

LAB 7 – Photosynthesis

Introduction

In order to survive, organisms require a source of energy and molecular building blocks to construct all of their biological molecules. The ultimate source of energy for almost all of life on Earth is the light that comes from the sun (*see the box on the next page for an example of organisms that do **not** depend on light as the ultimate source of energy*).

Photosynthesis and **cellular respiration** are two of the most important biochemical processes of life on Earth. Both are a series of reactions that are catalyzed by unique enzymes at each step. Although it is somewhat of an oversimplification to describe them as “opposite” sets of reactions, for introductory purposes we can think of them as such.

Photosynthetic (“light” “forming”) organisms are those that can take simple molecules from the environment such as **carbon dioxide** (CO₂) and **water** (H₂O), and using the energy of the sun, create their own **biological macromolecules** such as carbohydrates, proteins, lipids and nucleic acids. You will note that the reactions of photosynthesis are both **endothermic** and **anabolic**, in that they require energy and use small molecules to make larger ones. These reactions take place in the **chloroplasts** of plant cells.

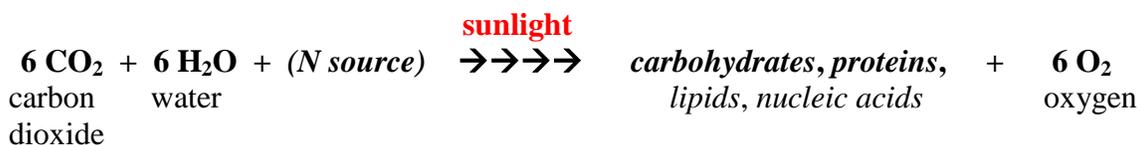
We generally *summarize* the series of reactions of photosynthesis in terms of the initial reactants and the final products - leaving out details of all the reactions in between. In introductory biology, we simplify what is happening by showing only the monosaccharide **glucose** as the ultimate organic molecule that is produced.

Simplified



In reality, the products of photosynthesis include the formation of all of the biological macromolecules the organism requires. In addition, photosynthetic organisms must have a source of nitrogen (e.g. fertilizer) to make its proteins and nucleic acids. In this lab, we will use the simplified equation above for our discussion.

Actual



Exercise 1 – Observing Photosynthesis via CO₂ Consumption

1. Label 4 screw cap tubes **1, 2, 3** and **4** with a marker and line them up in order in a test tube rack.
2. Obtain 3 pieces of *Elodea* about 6 cm in length and 2 pieces of aluminum foil.
3. Fill a 250 ml Erlenmeyer flask to the 100 ml line with tap water, then add 5 ml of phenol red solution to the flask. Swirl to mix.
4. Take a clean straw and blow bubbles carefully into the solution until it turns a distinct **orange/yellow**. *Note: the yellow color indicates an increase in the level of CO₂.*
5. Place a 6 cm *Elodea* stem with leaves in tubes #1, 2 and 4. Tube #3 will not have any *Elodea*.
6. Fill tubes #1, 2 and 3 with the CO₂ enriched phenol red solution.
7. Fill tube # 4 with tap water and add 0.5 ml of stock phenol red solution.
8. Tightly screw the caps on each tube and record the color of each tube on your worksheet.
9. Wrap a piece of aluminum foil around tubes #2 and #4, being sure to cover the bottom so that no light enters these tubes.

The chart below summarizes the contents of each tube:

	Tube 1	Tube 2	Tube 3	Tube 4
CO ₂ enriched phenol red solution	X	X	X	
H ₂ O plus phenol red				X
<i>Elodea</i> stem with leaves	X	X		X
Aluminum foil (to block light)		X		X

10. Turn each tube upside down (to maximize light exposure) and place the rack of inverted tubes directly underneath the lamp at your lab bench.
11. Record the **starting time** on your worksheet, then turn on the lamp and allow the experiment to run for two hours.
12. Write your hypothesis regarding this experiment on your worksheet, and identify the independent variable, dependent variable and control.
13. After 2 hours have passed, record the colors of each tube on your worksheet, analyze your data and answer the corresponding questions.

Move on to the next experiment while this experiment continues...

Part 2: Isolation of Plant Pigments

A **pigment** is a molecule that absorbs light. White light contains all of the different colors of the visual spectrum. This can be observed in a simple rainbow during a rain storm or by using a prism that splits white light into its various colors.

Why does a shirt appear red? The red shirt has a pigment molecule that we call a dye that *absorbs* all of the other colors of the visible spectrum (blue, green, yellow, etc.), but *reflects* back the red waves of light.

In plants, there are two categories of pigments used for photosynthesis: primary pigments and accessory pigments. The **chlorophylls** are the primary pigments of photosynthesis, with two types called **chlorophyll a** and **chlorophyll b**. The chlorophylls are *green* pigment molecules. What does this mean? Chlorophyll *absorbs* blue, red, orange, yellow, etc.....light, but *reflects* green light. On the other hand, accessory pigments collectively called **carotenoids** are red, yellow or orange – they absorb all of the other colors. You can see these colors on trees in the northern states, and locally as well, in the fall before they drop their leaves. They serve to broaden the spectrum of light absorption in plants and they protect the plant from harmful or excessive rays of sun.

Chromatography (“color” “measure”) is a technique that allows us to separate different molecules from a mixture based on differences in **solubility**. Some compounds do not like to dissolve in water. These are called **hydrophobic** (“water” “fearing”) compounds. On the other hand, some molecules are **hydrophilic** (“water” “loving”), meaning they like to dissolve in water. You should note that these properties are not absolute. For example, it is possible for one compound to be *slightly* hydrophobic and a different compound to be *extremely* hydrophilic.

The golden rule for solubility is: “*Like dissolves in like.*” In other words, a hydrophilic compound will be more soluble in a liquid that is also hydrophilic. Likewise, a hydrophobic compound will be more soluble in a liquid that is hydrophobic.

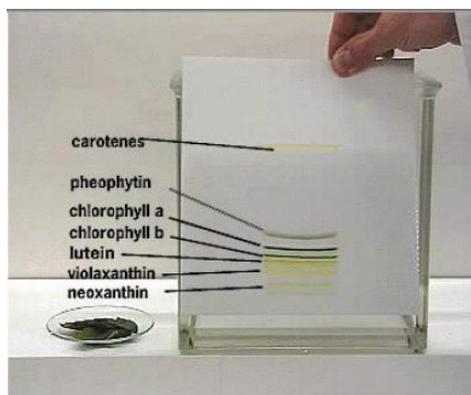
Chromatography is a method of separating and isolating molecules based on their level of hydrophobic or hydrophilic properties. In **paper chromatography**, we create a “molecular race track” in which molecules move through a piece of filter paper, carried along by a wave of liquid solvent. Those pigment molecules that have the highest solubility in the liquid solvent used will be “carried along” through the paper the fastest. Those pigments that are least soluble in the solvent will move more slowly or not at all.

The various plant pigments have differing degrees of *hydrophobicity*. Therefore, if we use a liquid solvent that is hydrophobic, different plant pigments will move at differing rates through the piece of paper as the liquid solvent is absorbed upward. In this way, individual pigments can be separated into bands on the filter paper.

In this experiment, you will use paper chromatography to separate the plant pigments from a plant with a green leaf (spinach) or one with a red leaf (*Coleus*) using a hydrophobic ether-based solvent.

Exercise 2 – Separation of Leaf Pigments Using Paper Chromatography

1. Obtain a mortar and pestle and a spinach leaf.
2. Tear up the spinach leaf and place a small number of spinach leaf fragments in the mortar, then add 2 ml of the solvent (60% isopropanol and 40% acetone) to the mortar.
3. Use the pestle to grind the leaves in the solvent. You should observe the formation of a liquid in the bottom of the mortar that contains the leaf pigments.
4. Acquire a 9 cm strip of chromatography paper. Use a pencil to *very lightly* draw a line 0.5 cm above one end of the chromatography paper.
5. Dip a small glass capillary tube into the pigment extraction solution at the bottom of the mortar to collect some of the solution.
6. Repeatedly “dab” the capillary tube with pigment solution *gently* across the filter paper just above the pencil line. The intention is to form a thin line or band of pigment solution that spans the width of the paper. Allow the first line to dry and then repeat this procedure three more times to concentrate the pigments along the paper.



7. Wash the mortar and pestle and repeat steps 2-6 using *Coleus* leaf fragments.
 8. Label one of the flat-bottom test tubes at your bench “S” for spinach and the other “C” for *Coleus*, and add 0.5 ml of the chromatography solvent (ether:acetone 95:1) to each tube. Note that this solvent is volatile (will evaporate quickly) and can be caustic to the eyes and nose.
 9. Carefully set each chromatography paper strip, with the line of pigments at the bottom, into the solvent at the bottom of the appropriate tube (“S” tube for spinach strip, “C” tube for *Coleus* strip). Make sure that the paper sits flatly on the tube bottom *and that the solvent is below the pigment line*.
 10. Cover each tube with plastic wrap and move them to the ventilation hood so that the vapors can be isolated from the rest of the room.
 11. Allow the tubes to sit in the hood until the highest band of pigments rises to within ~1 cm of the top of the paper which should take about 15 – 30 minutes.
 12. On your worksheet, draw the lines of pigments you observe on each filter paper and label the color of each line. Alternatively, you can take a picture of the filter papers which you can print and paste on your worksheet.
 13. Answer the corresponding questions on your worksheet.
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Part 3: Photosynthesis & O₂ Production

Refer to the overall reactions of photosynthesis in the introduction and you will see that the only gaseous *product* of photosynthesis is oxygen gas or O₂. Thus if you can detect gas production in plant material if you can be confident that the gas is O₂ produced by photosynthesis, in particular if the gas production is dependent on light. The next exercise involves a very simple but clever method to detect gas production in plant material, specifically small pieces of spinach, as they are exposed to a light source.

To perform this experiment you will produce numerous small circular spinach discs, remove any residual gas from the spinach discs, expose them to various levels of light, and determine the proportion of discs that begin to float due to gas production.

Exercise 3A – Observing & Measuring Photosynthesis via O₂ Production

Preparing the spinach discs:

1. Obtain several spinach leaves from the front of the lab.
2. Place the spinach leaves on the cutting board at your lab bench, and using the metal corer at your lab bench punch at least 50 or so discs from the leaves. This will go faster if you punch through multiple layers of leaves.
3. Transfer all of the spinach discs to the 250 ml filter flask at your bench.
4. Add 0.2 M sodium bicarbonate (NaHCO₃) to the flask up to the 100 ml line, and swirl to make sure all the spinach discs are in the liquid.
5. Place the rubber stopper on top of the flask and make sure the whole is sealed with a piece of masking tape.
6. Connect the flask to the vacuum spout with the rubber hose provided and turn on the vacuum.
7. Wait until the liquid begins to bubble vigorously and then stops. This may take several minutes, and when complete your spinach discs will be “degassed”.
8. Turn off the vacuum and peel back the tape on the rubber stopper to let air into the flasks. Most of the spinach disks should then sink to the bottom.
9. Give the flask a swirl and then immediately pour the bicarbonate solution with the spinach discs into the glass bowl at your bench. If you pour after swirling the discs should not stick to the side.
10. Proceed to setting up your experiment, and store any leftover spinach discs in a dark place such as a drawer.

Setting up the experiment:

1. Label the three glass petri dishes (the base, not the lid!) **A, B** and **C**.
 2. Fill the base of each petri dish ~2/3 full with 0.2% NaCHO₃.
 3. Use tweezers to transfer 10 **completely submerged** (i.e., on the bottom of the bowl) spinach discs to each petri dish. Each disc should be completely flat on the bottom of the petri dish before beginning the experiment.
 4. Place a lid on each petri dish and put them in the following locations:
 - A – in a closed drawer at your work bench (this is your “no light” control)
 - B – leave on your bench top far from the lamp
 - C – position directly under the lamp and turn the light on
 5. Leave each dish of spinach discs in their respective locations for 20 minutes.
 6. Count the number of discs that are floating or on edge (i.e., more buoyant due to O₂ production).
 7. Record the results on your worksheet, graph the data (% floating vs source of light), and answer any associated questions on the worksheet.
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Exercise 3B – Design an experiment

In this exercise you and your group will design a new experiment based on the previous one. In this experiment you will test the effect of different colors (i.e., different wavelengths of visible light) on photosynthetic activity as assessed in the previous experiment.

1. As a group, come up with a hypothesis regarding the effect of the different color filters at your lab bench (red, green and blue) on photosynthetic activity. Write the hypothesis on your worksheet.
2. Design an experiment to test this hypothesis. On your worksheet, briefly describe your experimental plan, and identify the independent variable, dependent variable and control.
3. Carry out your experiment, record and graph the results on your worksheet, and write your conclusion.

Laboratory 7 worksheet – Photosynthesis

Name: _____ Group: _____ Date: _____

Exercise 1 – Photosynthesis & CO₂ consumption Start time: _____ End time: _____

State your hypothesis below and identify the indicated components of this experiment:

Hypothesis:

Independent variable:

Dependent variable:

Control:

Results:

	Tube 1	Tube 2	Tube 3	Tube 4
Color at Start of Experiment				
Color at the End of the Experiment				
Was there a color change?				
Did Photosynthesis take place?				

What does a color change from red to yellow indicate about the relationship between pH and CO₂ level?

What happened with the color in tube #3? Explain these results based on its contents and light exposure.

Explain any color change in tubes #2 and #4 in terms of the contents and light exposure for each vial.

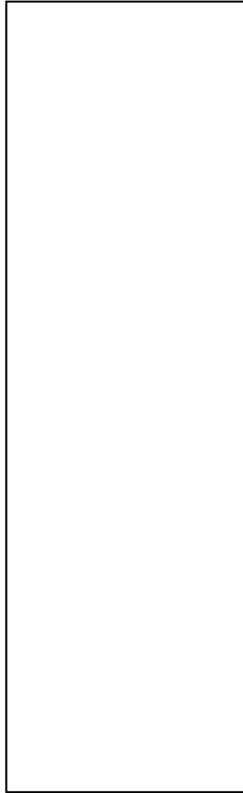
What happened to the color of tube #1? Explain these results based on its contents and light exposure.

Did these results support your hypothesis? Explain.

Exercise 2 – Separation of leaf pigments by paper chromatography

Use a ruler to measure the distance the pigment lines moved from the starting line. Draw a picture of your chromatography paper in the box below

SPINACH



of different pigments _____

COLEUS



of different pigments _____

What plant pigment molecules might account for the different color pigments you observed?
(If necessary, refer to your lecture notes or textbook)

Exercise 3A – Photosynthesis & O₂ production

State your hypothesis below and identify the indicated components of this experiment:

Hypothesis:

Independent variable:

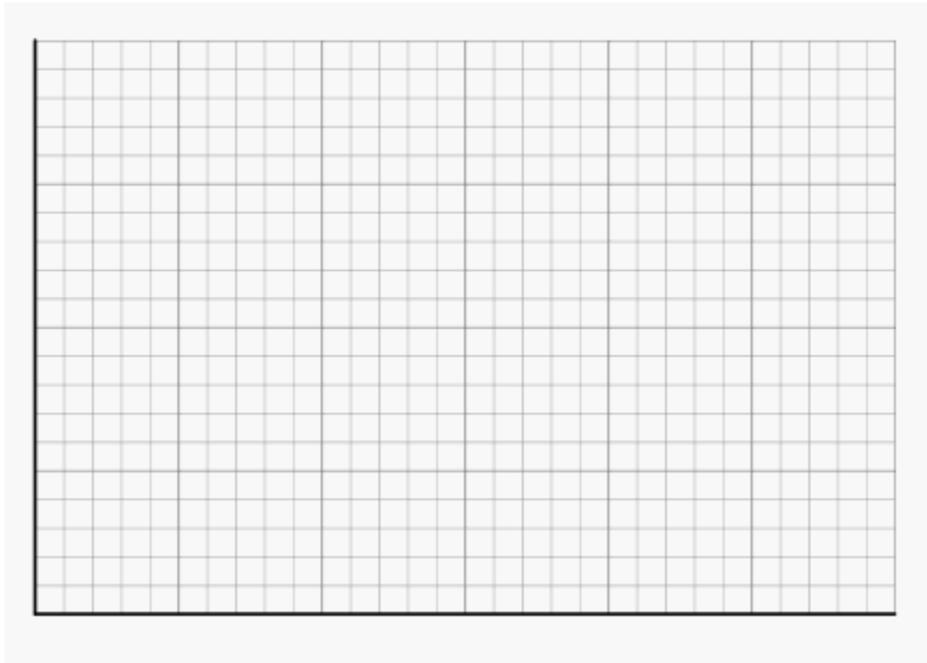
Dependent variable:

Control:

Results:

light source	total # of discs	# of floating discs	% floating discs
no light (dark)			
room light			
lamp			

Graph your results on the grid below:



Did these results support your hypothesis? Explain.

Exercise 3B – Design an experiment

Briefly describe or outline the design of your experiment below:

State your **hypothesis**:

Identify the indicated components of your experiment:

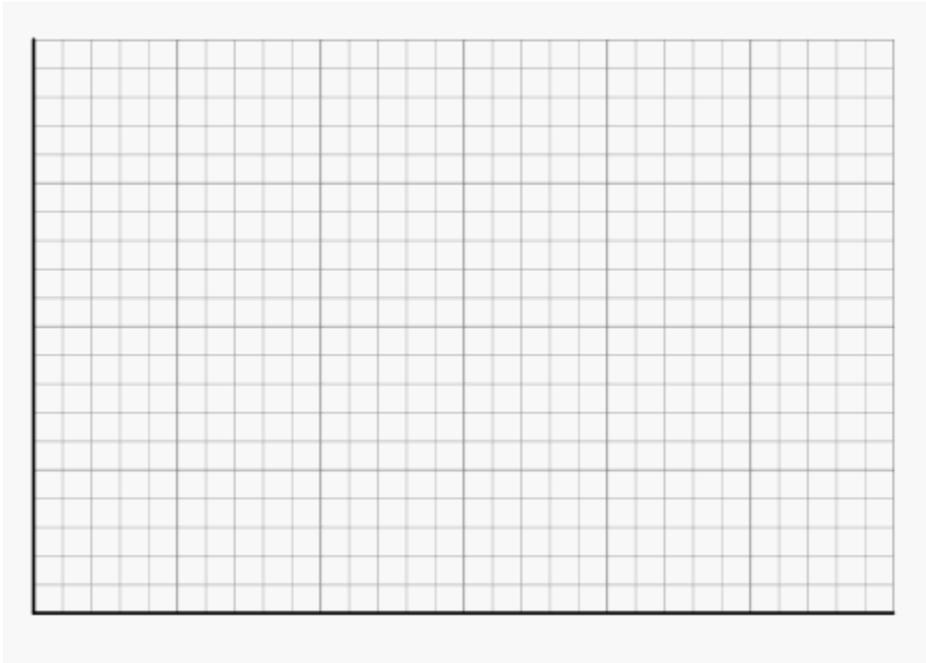
Independent variable:

Dependent variable:

Control:

Draw a chart or table and record the results of your experiment below:

Graph your results on the grid below:



Did these results support your hypothesis? Explain.