

Biology 3A Lab 8 Photosynthesis

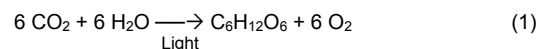
PLEASE BRING A USB THUMBDRIVE TO DOWNLOAD YOUR DATA

Objectives

- To observe the spectral absorbance of a mixed chlorophyll sample
- To separate and identify several common plant pigments using paper chromatography
- To investigate the rate of oxygen production during photosynthesis
- To measure the effect of wavelength of light on photosynthetic rate
- To investigate starch production and storage in a single leaf

Introduction

Photosynthesis can be summarized in a fairly simple equation:



This reaction actually occurs in two phases. First, light energy from the sun is captured by the pigment chlorophyll. This first step is often called the light reaction. The next phase does not require light. In this phase carbon (from carbon dioxide) is fixed into glucose. Both of these reactions occur in the chloroplast of plant cells.

In the chloroplast, the chlorophyll is found in the membranes of the grana. When struck by photon of the appropriate wavelength the chlorophyll gives up outer shell electrons to the electron transport chain located in the thylacoid membrane of the chloroplast. The energy in these electrons is used to drive the reaction



by chemiosmosis. In addition high-energy electron are also transferred to NADP, an electron carrier, to create NADPH. The byproduct of this set of reactions is oxygen gas, released from the leaf stomates into the atmosphere.

The ATP and the NADPH produced in the light reaction are used as energy sources to drive reactions that build macromolecules. In these reactions, called the Calvin Cycle (or dark reactions), carbon dioxide is fixed into organic molecules using the energy from ATP and the hydrogen atoms from NADPH. In most plants, the product of the Calvin Cycle is glyceradldehyde-3-phosphate, which is converted into many compounds including glucose and its polymers, starch and cellulose.

Important Note on Timing in This Lab

In this lab several steps require measured time periods. Unless you are using two lab periods, you must set up some procedures, then complete other procedures, and then return to the original procedures. Plan your time carefully. *We expect to complete the entire lab in one period.* Here is a suggested time line for the lab

1. Set up and begin exposure of the photosynthetic "photograph."
2. Separate plant pigments and set up the paper chromatograms
3. Run the chlorophyll spectral analysis
4. Read the paper chromatograms
5. Develop the photosynthetic "photograph"
6. Set up and run the oxygen production by spinach measurements
7. Set up and run the manometer experiment (effect of wavelength on Ps)

A. Photosynthetic "Photography"

As pointed out above, in the light dependent reactions of photosynthesis, photon energy is absorbed by pigment molecules and transferred to a reactive chlorophyll *a* molecule. In order to illustrate light as a reactant for photosynthesis, you will use geranium leaves that have been kept in the dark for two days prior to the lab (in order to deplete carbohydrate stores). Photosynthetic rates should vary as a function of light intensity, thus the production and storage of starch should also vary as a function of light. In fact, the rate may vary over the surface of a single leaf, where one part of the leaf is exposed to more light than another! In the macromolecules lab you learned the iodine test for starch. Today we'll apply that method to a whole leaf subjected to different light intensities.

Exposure Procedure:

1. Soak a piece of black cloth in a 0.1M sodium bicarbonate solution.
2. Sandwich 1 leaf with petiole (stalk) and saturated cloth between 2 glass plates making sure the leaf is flat.
3. On the outside of the glass, on the leaf side, place the negative of the photograph. Secure the entire "sandwich" with rubber bands near the edges of the glass plates. Place this on top of a small beaker of lukewarm water so that the petiole at the bottom sits well under the water surface.
4. Use the slide projector to create bright light to expose the image onto the leaf for **1 hour**.

Development Procedure:

1. After at least one hour, carefully remove the leaf from the glass plates. Place the leaf in boiling 70% alcohol for three to five minutes to leach out the pigments. The treated leaf should be brownish-white in color.
2. Place leaf in boiling water for 3 minutes to rinse.
3. Place leaf in a Petri dish and cover with iodine. It takes at least 2 – 3 minutes to develop, turn the leaf over to see the image.

D. Wavelength and Photosynthetic Rate

Thus far we have investigated the increase in the rate of photosynthesis as a function of light intensity. However, from our spectral analysis it is clear that wavelength of incident radiation will also affect photosynthetic rate. In this experiment we will estimate the photosynthetic rate by measuring the rate of oxygen production of the plant. It should be noted, however, that this is not an exact measure of photosynthetic rate because some of the oxygen produced used by the plant cells is used in the process of aerobic respiration.

Procedures:

GLX Setup

1. Plug the end of the Dissolved Oxygen probe cable into the connector on the top of the PASPORT Dissolved Oxygen Sensor.
2. Connect the Dissolved Oxygen Sensor into Port 1 on the top of the Xplorer GLX (Figure 2).
3. The Graph Screen will automatically open with Dissolved Oxygen (mg/L) versus Time (s).



Figure 1. Dissolved O₂ probe & DO sensor



Figure 2: Connect sensor to GLX


Equipment Setup

1. NOTE: Student groups in the front of the class will shine the light towards the whiteboard. Students in the back of the class will shine the light towards the back wall (where the microscope cabinet is located). This is to ensure that another groups light sources does not have an effect on your data. NOTE: The overhead room lights also need to be turned off.
2. Place the photosynthesis tank on the magnetic stirrer (Figure 3) and attach the light source to a ring stand.
3. Mix 4 drops of green food coloring with 1200 mL of DI water. Place the green colored water into the outer chamber of the tank. The water helps to maintain the temperature of the inner tank and will only allow a particular wavelength of light to be absorbed by the plant
4. Place the light meter into the inner chamber making sure that light sensor is facing the light source and is not tilted upward or downward. You can wedge some paper towels to make the light sensor stay vertical.
5. Turn on the light and adjust the distance so that the light meter reads 1000 lux.
6. Put a stir bar into the photosynthesis tank that contains 325 mL of 0.1 M NaHCO₃ solution and the 12 cm sprig of *Elodea* plant. Put the large two-hole stopper into the top of the tank.
7. Remove the storage bottle from the end of the Dissolved Oxygen (DO) probe and *carefully* insert the end of the probe through the larger opening in the two-hole stopper.
8. Make sure that the metal band on the Dissolved Oxygen probe is below the surface of the water in the tank and that no air bubbles are trapped on the end of the probe.
9. Put a #3 rubber stopper into the other hole in the two-hole stopper.
10. Allow the plant to stabilize for 5 minutes (There is not set distance for each different color of light. The distances are based upon the illuminance in lux. Please see the instructor if you have any questions).



Figure 3: Setup

Record Data

1. Turn on the magnetic stirrer to a moderate speed so the water circulates in the tank.
2. After the 5 minute stabilization, press the Start key  on the GLX and record data for 10 minutes.
3. After 10 minutes, turn the lamp off and carefully change the green colored water to red by removing. Yes, you will need to remove the stir bar, *Elodea* and water.
4. Mix 7 drops of red food coloring with 1200 mL of DI water. Place the red colored water into the outer chamber of the tank. The water helps to maintain the temperature of the inner tank and will only allow a particular wavelength of light to be absorbed by the plant
5. Place the light meter into the inner chamber making sure that light sensor is facing the light source and is not tilted upward or downward.

6. Turn on the light and adjust the distance so that the light meter reads 1000 lux.
7. Put a stir bar into the photosynthesis tank that contains 325 mL of 0.1 M NaHCO₃ solution and the 12 cm sprig of *Elodea* plant. Put the large two-hole stopper into the top of the tank.
8. With the light on, allow the plant to stabilize to the red colored solution for 5 minutes. Record data with the plant in behind for 10 minutes.
9. After 10 minutes, turn the lamp off and carefully change the red colored water to blue as before.
10. Mix 7 drops of blue food coloring with 1200 mL of DI water and complete the same procedures as #4 above for the blue dyed water.
11. After 10 minutes, turn the lamp off and carefully change the blue colored water to DI water. Do not pour out the NaHCO₃ in the inner chamber. Remove the DO probe and use the other #3 stopper.
12. Place the light meter into the inner chamber making sure that light sensor is facing the light source and is not tilted upward or downward.
13. Turn on the light and adjust the distance so that the light meter reads 1000 lux.
14. With the light on, allow the plant to stabilize to the DI water solution for 5 minutes. Record data with the plant in behind for 10 minutes. Turn off the magnetic stirrer.

PLACE YOUR DATA ON THE COMPUTER

1. Collect all class data (O₂ production for green, red, blue and white light). Using Excel, graph the effects of wavelength on photosynthetic rate.
2. Your instructor will provide absorbance spectra for each of the colored water. Create another graph in which you overlay the chlorophyll spectral absorbance data (from Part B2) with the photosynthetic rates at three different wavelengths (from Part D).
3. Clean up the equipment as instructed.
4. Do not let the magnetic stir bars go down the sink!
5. Wash and dry the photosynthesis tank.

A. Photosynthetic Photography

1. Do you see the image in the leaf? Is the image a positive or negative of the original? Explain your answer to this question.

D. Wavelength and Photosynthetic Rate

| Total O ₂ Production (mg/L) | Color of Light | | | | |
|--|----------------|-----|------|-------|------|
| | Green | Red | Blue | White | Dark |
| Initial O ₂ | | | | | |
| Final O ₂ | | | | | |
| Change in O ₂ | | | | | |

10. In general, what is the effect of wavelength on the rate of oxygen production? Include this in the figure caption for #11C.

11. Using the whole class data set (available on the web) create an Excel graph that relates light color to oxygen production and wavelength.

- First let's express oxygen production as a rate. Divide the **mean** (from all sections) oxygen production for each colored water by the total time for the experiment, 10 minutes. What are the units for this rate?
- Now, examine the spectral analyses provided for each of the colored water used in this experiment. For each color choose a major transmitted wavelength range downloaded from the website.
- Finally, create an Excel graph in which you show the absorbance spectrum of a typical green plant pigment extract (from exercise B2 above) and the rate of oxygen production vs. wavelength.

Suggestions for 11C above:

- White light and darkness don't fall on this graph, so do not use those data
- Graph the data from B2 using a line graph (Wavelength will be on the x-axis and on one y-axis place the absorbance values
- Use Oxygen Production in ml/min which you calculated in 11a.
 - On the other y-axis place the mean oxygen production values calculated from 11a.
 - You will need to have these three values at a particular wavelength associated with the wavelengths from B2 in order to be able to graph them correctly. Please take notes on how to do this.
 - You will fit the three bars to the oxygen production