Activity: Effect of Light Wavelengths on Photosynthesis
Teacher Tips

Material Justifications and Explanations

It may help to demonstrate steps to ensure experiment success; you may choose to demonstrate more or fewer steps based on the level of your students and their experience with the equipment and methodology.

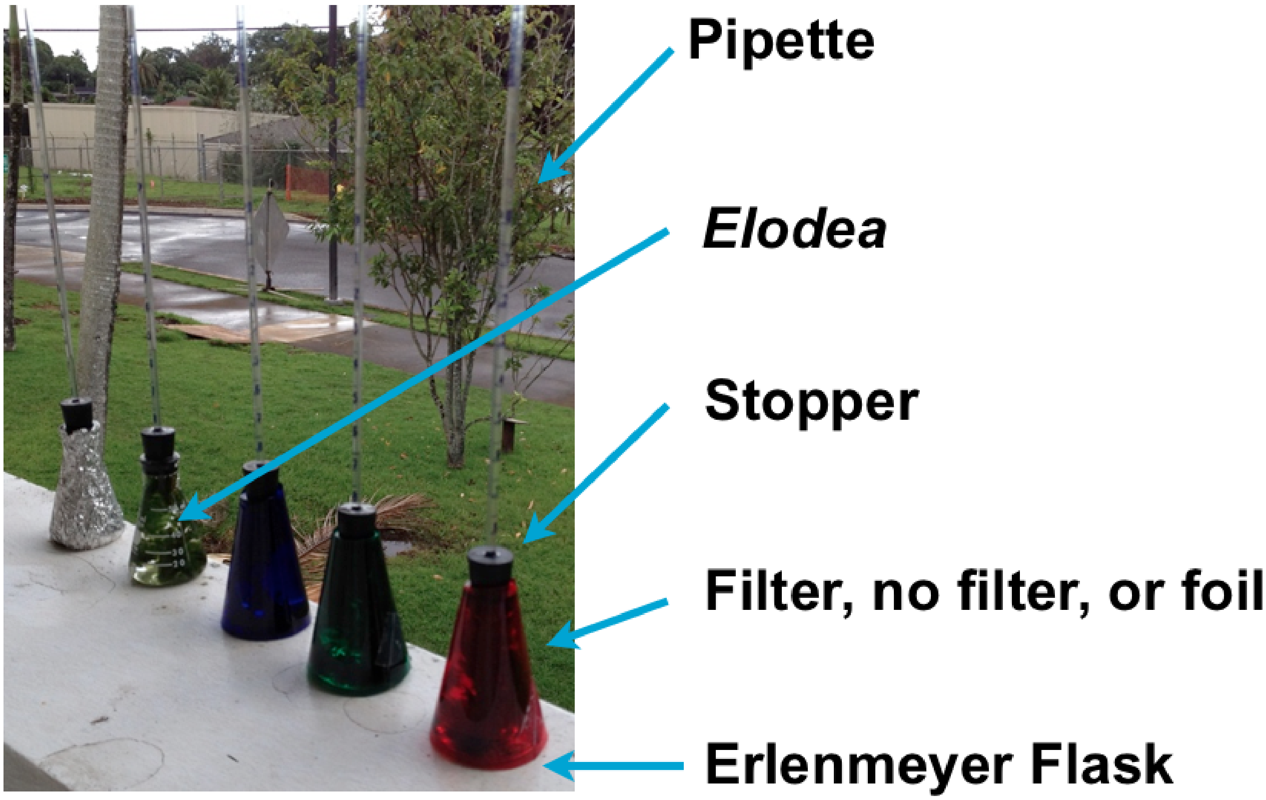


Fig. 1. Elodea experiment.
Image by CRDG.

Elodea

Elodea is a freshwater plant (*not* an algae) that has a high metabolic rate (it photosynthesizes quickly).

- Buy *Elodea* the day before the experiment if possible. Most aquarium stores have this plant. *Elodea* is often sold under the common name *Anacharis.* Buy bright green, "bushy" (many leaves per section of stem) looking *Elodea*. *Elodea* is often sold in "short" or "long" branches. It does not matter how long each *Elodea* branch is, it is more important that the *Elodea* are "bushy", the bushier the plant, the shorter lengths you will need for the experiment.
- Transfer the plants to a fresh container of tap water outside or near a window. Plants should have enough room to spread out. If you have an aquarium

bubbler, place it in the container of water. If you do not, plants should be fine for a day or two if you replace the water once a day.

- To standardize *Elodea* as much as possible between flasks, encourage students to use similar looking pieces of plant (e.g. similar amounts of leaves, or "bushiness").
- We recommend using fresh *Elodea* for each class. Adding baking soda to the water increases the pH of the water, making it more basic, and this may affect plant physiology. If you need to re-use *Elodea* for a class later in the day, place it back in fresh water between classes. The entire second class should use the "older" *Elodea*; do not mix "fresh" and "old" *Elodea* in the experiment.

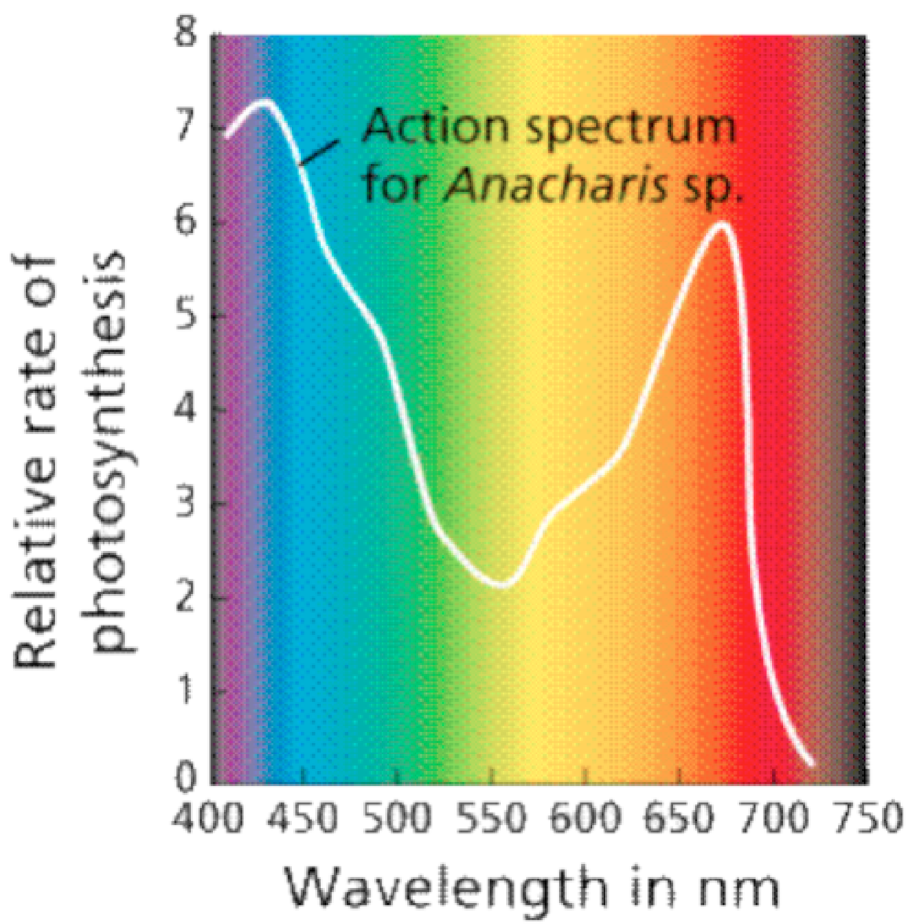


Fig. 2. Action spectrum for *Elodea*
Image from Purves et al., Life: The Science of Biology, 4th Edition, by Sinauer Associates (www.sinauer.com) and WH Freeman (www.whfreeman.com)

- The above figure is the action spectrum for *Elodea*. This spectrum is not specific to any one pigment. It is a combination of all of *Elodea*'s pigments. Based on this spectrum, we would expect Elodea to photosynthesize fastest when exposed to blue and red light, and slowest when exposed to green light (but please read about specific results to expect in the section on filters).

Disposing of Elodea

- Elodea is an invasive, noxious weeds in many places.
- Please adhere to local guidelines regarding the purchase and transport of non-native species.
- Please dispose of aquarium plants properly and never release them into the wild (even a toilet or drain).
- After classroom use, please kill all aquatic plants by freezing them before disposing in the garbage.
- Source: http://berkshirebiological.com/care-handling/

Erlenmeyer flasks

- The shape of the Erlenmeyer flasks prevents the baking soda solution from heating up as quickly as more cylindrical glassware (e.g. test tubes). Heating the water increases the photosynthesis rate up to a certain temperature and then will negatively affect the plant physiology. Thus, temperature may override the affect of the colored filters if your glassware has a small volume of water.
- Erlenmeyer flasks can withstand slight wind buffeting without falling over when placed outside.
- Larger Erlenmeyer flasks will also work (e.g. 100 mL) but will require larger amounts of *Elodea* and sodium bicarbonate solution.
- Arrange *Elodea* in flasks so it is not overly crowded. If the *Elodea* is crowded it will self-shade and limit the amount of light reaching all parts of the plant, decreasing photosynthesis rates. One way to ensure this is to break the *Elodea* branches into shorter pieces and arrange them in the flask with a skewer.

Rubber stoppers

- For 50 mL Erlenmeyer flasks we recommend rubber stopper size #2. Although stopper size #1 better fits the 50 mL flasks, the hole size of stopper #2 (5 mm) is the right size for the pipettes used in this experiment. Smaller stoppers are more difficult to insert and remove pipettes from and are more likely to cause pipettes to break. If you use different pipettes, you may have to use different stoppers.

Pipettes

- We tested this activity using 1 mL (with 1/100 gradations) capacity disposable serological pipettes. These are considered “one-time” use medical pipettes. We chose these pipettes because they are very cheap and fit our suggested sized equipment. Depending on the brand, these pipettes can be easier to break than traditional pipettes (one reason we recommend inserting and pulling them out of the stopper using a dry towel). If the brand of pipettes you are using has lines that are easily rubbed off, we recommend handling the pipettes from the end with no markings. (You can also varnish the pipettes to prolong their life.) If your pipettes have lines that are easily rubbed off, do not rinse them as this will hasten the removal of the markings. When the experiment is completed, simply stand up

the pipettes (e.g. in a tall container) and let air-dry. Handled carefully, they can last through a number of experiments.
- If you choose to use a different sized pipette, you may have to adjust the stopper size and modify the length of the experiment (see below for details on Procedure #6).
- Place the pipettes *tapered tip down* into the rubber stopper. This not only makes the pipette easier to insert, it means that the pipette does not need to be pushed very far into the stopper because the markings start close to the tapered end of the pipette.
- We recommend inserting the pipette until the water level has reached somewhere between the 0.8 and 0.7 mL mark on the pipette. At this depth the pipette should still be easy to insert and pull out, but should remain firmly in place. Do not force a pipette into a stubborn stopper – try a new pipette or stopper instead. It may be helpful to have your students standardize the starting level the water in the flasks by setting each meniscus to the same number.
- Depending on your equipment, you may want your students to check for leaks at after inserting the pipettes, the water level should not be dropping.

Light Filters
- Color filters are used to selectively allow certain colors (or wavelengths) of light to reach the *Elodea*. Most color filters are made for theaters to create lighting effects. When a full spectrum light shines on a filter, colors are created when certain wavelengths of light are blocked. For example a red filter absorbs blue and green light, allowing only the red wavelengths to be transmitted.
- However, filters often allow more wavelengths to pass through than you might expect. For example, a filter that looks “blue” might transmit purple and red wavelengths as well. (We suspect this is probably because color mixtures are more flattering on actors!) This makes it important to research the companies that make the filters and examine the spectra and transmission readings of the filters you are going to use.

  Wavelengths in nanometers:
  - White light (400 – 700 nm)
  - Red light (400 – 620 nm)
  - Green light (425 - 575 nm)
  - Blue light (440 – 490 nm)

- We have researched and tested this activity with Roscolux filters (http://www.rosco.com/filters/roscolux.cfm#colors) and suggest the colors in Table 1.1 as they have absorbance spectrum relatively consistent with our target experimental colors (red, blue, and green).
- Filters also have a transmission value, for example a filter with a “trans” of 40% means that 40% of the overall light transmission is allowed to pass through the filter. Transmission of light is related to the intensity of light. A filter with a high

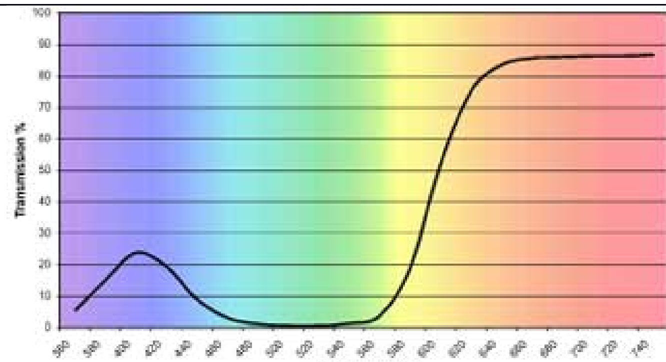
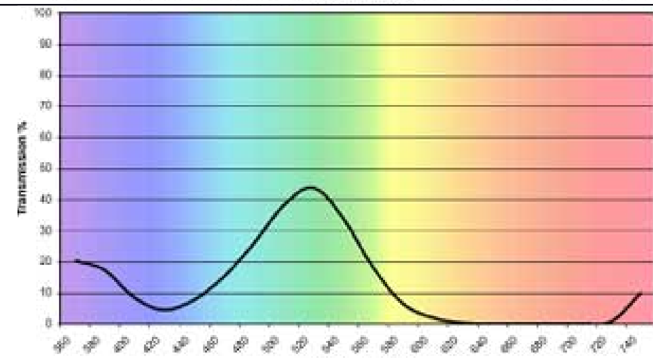
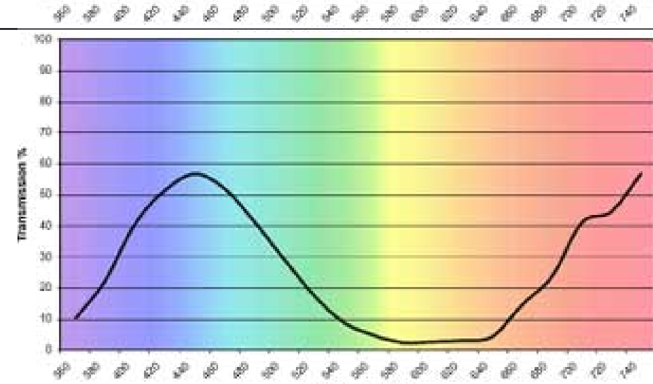
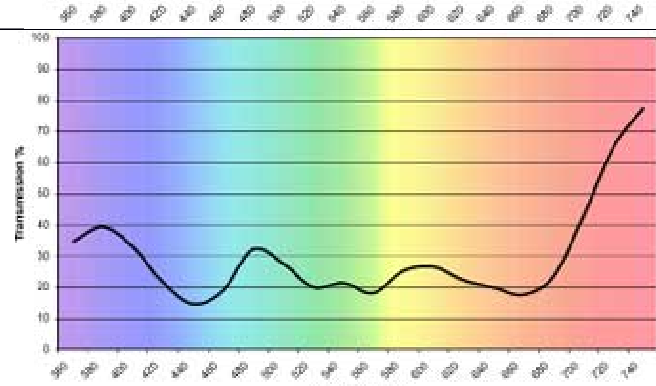
transmission value allows more light energy to pass through the filter, reach the plant, and increase the photosynthetic rate compared to a filter with a low transmission value. To be able to compare results across colors, it is important that filters have similar transmission values. The filters we have suggested are as similar as possible given the options available.

- In our experience, the plant in the clear vial will produce the most gas, followed by the plant with the red filter, then blue, and finally green. The gas production difference between the blue and green filters is not as large as expected using our suggested filters. This is partially due to the transmission difference. For students not ready to tease apart variables in this experiment, we recommend using only red and green filters (with the foil, white light, and no *Elodea* controls). The medium grey filter decreases all wavelengths roughly equally and may serve as a good additional "white light control".

Sunlight

- Filters work best when exposed to the entire spectrum of wavelenghts. The only way to ensure the filters are exposed to the full spectrum is to use sunlight.
- Traditional light bulbs (e.g flood lights) may look white but do not emit the entire visible spectrum. For example, a light bulb may not emit wavelengths in the green range, therefore a green filter would not work. This is true even of "full spectrum" light bulbs.
- On a very sunny, warm day, the sodium bicarbonate solution may heat up enough to cause dissolved gas to come out of solution. Monitoring this, as well as temperature and pressure effects caused by sunlight warming and expanding the water, is one of the purposes of the control flask with no plant.

Table 1. List of filters (Roscolux brand) used in the *Effect of Light Wavelengths on Photosynthesis* activity. Filter, transmission % of light, and transmission spectrum.

Filter Number	Filter Name	Transmission (%)	Spectral Energy Distribution Curve*
24	Scarlet	22	
94	Kelly Green	25	
84	Zephyr Blue	14	
98	Mediu m Grey	25	

*The Spectral Energy Distribution Curve (SED) is a graphic representation of a color and describes the wavelengths of color transmitted through the individual filters. The area under the curve is transmitted. SEDs are provided by the filter manufacturer.

How long experiment should run – Procedure #13

The experiment should run until the clear flask with plant (the flask we expect to photosynthesize the most) pipette's water level is close to the end of the pipette markings. To allow group comparisons:

- Note the time when the first, fastest, group puts their flasks in the sunlight.
- Monitor this group's clear control carefully.
- When the water level hits the top of the markings, note the time and end this group's experiment.
- Announce this time interval (e.g. 15 min). All groups should run their experiments for the same amount of time.

Other time considerations:

- On a sunny day with the baking soda concentration listed in the procedure this experiment should take about 10 - 15 minutes. On a cloudy day this may take as long as 50 minutes. This lab should take place on a sunny day to ensure the experiment can finish by the end of class.
- You can speed up the photosynthesis reaction by adding more baking soda to your solution (see baking soda section).
- We recommend pilot testing to determine approximately how long you will run your trials, especially if you are modifying equipment. Time will depend on sunlight intensity, the size of your flask, amount of *Elodea*, and baking soda concentration.

Option: Students read off water volumes in the pipettes every 5 or 10 minutes.

Baking soda concentration

- Baking soda (sodium bicarbonate, $NaHCO_3$) is added to water to provide an abundant supply of carbon for photosynthesis. Baking soda is a base ($NaHCO_3 + H_2O \rightarrow H_2O_3 + OH^- + Na^+$). If no baking soda is added, the amount of dissolved carbon dioxide in the water in a small flask will quickly run out when the *Elodea* is rapidly photosynthesizing. If you do not add any baking soda to the water, the photosynthesis reaction will not proceed quickly enough to allow the activity to be completed in a class period.
- The solubility of baking soda (sodium bicarbonate) in water is 96 g/L (20 °C). We are suggesting an approximately 25% saturated solution (25 g/L). You can speed up the photosynthesis reaction by adding more baking soda. However, more baking soda will make the solution more basic and may affect plant physiology and thus results.

Extras

- We recommend having extra flasks, stoppers, and filters on hand for students who want to test out additional hypotheses.
- Because students are working with glass, make sure you follow all recommended safety guidelines for handling glass and broken glass.

Suggestion for class data chart

- Determine the mean (and for older students, standard deviations) for each treatment. Make a bar chart of these values.

Background Information

Depending on the content knowledge of your class, you may need to introduce or review some basic information about photosynthesis prior to beginning the experiment.

Brief Review of Photosynthesis

Photosynthesis ($6\ CO_2 + 6\ H_2O$ + light energy --> $C_6H_{12}O_6 + 6\ O_2$) takes energy from the sun (light) and turns it into stored energy (fixes carbon, $C_6H_{12}O_6$ is the chemical equation for a sugar carbohydrate). This process releases oxygen. Oxygen is needed for aerobic respiration (e.g. in humans).

Photosynthesis occurs in two stages (see the figure below). In the first stage, light reactions capture the energy of light and use it to make the energy-storage molecules (ATP and NADPH). In this stage, the oxygen given off comes from *water*. In the second stage, the light-independent reactions (the Calvin cycle) use the energy-storage products to capture and reduce carbon dioxide, transforming it into sugar.

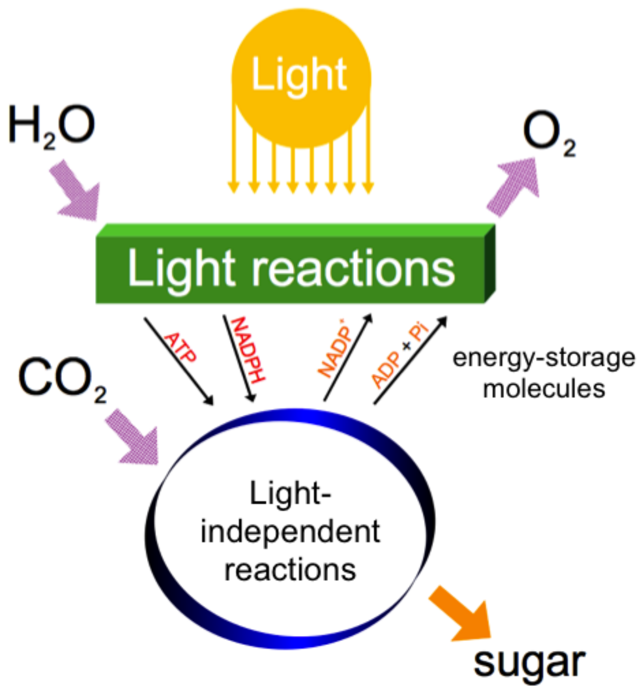


Fig. 3. Photosynthesis overview.

Image by Daniel Mayer (mav) - original image https://en.wikipedia.org/wiki/File:Simple_photosynthesis_overview.svg

Today, the atmosphere consists of approximately 78% nitrogen (N_2), 21% oxygen (O_2), 0.03% carbon dioxide (CO_2) and miscellaneous gases (e.g. H_20 and CH_4). The early atmosphere of earth was very different. Notably, there was much less oxygen, so the first life on earth was probably anaerobic. The oxygen produced by photosynthesis, initially by cyanobacteria and eventually by more complex multicellular plants, supplied most (~98%) of the oxygen to the early atmosphere. Today, between one-third and one-half of the oxygen going into the atmosphere is produced in the ocean.