

Lab 12: Photosynthesis

Objectives:

Upon completion of this topic you should:

1. Understand the processes of photosynthesis and respiration.
2. Know what pigments are involved in photosynthesis and how they can be studied using chromatography and the spectrophotometer.
3. Understand the relationship between stomata and photosynthesis and respiration.
4. Be able to explain the relationship between light intensity and photosynthesis.

Safety Issues:

There are several safety issues in this lab—so, let's be careful.

We will be heating ethanol in a boiling water bath—exercise caution!

We will be using acetone and petroleum ether. Both are toxic and flammable—again, be careful!

Introduction:

The exercises in this Lab are designed to develop an understanding of the relationship between photosynthesis and respiration. The exercises also emphasize the importance of the different components of the photosynthetic process, specifically: light, carbon dioxide, and chlorophyll. Photosynthetic activity can be measured by the amount of sugar or oxygen produced in the cells or tissues of green plants. Because starch is formed (by dehydration synthesis) from simple sugars produced during photosynthesis, the presence of starch will be used in most experiments to verify photosynthetic activity. The net chemical reactions for photosynthesis and starch synthesis are provided below.

In the presence of chlorophyll



Lab Exercises:

PART A. THE RELATIONSHIP BETWEEN PHOTOSYNTHESIS AND RESPIRATION

EXERCISE #1: PHOTOSYNTHESIS AND CELLULAR RESPIRATION IN *Elodea*

In this exercise, you will be conducting experiments that use the dye Phenol Red as an indirect indicator of whether photosynthesis and/or cellular respiration is occurring in *Elodea* plants. The experiments investigate the effect of light on these processes.

1. Demonstration:

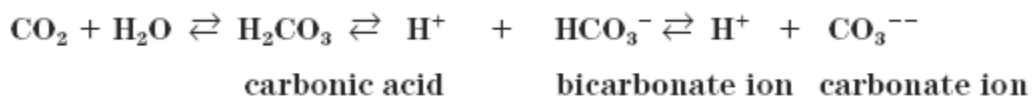
Phenol Red as a pH indicator

As you all remember, pH is a measure of the free hydrogen ion concentration $[H^+]$. The greater the $[H^+]$, the lower the pH value. Water has a pH value of 7 and is referred to as “neutral,” even though water does contain free hydrogen ions. Acids contain a higher $[H^+]$ than pure water, and therefore have a pH value less than 7. Bases contain a lower $[H^+]$ than pure water, and therefore have a pH value greater than 7.

Phenol red is an organic dye that undergoes a color change when placed in different pH solutions. That is, the color of the dye depends on whether the solution is acidic, neutral, or basic. The colors of phenol red at different pH's are as follows:

acidic solution (pH < 7)	→	yellow
neutrality (pH = 7)	→	orange-pink
basic solution (pH > 7)	→	dark pink

Because phenol red can be used to detect changes in pH, it can also be used as an indirect method of detecting changes in the amount of CO_2 dissolved in a solution. When CO_2 is added to a solution, some of it reacts with water to produce an acid called carbonic acid. In turn, some of the carbonic acid dissociates to increase the $[H^+]$ in the solution. Therefore, if the amount of CO_2 in a solution is increased, the $[H^+]$ increases (pH is lowered) and the solution becomes more acidic. It also follows that if the amount of CO_2 in a solution is lowered (e.g., by removal of CO_2 from the solution), the $[H^+]$ will decrease (pH increases) and the solution becomes more basic. The chemical reactions involved are shown below.



You should note that the reactions are reversible. Therefore, if CO_2 is added to the solution, the reactions are driven to the right (H^+ increases). If CO_2 is removed, the reactions are driven to the left (H^+ decreases). From what you know about the process of photosynthesis and respiration, indicate which uses CO_2 and which releases CO_2 .

pH of Distilled Water, Industrial Water, and Industrial Water + CO_2

The instructor will demonstrate the use of phenol red as a pH indicator. A few drops of phenol red may be added to a beaker of distilled water and to a beaker of tap water to show differences in pH. In addition, blowing into the tap water demonstrates the effect of CO_2 on pH.

Table 1. Phenol red, water, and CO_2

Solution Tested	Color of Solution	Condition of Solution (acidic, neutral, basic)
Distilled Water		
Tap Water		
Tap Water + CO_2		

2. *Elodea* Experiments

Experiment 1: *Elodea* in the Light

Fill one of two flasks with 250 ml of **tap** water and add 10 drops of phenol red. Make the water in the flask **slightly acidic** by **gently** breathing into the flask while swirling it. Repeat if necessary to get a **slight** color change from neutrality, but do not make the solution too acidic (yellow). Record the color of the solutions in Table 2.

Now, pour half of the water (125 ml) from this flask into a second flask. Place a 20 cm piece of *Elodea* **in one flask only**. If a 20 cm piece is not available, put in two 10 cm pieces. Make sure that the *Elodea* is **completely submerged**. You may want to use a glass rod or something similar to gently push every piece of the *Elodea* under water. Place both clearly labeled flasks under the light table. Examine the flasks near the end of the laboratory period and record your results in Table 2.

Experiment 2: *Elodea* in the Dark

Fill one of two flasks with 250 ml tap water and add 10 drops of phenol red indicator. Record the color of the solutions in Table 2.

Now, pour half of the water (125 ml) from this flask into a second flask. Place a 20 cm piece of *Elodea* **in one flask only**. If a 20 cm piece is not available, put in two 10 cm pieces. Make sure that the *Elodea* is **completely submerged**. Mark your flasks so that you can identify them at the end of the laboratory period. Place both flasks **in the warm dark place** (in the covered empty water bath). Examine the flasks near the end of the laboratory period and record your results in Table 2.

Please note: in the event that the industrial water in the lab is not neutral (at times in the past, the water from the tap has been slightly acidic), you may have to obtain water for your experiment from an alternate source. Your instructor will give you directions.

Table 2. Results of *Elodea* Experiments

Experiment #1: Flasks in the Light	Experiment #2: Flasks in the Dark
Flask with <i>Elodea</i>	Flask with <i>Elodea</i>
Color of initial solution = _____ (before putting the flask in the light) (Note: the flask contained industrial water + CO ₂)	Color of initial solution = _____ (before putting the flask in the dark) (Note: No CO ₂ was added to the industrial water)
Color of final solution = _____ (at the end of the experiment)	Color of final solution = _____ (at the end of the experiment)
Flask without <i>Elodea</i>	Flask without <i>Elodea</i>
Color of initial solution = _____ (before putting the flask in the light) (Note: the flask contained industrial water + CO ₂)	Color of initial solution = _____ (before putting the flask in the light) (Note: No CO ₂ was added to the industrial water)
Color of final solution = _____ (at the end of the experiment)	Color of final solution = _____ (at the end of the experiment)

Questions (answer in your lab notebook):

1. Why were the flasks without *Elodea* used in Experiments #1 and #2?
2. In experiment #1, why did blowing into the tap water containing phenol red produce a color change? [Note: you should discuss what was in the exhaled air; why it was in the exhaled air; and how did it produce the color change].
3. Describe and explain the results that were obtained in Experiment #1. [Your answer should consider the following: What metabolic process or processes were occurring in the flask with *Elodea* placed in the light? What effect did these processes have on CO₂ levels in the flask? How and why did this occur (i.e., give the actual chemical reactions)? How and why did the change in CO₂ levels produce a color change (again, give the chemical reactions)?]
4. Describe and explain the results that were obtained in Experiment #2. [Your answer should consider the following: What metabolic process or processes were occurring in the flask with *Elodea* placed in the dark (give the actual chemical reactions)? What effect did these processes have on CO₂ levels in the flask? How and why did this occur? How and why did the change in CO₂ levels produce a color change (give the actual chemical reactions)?]

Please note: DO NOT DISCARD THE USED *Elodea* FROM YOUR FLASKS! Pull it out and return it to the *Elodea* stock tray/tub. If you discard it, the next class will not have any. We recycle it.

Background for Exercise 3 – Gas exchange in leaves

In order to carry out photosynthesis, plants need to take in CO₂ and release O₂ through their leaves. Most of the leaf is covered with an impervious wax layer—the cuticle—that prevents water loss, but also prevents gas exchange. In order to allow gas exchange, plants have evolved with openings in through this impervious layer—the stomata. The opening and closing of stomata is carefully regulated by plants, especially under dry conditions since, in allowing CO₂ and release O₂ exchange water vapor is also lost from the leaf. This opening and closing is controlled by guard cells, specialized cells that surround the opening (two guard cells per stoma). To open stomata, signals cause the guard cells to take up water and increase in turgidity (or tightness) as they fill with water. The walls on these cells are thicker on the inside of the pore than on the outside. As a result, with increasing turgidity they swell and bend—causing the pore to open. In this exercise we'll do an epidermal peel and take a look at stomata.

Materials for Exercise 3:

Compound microscope
and cover slips

Leaves from a jade plant (or others)

Microscope slides

Procedure for Exercise 3:

1. Obtain two slides and cover slips.
2. Obtain several leaves.

3. As demonstrated by the instructor, snap the leaf in half leaving the epidermis on the underside of the leaf unbroken. Carefully peel the epidermis from the lower side of the leaf by gently separating the two halves.
4. Transfer a portion of the epidermis to a slide, add a drop of distilled water, and cover with a cover slip.
5. Observe the leaf under low power first and locate a stoma. Increase the power you are using to observe and try to differentiate the guard cells.
6. Sketch a stoma with guard cells in your lab notebook.
7. Prepare a second peel and add a drop of 0.5 M NaCl to this prep instead of distilled water. What do you predict will occur with the stomata?
8. What do you observe?

Background for Exercise 4—What's necessary for photosynthesis and starch accumulation?

In preparation for this experiment, days ago foil was placed over sections of leaves on variegated geranium plants. Variegated means that not all of the leaf is green. The variegated geranium leaves you will be using have a white border that lacks the pigments found in chloroplasts. A piece of foil has been shielding a portion of the leaf for some time, preventing the underlying cells from receiving light. After sketching the outline of your leaf, the foil, and the variegation, you will first remove the chlorophyll and other pigments of the leaf by boiling it in alcohol, and then you will add iodine potassium iodide (IKI) to stain the starch stored in a leaf.

Materials for Exercise 4:

1 clean 250 ml beaker; 1 clean 500 ml beaker	Alcohol solution (80% ethanol)
Variegated geranium leaf with a section wrapped in foil	Petri dish
Hotplate and tongs	IKI solution

Procedure for Exercise 4:

1. Obtain a 500 ml beaker and a 250 ml beaker.
2. Fill the 250 ml beaker half full with alcohol.
3. Fill the 500 ml beaker half full with tap water and then create a water bath for the alcohol by placing the beaker of alcohol into the 500 ml beaker.
4. **Before continuing to 5, practice using the tongs to move the beaker of alcohol in and out of the water bath. We do not want any burns to occur in the next steps!**
5. Place the beakers on your hotplate and then turn it on. **(Warning: do not place the beaker of alcohol directly on the hotplate; it should only be heated in a water bath)**
6. Have one lab member in charge of the hotplate. The setting on the hotplate should be adjusted so that the water in the beaker stays at a gentle boil.
7. Obtain one foil-covered leaf from a variegated geranium plant at the side of the room.
8. **Before removing the foil**, sketch a simple outline of the leaf in your lab notebook. Do not use shading or color, just make a simple basic outline. Next, using dotted-lines, sketch the outline of both the foil and the boundaries of the green and white colors. Later you will shade in the areas that were stained with IKI.

9. Remove the foil (you sketched its location, right?) and paper clip. Please place the foil and clips in the designated container for reuse.
10. Place the leaf in boiling alcohol for about 5 minutes. Note which pigments (colors) are removed in the hot alcohol.
11. After the leaf has been bleached of its colored pigments, place it in a Petri dish and cover it with IKI solution. On the same sketch you made for step #7, record the location of the starch by shading in all the areas stained dark by the IKI.
12. Clean up. Make sure to remove all grease pencil marks and rinse out all glassware. The leaves can be thrown away but keep the foil and paperclips.

Exercise 5: Light Intensity Demonstration

The intensity of sunlight striking the surface of the earth varies from hour to hour as well as from season to season. What effect, if any, does this variation have on photosynthesis?

In this demonstration, the amount of O₂ released by the plants will be used as an indication of photosynthetic activity; the greater the amount of O₂ the more photosynthetic activity.

1. Four pieces of *Elodea* with freshly cut stems were placed into small test tubes.
2. These small test tubes were placed upside down inside larger test tubes containing an aqueous solution of 0.25% sodium bicarbonate. Precautions were taken so that no air bubbles were trapped inside either tube.
3. These tubes were then placed in a test tube rack, which was then placed near a light source so that the tubes were not equidistant from the light source.
4. Examine the tubes and observe any difference in the size of bubbles of O₂ that have collected.
5. What does this experiment demonstrate about the effect of light intensity on photosynthesis?

PART B. CHLOROPLASTS AND THEIR PIGMENTS

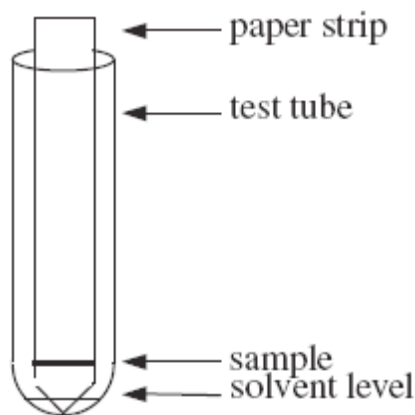
Exercise 1: Separation of Pigments in Spinach Leaves Using Paper Chromatography

One technique used to demonstrate the presence of individual pigments in a mixed solution is **paper chromatography**. In the process of chromatography, the sample to be analyzed is applied to a paper strip. This paper strip is then placed in a small volume of solvent and the solvent is allowed to move up a paper strip by capillary action. As the solvent passes through the portion of the paper to which the sample has been applied, it will begin to carry the sample with it as it advances up the paper strip. Often some components of the sample will be carried more readily by the solvent than others. It is this effect that we will use to separate the different pigments in chloroplasts. Half of the class will concentrate on the chromatography portion while the other half continues with the spectrophotometry portion.

Procedure:

Colored Solution. Before analyzing chloroplast pigments we will practice chromatography using the colored solution (ink or food coloring) provided.

1. Obtain two strips of special filter paper. Be careful in handling this paper. For best results you should not put your fingers on the flat surface, particularly near the pointed end. Handle it by holding it at the edge or at the blunt end of the strip.
2. Using a capillary tube, paint a *very narrow* line across one of the filter paper strips, about 2 cm from the pointed end.
3. Next, suspend the strip into a test tube with a small amount of distilled water in it. Only the pointed tip of the paper should be submerged. Make certain that no water is splashed up on the sample and that the sample is not submerged.
4. Hold the paper strip in place by corking the open end of the test tube and place it in a rack for about five minutes. The setup is diagramed below.



RECORD YOUR RESULTS IN YOUR LAB NOTEBOOK

Chromatogram of colored solution

CAUTION! All solvents used in this exercise are toxic and flammable. Use extreme care in handling them.

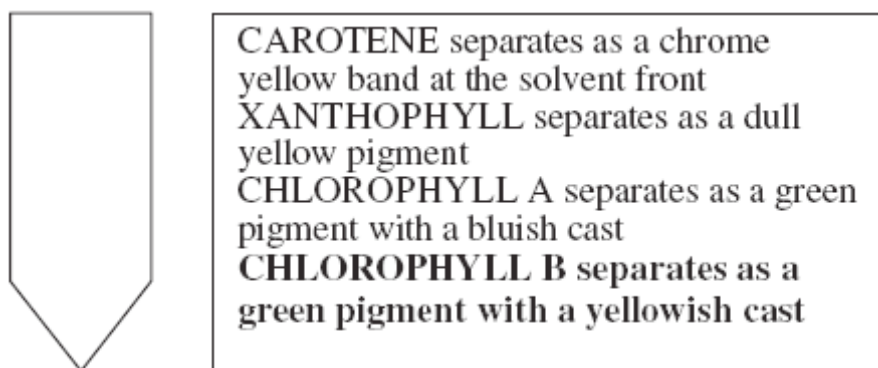
Spinach Pigments

1. Prepare a concentrated chloroplast pigment extract by grinding some dried spinach leaves in a very small amount (about 10 ml) of 85% acetone. It will help if you grind the dry spinach to a fine powder *before* adding the acetone. Be sure to use the proper reagent. This step calls for ACETONE, *not* acetone petroleum ether. Use just enough acetone to obtain a very concentrated extract.
2. Grind in mortar with pestle until the extract is intensely dark green. (nearly black).
3. With a capillary tube, paint a narrow line of pigment across the filter strip about 2 cm above the pointed end.
4. Use a hair dryer to speed drying, and then re-apply the pigment extract.
5. Repeat this process about five times allowing for thorough drying between layers.

- Obtain a large test tube. To ensure that there is no water in the tube at all, rinse the tube with a small amount of acetone.
- Place 20 drops of acetone petroleum ether solution into the thoroughly dry test tube.
- Place your test tube with the acetone-petroleum ether solution into the test tube rack and then carefully suspend the paper strip so that only the pointed tip is in the solution. Be careful not to splash the solvent up on the filter paper.
- Hold the paper strip in place with a stopper. Set the experiment aside for about ten minutes.

RECORD YOUR RESULTS IN YOUR LAB NOTEBOOK USING A DIAGRAM LIKE THAT BELOW.

Chromatogram of spinach pigments

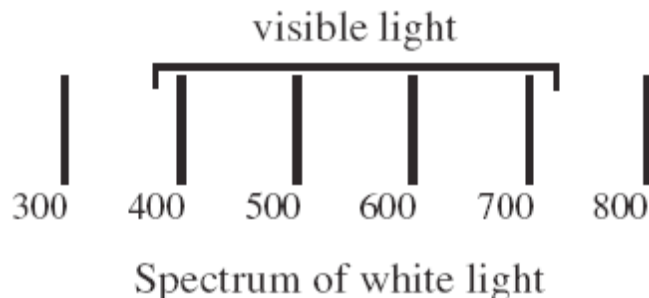


Exercise 2: Pigments and Light Energy

When white light passes through a glass prism, as in a **spectroscope**, a spectrum of different wavelengths becomes visible. If a colored solution is placed between the source of white light and the spectroscope it will absorb some wavelengths of white light and transmit others into the spectroscope. The colors of the wavelengths that are totally absorbed will no longer be visible; instead, vertical “black” **absorption bands** will appear. The “blackness” indicates no light in the band region.

Procedure:

- Examine the normal spectrum of white light. In your lab notebook, record the colors seen from using a diagram that is similar to the one below.



2. Place a solution of spinach pigment (chlorophyll plus the other pigments you saw on your chromatogram) between the light source and the spectroscope. Now examine the spectrum. Move the solution in and out of the light path to compare the absorption spectrum of the spinach pigments with the spectrum of white light. All of the pigments seen with the chromatogram are involved and much of the absorption in the blue end of the spectrum is due to the yellow xanthophylls.

What portions of white light does chlorophyll absorb?

What portions of white light are not absorbed by chlorophyll?

Why do most leaves appear green to us?

3. Without using the spectroscope, observe the color of the pigments when the bright light source is placed very close to the tube. What you see is fluorescence and is due to the release (from chlorophyll) of the light energy absorbed by the chlorophyll. This light energy cannot be passed on because the chlorophyll has been removed from the photosynthetic apparatus of the intact cell.

NOTE: Be sure you have washed all glassware and leave the laboratory with everything clean and in place. DO NOT throw any plant material into the sinks. Remember to recycle the *Elodea* from the flasks (put it back into the *Elodea* bucket under the light table).

Clean Up Procedures

Be sure to follow directions regarding the accumulation and disposal of hazardous waste in today's lab. As always, **you are required to follow the specific instructions for clean up provided for you at each lab bench.** Remember that you must remove any markings from the glassware and beakers. Use paper towels or lab wipes to remove all grease pencil markings. Also remember that you must return all the glassware and supplies you used back to your tray. Check to be sure you have not left any beakers, test tubes, etc., at the sinks, the side benches, inside the warm dark place or under the light table. All supplies are to be in your trays at the end of the lab. **DO NOT DISPOSE OF ANY PLANT MATERIAL IN THE SINKS.**