Biology 196 Laboratory
Photosynthesis

Laboratory Objectives
After completing this lab you should be able to:

- Write the chemical equation for photosynthesis; describe the origin and destination of the reactants and products.
- Within the *Elodea* leaf, identify major cell types, intracellular structures and molecules involved in photosynthesis.
- Quantitatively measure the production of the oxygen by-product and plot the data in graphical form.
- Use control groups and a colorimetric assay to reveal the role of carbon dioxide in photosynthesis

Photosynthesis[1]

Background
What would life on Earth be like without photosynthesis? It might resemble the surface of Mars, where no photosynthesis is known to occur. A world without photosynthesis would probably have more carbon dioxide and less oxygen in its atmosphere, as is the case on Mars. A vast array of life on Earth depends upon oxygen, and green plants are a primary food source for many living things, including humans. Without photosynthesis, the diversity of life on our planet would be very different—and potentially, nonexistent.

Photosynthesis is not just a simple process of oxygen production. It is an elegantly complex process that combines carbon dioxide, water, and energy from sunlight to produce food for green plants in the form of water, oxygen, and a single-sugar molecule called glucose. The molecule required for photosynthesis to occur is **chlorophyll**, a green pigment contained in plant cell organelles called **chloroplasts**. Chlorophyll absorbs light energy and ultimately converts this radiant energy into chemical energy. The simple **redox** equation for photosynthesis is:

$$6CO_2 + 12H_2O \xrightarrow[\textit{chlorophyll}]{\textit{light}} C_6H_{12}O_6 + 6H_2O + 6O_2$$

Photosynthesis involves a series of complex, interrelated reactions that can be grouped into two major categories. The first set of reactions is termed the **light reactions**. Here, light energy excites a chlorophyll molecule housed within a chloroplast. Through various interactions with water and other molecules during the light reactions, oxygen gas (O_2) is released. Other products of the light reaction move on to the next set of reactions, termed the **Calvin Cycle**. Here, these products and carbon dioxide (CO_2) from the environment interact to form **monosaccharides**, single-sugar molecules. The monosaccharides produced are glucose molecules and are utilized in other important cellular activities, such as cellular respiration.

Some parts of plants lack the ability to photosynthesize and are unable to produce glucose. These areas receive glucose from photosynthetic parts of the plant in the form of sucrose, a **disaccharide** formed from the joining of a glucose and fructose molecule. If there is an ample supply of glucose or sucrose in plant cells, they may form **polysaccharides** (made up of many bonded sugar molecules) in the form of **starch**. Starch can then be stored where needed and can be later utilized by the plant. For example, when photosynthesis is halted, such as when light or carbon dioxide is unavailable, the plant cells can break down the starch molecules into glucose monomers

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that can then act as fuel for cellular reactions. Starch may also be broken down into glucose molecules that can be transported to other plant cells.

During this activity, you will conduct three experiments to demonstrate the involvement of chlorophyll, light, oxygen (O_2), and carbon dioxide (CO_2) in photosynthesis. By performing a starch test on *Elodea* leaves, you will explore the importance of light and chlorophyll in photosynthesis and how these variables affect final production of carbohydrates within the plant. Through experimentation with *Elodea*, you will also measure oxygen production and observe carbon dioxide usage during photosynthesis.

Activity #1: Chlorophyll and Light

Materials:

- 2 Elodea leaves (1 from the dark and 1 from the light)
- 2 microscope slides
- coverslips
- permanent marker or glass marking pencil
- iodine-potassium iodide
- water and droppers
- paper towels or Kim wipes
- timer

Procedure:

Iodine-potassium iodide has the property of staining starch blue-black. If starch is not present, the iodine solution will remain yellow-brown. Iodine-potassium iodide will stain hands and clothing. Practice safe laboratory procedures when performing this activity, and you may consider wearing gloves.

1. Obtain two microscope slides from your drawer. Use a permanent marker to label one slide with an “L” and the other with a “D” in the lower right-hand corner.

2. Obtain two leaves. One leaf was incubated in the dark for an extended period (“D”) while the other was exposed to light (“L”). Both were then soaked in isopropyl alcohol for 24 hours to disrupt the **cell walls** of the plant’s cells.

3. Place each leaf on the corresponding labeled slide. Add 1 or 2 drops of water on each slide.

4. Place a coverslip over each leaf, and examine both leaves under the low and high (10-40x) power objectives of your microscope. Examine the central, **main vein** toward the base of each leaf.

5. In the Activity 1 question section, sketch a portion of each leaf as it appears under high magnification.

6. Remove coverslip and gently blot the leaves with a Kim wipe or paper towel.

7. Using the 7 mL pipet, place 2 drops of iodine-potassium iodide onto each leaf. Replace coverslip and return to your desk.

8. Wait 5-10 minutes and examine both leaves again under low and high power. Again, examine the central, main vein.

9. Sketch a portion of each leaf after staining under high power.

Activity #2: Oxygen

Materials:

- *Elodea*
- 2 test tubes
- 2 stoppers
- 2 2-mL pipets
- test tube rack
- sodium bicarbonate
- electronic balance
- weigh boats or weigh paper
- stirring rods
- graduated cylinder
- ruler
- permanent marker
- water
- timer

Procedure:

1. Measure three grams of **sodium bicarbonate** and add it to a clean, empty graduated cylinder. Add deionized water up to 100 mL, creating a 3% sodium bicarbonate solution. Stir until the sodium bicarbonate dissolves completely. If there are bubbles in the solution, continue stirring until they are released. Sodium bicarbonate is an additional source of carbon dioxide for the *Elodea*.

2. With a ruler and a marker, measure and mark 2.5 cm below the rim of both of your test tubes.

3. Obtain a 12-cm sprig of *Elodea* and add it to one test tube.

4. Fill both test tubes to the 2.5 cm mark with the 3% sodium bicarbonate solution.

5. Dip the tip of a 2-mL pipet into the sodium bicarbonate solution. Insert the tip of this pipet, which should retain a drop of water, into the hole at the top of the stopper. Push down to make a secure seal. The pipet tip should not push substantially through the end of the stopper.

6. Place the stopper into the mouth of the test tube. Slowly push down on the stopper with both thumbs The water drop in the pipet should rise. Remove your thumbs. After falling down the pipet slightly, the water drop should come to rest. If the water drop continues to fall, there is a leak, and you should carefully push the stopper farther into the tube.

7. Repeat steps 5 and 6 for the test tube without *Elodea*.

8. Place both test tubes under a bank of light or in sunlight. In Table 1 in the question section for Activity 2, record the initial level of the water drop in each pipet. As photosynthesis occurs, oxygen is produced and pushes the water drop up the pipet. Any change in the control tube is due to temperature or pressure changes or leakage.

9. Record the level of the water drops in each pipet every 10 minutes for 1 hour.

10. Answer the questions for Activity 2.

Activity #3: Carbon Dioxide

Materials:

- *Elodea*
- 2 vials with caps
- marker
- bromothymol blue solution
- light source
- paper towels
- timer

Procedure:

The chemical **bromothymol blue** is an indicator that appears blue in an alkaline (base) medium and yellow-green in an acid medium. CO_2 has been added to the bromothymol blue. Carbon dioxide (CO_2) in solution forms **carbonic acid** (H_2CO_3), turning the bromothymol yellow-green. If carbon dioxide is removed from the solution, the solution will change to dark green and then to blue. Bromothymol blue will stain your hands and clothing. Practice safe laboratory procedures when performing this activity, and consider wearing gloves.

1. With a permanent marker, write your initials (or group symbol) on both of the capped vials.

2. Obtain an 8-cm sprig of *Elodea* and place it in one of the vials provided.

3. Over a paper towel, fill both vials to overflowing with the yellow-green bromothymol blue solution and cap tightly. Take care not to spill on your hands and thoroughly clean up any spills.

4. Place both vials in sunlight or strong light source.

5. Wait one hour, and then answer the questions for Activity 3.

Photosynthesis Laboratory Questions

Questions for Activity 1: Chlorophyll and Light

1. Sketch a section of each leaf, before and after staining with iodine-potassium iodide, on high power. Include the central, main vein.

***Before* adding iodine-potassium iodide:**

"Light" Leaf

"Dark" Leaf

***After* adding iodine-potassium iodide:**

"Light" Leaf

"Dark" Leaf

2. What did this experiment reveal about the role of light and chlorophyll in the process of photosynthesis?

3. Some plant cells do not contain chloroplasts or chlorophyll. Give a couple examples of these cells and explain why they may not possess these photosynthetic pigments.

Questions for Activity 2: Oxygen

1. As you monitor the oxygen levels, record your data in Table 1. To calculate the change in volume, subtract the recorded pipet measurement from the initial pipet measurement. To calculate the corrected change in volume, subtract the change in volume without *Elodea* from the change in volume with *Elodea*. The corrected change in volume is the adjusted oxygen production for *Elodea*.

Time	Pipet Measurement with *Elodea* (mL)	Change in Volume with *Elodea* (mL)	Pipet Measurement without *Elodea* (mL)	Change in Volume without *Elodea* (mL)	Corrected Change in Volume (mL)
Initial		0.00		0.00	0.00
10 min					
20 min					
30 min					
40 min					
50 min					
60 min					

2. On the next page, graph the adjusted production for *Elodea* (corrected change in volume). Plot the independent and dependent variables on the correct axes. Include a title and supply the following information:

   a. The independent variable is: ________________________________.

   b. The dependent variable is: _________________________________.

3. Briefly describe an experiment that allows you to study the influence of some environmental variable on the rate of oxygen production in *Elodea*. State your hypothesis for your experiment.

Title: ______________________________________

Questions for Activity 3: Carbon Dioxide

1. Compare the color of the two solutions. What happened?

2. What does this reveal about the role of carbon dioxide in photosynthesis?

3. What would you have expected to happen if the vials were instead placed in a dark room at the beginning of the experiment? Explain your answer.

4. What is the purpose of the vial that does not contain *Elodea*?

Study Checklist

To perform well on the quiz, you need to have a thorough knowledge of the following:

- You should know and understand all of the terms which appear in **boldface** type.
- Use your textbook to provide a more full understanding of the photosynthetic light reactions and Calvin Cycle.
- What are redox reactions, and which components of the photosynthetic reaction are oxidized/reduced?
- What are the major structures involved in photosynthesis in the plant tissue, and within the cells?
- Describe the origin and destination of the molecules of O_2 and CO_2.
- What happens to CO_2 when dissolved in water that allows the use of bromothymol blue as an indicator of its presence?
- Why do you need to assay negative control samples as well as your experimental samples?