LAB 8 – Photosynthesis

Objectives

1. Assess CO_2 consumption and O_2 production during photosynthesis.
2. Analyze the role of light in photosynthesis.
3. Examine the effect of different wavelengths of visible light on 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** (CO_2) and **water** (H_2O), 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 can *summarize* the series of reactions in 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.

$$\underset{\text{carbon dioxide}}{6\,CO_2} + \underset{\text{water}}{6\,H_2O} \xrightarrow{\text{sunlight}} \underset{\text{glucose}}{C_6H_{12}O_6} + \underset{\text{oxygen}}{6\,O_2}$$

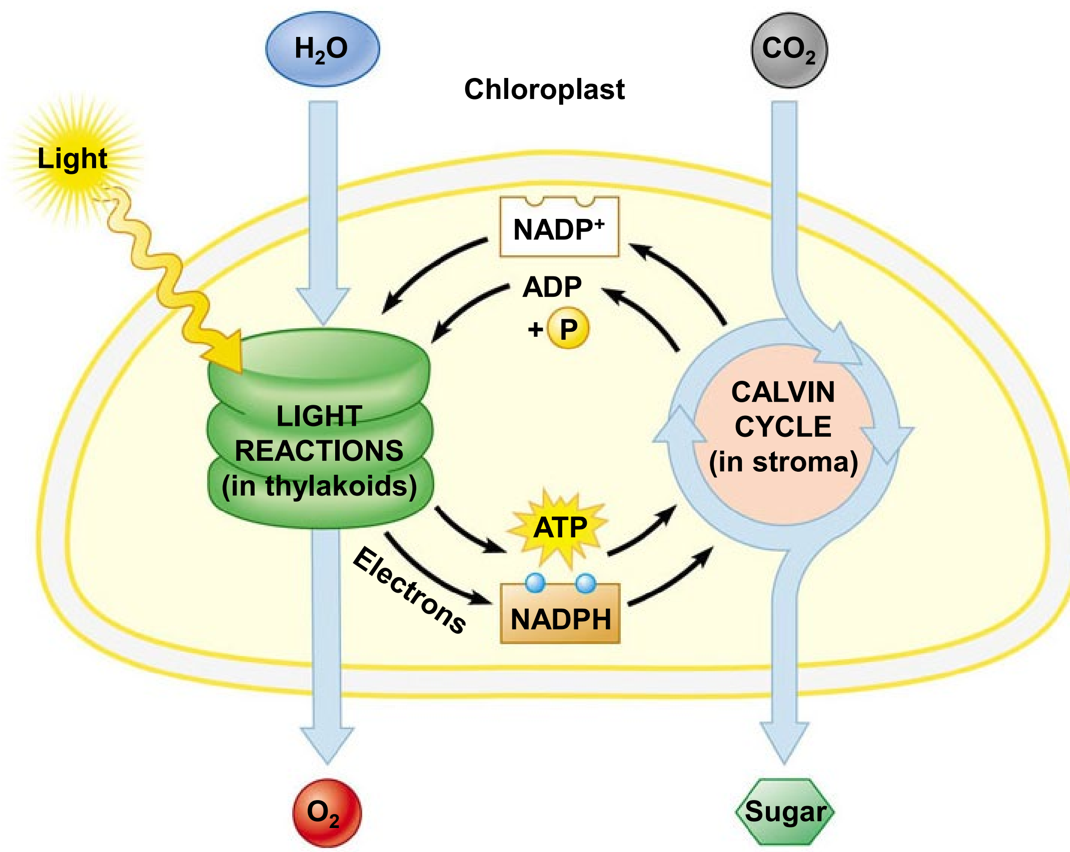
In reality, the products of photosynthesis include the formation of all of the biological macromolecules the organism requires. For example, photosynthetic organisms must have a source of nitrogen (e.g. fertilizer) to use photosynthetic products to make proteins and nucleic acids. You should also note that one of the *products* of photosynthesis is **oxygen**. Essentially all of the oxygen in our atmosphere comes from the process of photosynthesis.

The chemical reactions of photosynthesis actually occur in two distinct stages – **the Light Reactions and the Calvin Cycle**:

1) The **Light Reactions** convert light energy to energy contained in **ATP** and **NADPH** while producing O_2 as a byproduct.

2) The **Calvin Cycle** (also known as the **Light-independent or Dark Reactions**) which uses energy from ATP, hydrogen from NADPH, and carbon from CO2 to produce energy-rich glucose and other organic molecules.

Both of these processes occur in chloroplasts and are summarized in the illustration below.



The exercises you will carry out today involve detecting evidence of O_2 production and CO_2 consumption as indicators of the Light Reactions and the Calvin Cycle, respectively. Be sure to refer to the illustration above as needed to ensure that you relate each exercise to the corresponding stage of photosynthesis.

Part 1: Photosynthesis & CO_2 Consumption

You have learned that photosynthesis involves the conversion of carbon dioxide and water into organic molecules such as glucose during the Calvin Cycle. In doing so, oxygen gas is produced during the Light Reactions while carbon dioxide is consumed during the Calvin Cycle.

In the first experiment, we will be using a plant called *Java Moss*. The experimental design involves observing changes in the concentration of dissolved CO_2 revealed by changes in pH. The variables to be examined in relation to carbon dioxide are the amount of light exposure and the presence of plant material.

When CO_2 concentrations increase in aqueous solution, it causes an **increase** in the concentration of H^+ ions, thus **decreasing** the pH value. This occurs through the formation of an intermediary compound called **carbonic acid**, which forms by the combination of CO_2 and H_2O as shown here:

$$CO_2 + H_2O \;<=====>\; \underset{\text{carbonic acid}}{H_2CO_3} \;<======>\; H^+ + \underset{\text{bicarbonate}}{HCO_3^-}$$

Thus the pH indicator **phenol red** will be used to reflect the amount of CO_2 present in the vials. Phenol red is **orange** when slightly acidic and **yellow** when more strongly acidic. This occurs when the concentration of CO_2, and thus the concentration of carbonic acid, is high.

Below pH 7 (acidic)	Neutral pH	Above pH 7 (basic)
Higher CO_2 Level	--------------	**Lower CO_2 Level**
YELLOW or ORANGE	**RED**	**PINK**

The relationship between dissolved CO_2 and pH can be summarized as **"higher CO_2 concentrations result in higher H^+ concentrations and thus lower pH values"**. Conversely, **"lower CO_2 concentrations result in lower H^+ concentrations and thus higher pH values"**.

As you carry out the following experiment, keep in mind that plants, like animals, have mitochondria and carry out cellular respiration which produces CO_2 as a byproduct. So a plant that is ***not*** actively photosynthetic (i.e., in the dark) will still carry out cellular respiration and thus may cause a net increase in dissolved CO_2 causing a drop in pH and a color change toward yellow. A plant that *is* actively photosynthetic, however, will consume more CO_2 in the Calvin cycle than is produced by cellular respiration. So if photosynthesis is occurring there should be a net decrease in dissolved CO_2 and a corresponding increase in pH as shown by a color change toward red.

Exercise 1 – Observing Photosynthesis via CO_2 Consumption

1. Label three screw cap tubes **1**, **2** and **3** with a marker and line them up in order in a test tube rack.

2. Use a graduated cylinder to add 100 ml of tap water to a 250 ml Erlenmeyer flask, then add 5 ml of phenol red solution to the flask. Swirl to mix.

3. Take a clean straw and blow bubbles into the solution and stop once it turns **orange** (not yellow). *Note: orange will allow you to detect pH changes in either direction (i.e., more acidic or basic).*

4. Add CO_2 enriched phenol red solution from the previous step to each tube until almost full.

5. Place equal & generous amounts of *Java moss* in tubes 1 & 2. Tube #3 will not have any plant material.

6. Tightly screw the caps on each tube and record the color of the solution in each tube on your worksheet.

7. Wrap a piece of aluminum foil around tube #2, being sure to cover the bottom so that no light enters this tube.

The chart below summarizes the contents of each tube:

	Tube 1	Tube 2	Tube 3
CO_2 enriched phenol red solution	X	X	X
Java Moss	X	X	
Aluminum foil (to block light)		X	

8. Place each tube side by side in an outside row of a rack and put a 1 liter beaker filled with tap water in front of the tubes. Position your lamp so that the light shines through the beaker of water before reaching the tubes (this will avoid overheating the samples). Turn on the lamp and make sure the light path is not blocked by labels on the beaker or any parts of the rack.

NOTE: make sure nothing is blocking the light path to the tubes (e.g., label on beaker or panel on rack)

9. Record the **starting time** on your worksheet.

10. Write your hypothesis on your worksheet regarding which tube should show evidence of the most Calvin cycle activity, and identify the independent variable, dependent variable and control.

11. After at least 1 hour has passed, record the colors of each tube on your worksheet, analyze your data and answer the corresponding questions.

NOTE: As you examine your tubes, remember that photosynthesis, specifically the Calvin cycle, will <u>decrease</u> the concentration of dissolved CO_2, and cellular respiration will <u>increase</u> the concentration of dissolved CO_2.

Move on to the next experiment while this experiment continues…

Part 2: Photosynthesis & O_2 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 O_2. This is the result of splitting water during the Light Reactions of photosynthesis. Thus if you can detect gas production in plant material you can be confident that the gas is O_2, 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 oxygen gas production.

Exercise 2A – Detecting O_2 Production during the Light Reactions of Photosynthesis

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 75 or so discs from the leaves (enough for Exercises 2A & 2B). 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 (with spout on the neck) at your bench.
4. Add 0.2 % sodium bicarbonate ($NaHCO_3$) 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 hole 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".

   NOTE: Sometimes the vacuum pressure is quite weak. If are having trouble getting your flask to bubble, consult your instructor.

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% $NaHCO_3$.

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, cover with a one liter beaker filled with water*, and turn the light on

   **The beaker of water is essentially a heat filter preventing the lamp from increasing the temperature of your sample. Without this temperature would be a 2nd independent variable.*

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 O_2 production).

7. Record the results on your worksheet, graph the data (% floating vs source of light), and answer any associated questions on the worksheet.

Exercise 2B – 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.

NOTE: Due to limited supplies you can use the results for dishes A and C from Exercise 2A as negative and positive controls, respectively.

Before you leave, please make sure your table is clean, organized, and contains all supplies listed below so that the next lab will be ready to begin. Thank you!

Supply List

- Marker pen or China marker
- 3 Screw capped test tubes in rack
- 1 piece of aluminum foil (try to recycle)
- 250 ml Erlenmeyer flask
- 100 ml graduated cylinder
- Bottle of phenol red solution, pipette and pipette pump
- Three 1 liter beakers
- Cutting board
- Cork puncher
- 250 ml side armed flask with stopper and tubing
- 0.2% sodium bicarbonate ($NaHCO_3$) in liter container
- 6 glass petri dishes
- Forceps
- Wooden Applicator stick
- Set of colored filters

NOTE: Dispose of phenol red waste in the fume hood, rinse off Java moss with tap water and put back where you got it from, and rinse out the tubes with tap water.

Laboratory 8 worksheet – Photosynthesis

Name: ___________________________ **Group:** ________ **Date:**_______________

<u>Exercise 1</u> – Photosynthesis & CO_2 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**
Color at Start of Experiment			
Color at the End of the Experiment			
Change in pH (more acidic, more basic, no change)			
Change in [CO_2] (increase, decrease, no change)			

*Explain any color change in each tube with regard to **<u>photosynthesis</u>** (or **<u>cellular respiration</u>**):*

TUBE 1:

TUBE 2:

TUBE 3:

Did these results support your hypothesis? Explain.

Exercise 2A – Photosynthesis & O_2 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.

<u>Exercise 2B</u> – 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.