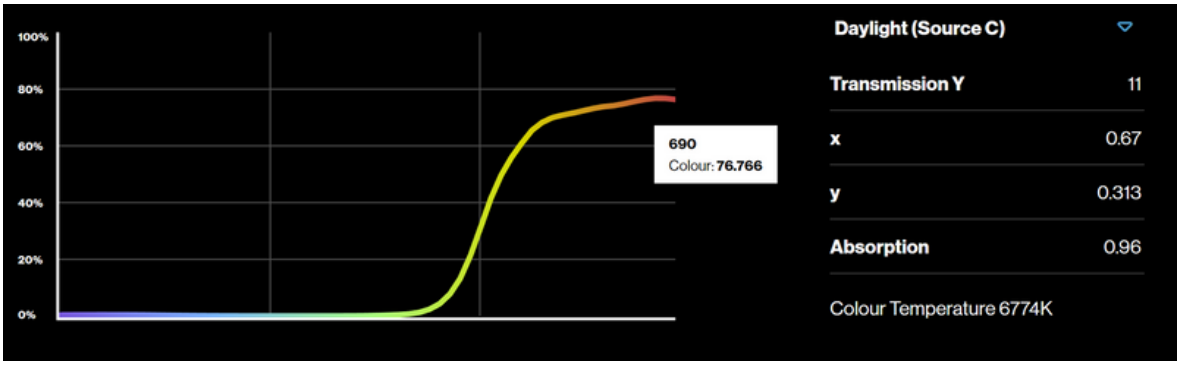
139 Primary Green



Filter 182 allows 11% of all light to transmit through the filter to the sample. The algae has access to a range of light wavelengths, centered on **690 nm**.

Compare colours Add to my list

182 Light Red



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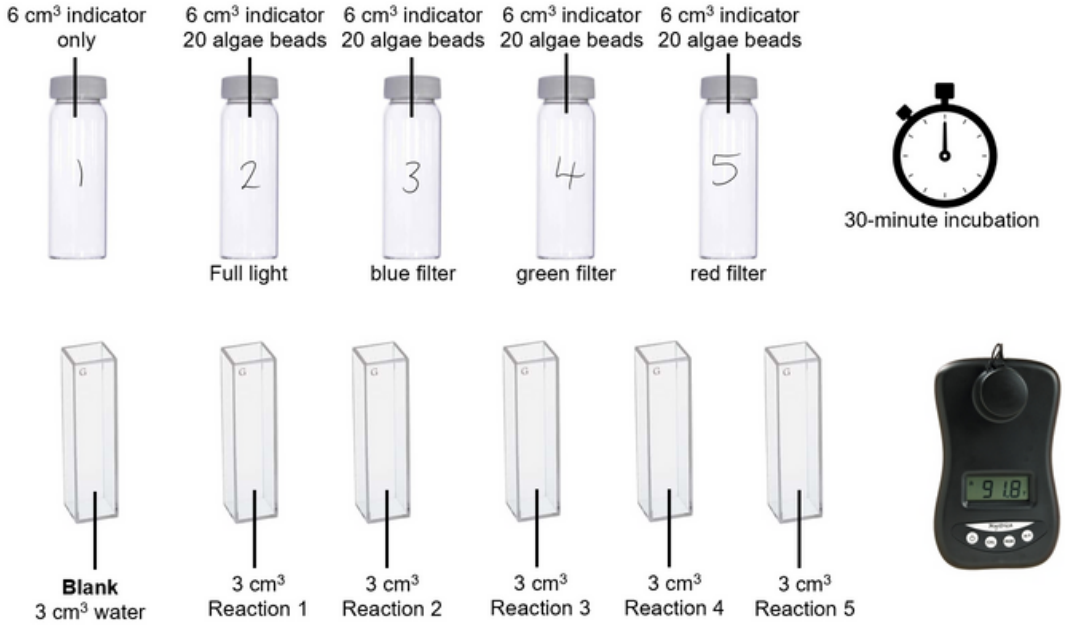


STEP-BY-STEP METHOD

Part 1 - Preparation of the algal beads

1. The *Scenedesmus quadricauda* must be grown in the provided enrichment medium (Darwin Biological) for 3-4 weeks.
2. For each pair, 50 cm^3 algal culture needs to be dispensed and concentrated, which is achieved by leaving them to stand or by centrifugation. Discard the supernatant and retain the concentrated algae. Steps 1 and 2 would typically carried out by a school science technician. See Figure 5 opposite.
3. Mix 3 cm^3 concentrated algae with 3 cm^3 2% sodium alginate. See image 1 in Figure 6 below.
4. Transfer this mixture to a syringe barrel, and allow the mixture to drop into a container of approximately 30 cm^3 2% calcium chloride. See **image 2** in Figure 6 below.
5. Swirl the calcium chloride solution regularly as the beads form. The result should be algae beads of uniform size containing approximatel equal quantities of algae.
6. The beads should be left for approximately 5 minutes to harden, washed in tap water (through a tea strainer) and then given a final rinse in distilled water. See **image 3** in Figure 6 below.
7. The beads can be stored in distilled water (for up to 6 months) until ready to use.

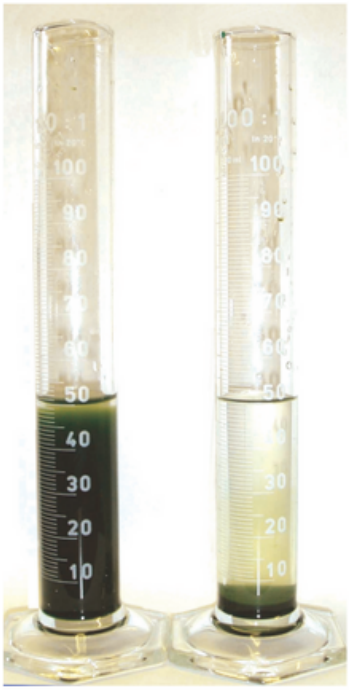


Figure 5: Concentrating the algae.

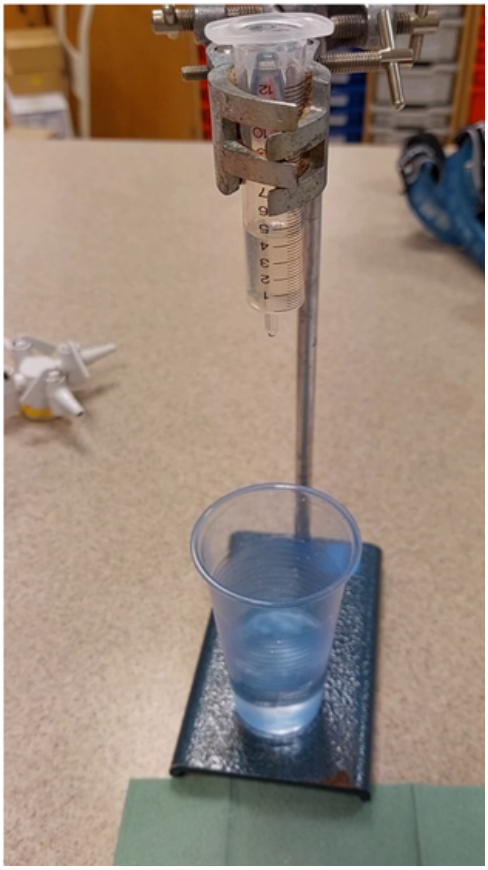



Figure 6: Steps in preparing immobilised algae. The barrel of a 10 cm^3 syringe is clamped into a retort stand, positioned above a container of 2% calcium chloride. The mixture of algae and sodium alginate is transferred into the barrel of the syringe and allowed to drop into the calcium chloride. As the drop makes contact with the calcium chloride, it forms a bead, which continues to harden over time. Allow approximately 5 minutes after beads have been produced before thoroughly rinsing with distilled water.

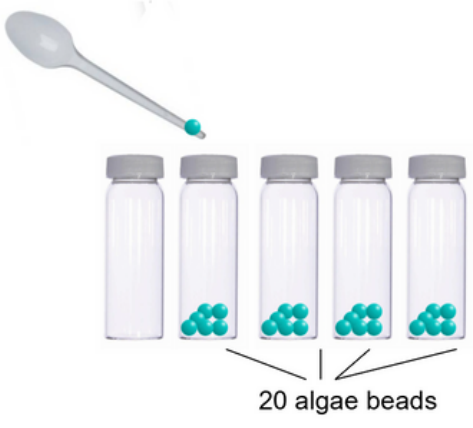
Part 2 - Photosynthesis experiment

1 Line up the empty bijou bottles. Add 2-3 cm^3 bicarbonate indicator to the first bottle and then transfer this to the second, and then the third, and so on.

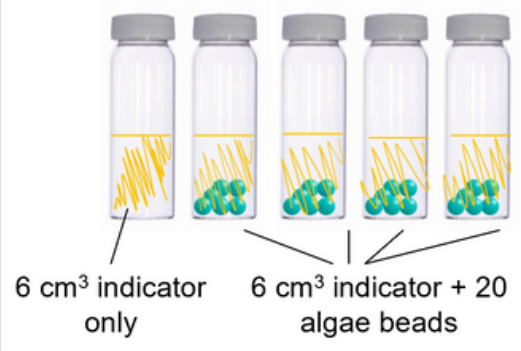
This is done to ensure that there are no contaminants in the bottle that will change the colour of the indicator before the biological material is added.

2 Pass the immobilised algae beads through the tea strainer and use the paper towels to dab dry from the underside of the strainer.

3 Using the spoon (if using a plastic spoon, there is often a handy groove on the underside of the handle to collect the beads), transfer 20 immobilised algae beads to four of the rinsed bijou bottles.



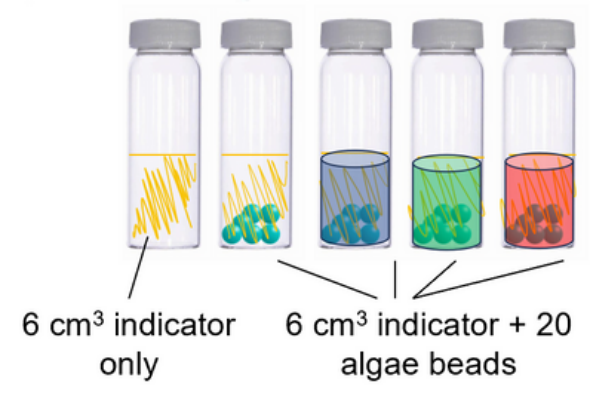
4 Fill all bijou bottles with bicarbonate indicator. Write your initials on the lid.



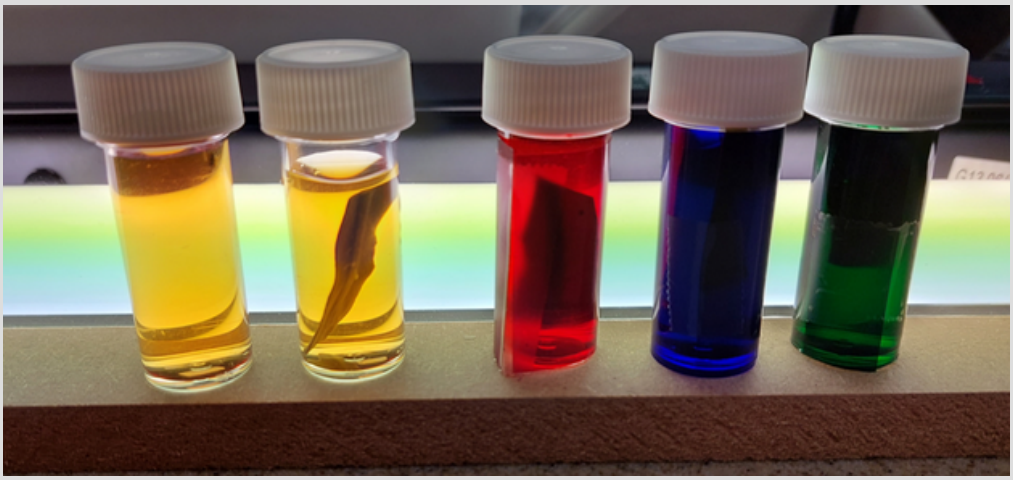
5 Place the pre-formed coloured filtered over bijou bottles 3, 4 and 5.

Bijou bottle 1 contains indicator only, allowing observation of the colour of indicator over the incubation period in the absence of biological material.

Bijou bottle 2 contains algae with access to wavelengths of light extending from approximately 400 - 700 nm.



6 Line all five bottles in front / under a fluorescent tube light and leave for 30 minutes.



7 Thoroughly mix the content of each bijou bottle.

Use the colorimeter to record the absorbance values of the solutions. These instructions refer specifically to using the Mystrica colorimeter, using the ***green*** diode (or 550 nm).

Ensure the colorimeter display reads "A" for absorbance and "G" for green diode.

8 Place a cuvette containing distilled water into the sample holder and press CAL to zero the colorimeter.

Note the direction of the light beam - ensure the cuvette is in the correct orientation. Record the absorbance - it should read 0.00.

Empty the cuvette and replace with the indicator from the bijou bottle which only containing indicator. Measure the absorbance and note it down.

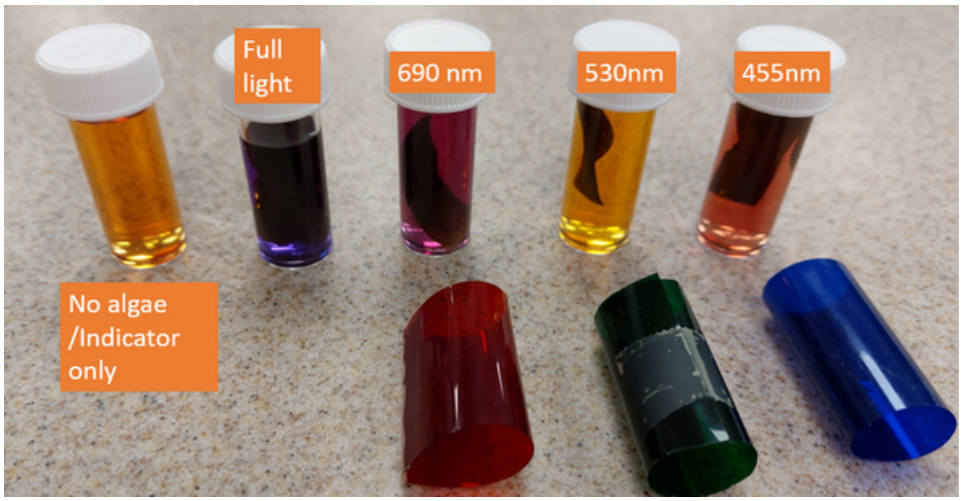
Measure and record the absorbance of the four remaining bijou bottles. The absorbance value of the indicator alone must be subtracted from all remaining values.

RESULTS

Present results in a table similar to the one below. The results can be then plotted as a line graph.

Absorbance from bijou 1 (indicator only): _____
Subtract this value from all other readings and present in the "Corrected absorbance" column

	Absorbance	Corrected absorbance



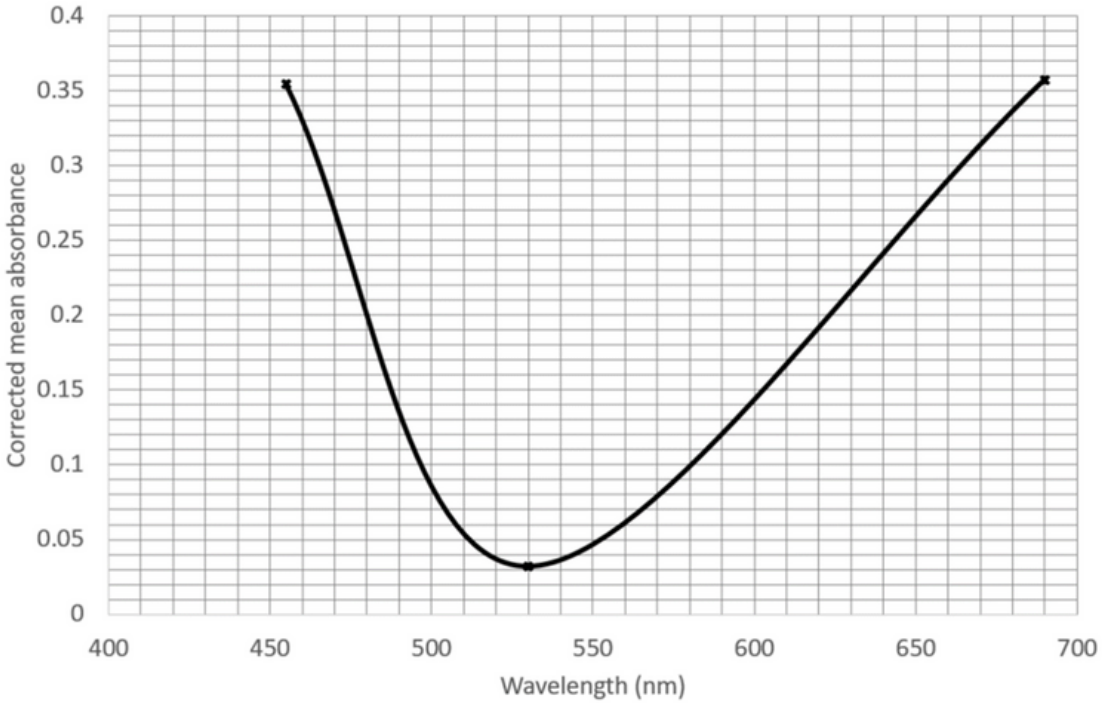
SAMPLE RESULTS

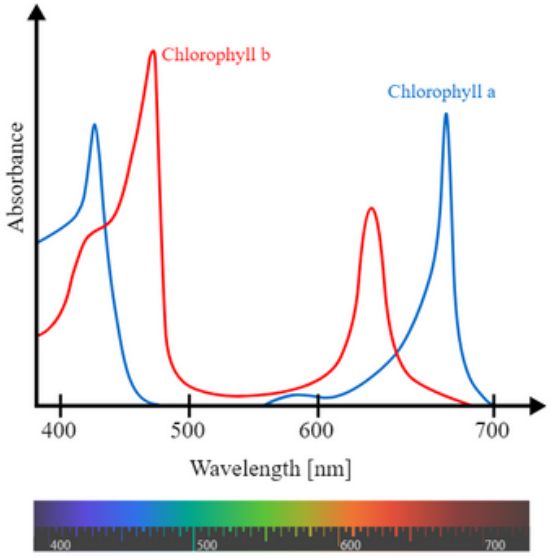
Wavelength of light (nm)	Absorbance				
	Run 1	Run 2	Run 3	Mean	Corrected mean
400-700	0.669	0.679	0.682	0.676	0.572
455	0.400	0.499	0.475	0.458	0.354
530	0.155	0.121	0.132	0.136	0.032
690	0.465	0.460	0.458	0.461	0.357

Absorbance of the indicator in the absence of algae = 0.104

The results show that peak photosynthetic activity is taking place when the plant has access to the full spectrum of visible light. In the presence of light of 455 nm and 690 nm, the plant will photosynthesis comparably. The plant did not photosynthesise in the presence of light with a wavelength of 530 nm.

Action spectra for photosynthesis activity in *Scenedesmus quadricauda*

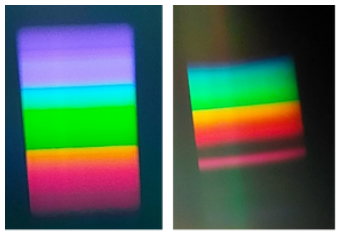




Absorption spectra for photosynthesis in plants. Image courtesy of Wikimedia Commons.

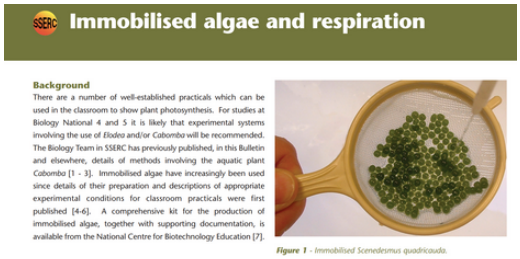
These sample results can be analysed alongside the absorption spectra (opposite), introduced on page 2. The absorption spectrum reveals that plants lack photosynthetic pigments that absorb photons of light with wavelengths between 500 – 600 nm approximately.

Learners can follow up on this observation by exploring the absorption spectrum of plants. This can be done using a simple spectroscope, as outlined in the CfE Higher course specification notes.

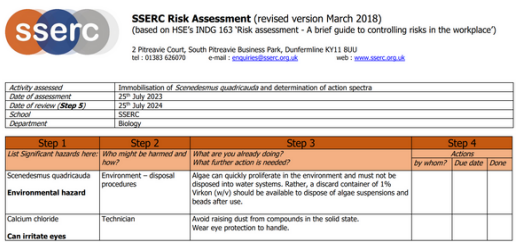


Visible light Through chlorophyll

SUPPLEMENTARY RESOURCES



SSERC bulletin (2012) available to download.



Risk Assessment

Powerpoint to download