

Physiological Dynamics in Animals and Plants - Laboratory 2 - Measurement of the Light Dependence of Photosynthesis

Introduction:

This laboratory is a modified version of an exercise developed by Dr. Diane C. Robertson, Biology Department, Grinnell College, Grinnell, Iowa 50112. All of the exercises developed by Dr. Robertson are available at:

<http://www.grinnell.edu/courses/bio/qubitmanual/>

Today's physiological phenomenon – The “light reactions” of photosynthesis:

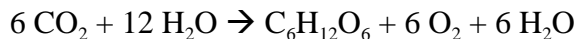
Photosynthesis is the single most important autotrophic (self-feeding) mechanism by which organisms, terrestrial and aquatic, capture solar energy from outside the earth and convert it to a form that provides the metabolic energy they need to survive, grow, and reproduce. In photosynthesis, radiant light energy is converted to chemical energy that is then used to synthesize sugars that are the food for plants and all other organisms that depend on plants to produce their food for them (heterotrophs).

A green pigment, chlorophyll, is the central light-absorbing pigment that makes this energy interconversion possible. Along with various other accessory pigments, chlorophyll is harbored in the chloroplasts of cells in the aerial portions of plants. Most chlorophyll is found in leaves, organs that are evolved to capture light.

In the “light reactions” of photosynthesis, light strikes these pigments and all of the absorbed energy is funneled to a special subset of chlorophyll molecules. This energy transfer causes the ejection of electrons from certain atoms in the chlorophyll molecules (the chlorophyll becomes oxidized). The ejected electrons are passed sequentially through a series of chloroplast proteins, reducing one, and then the next, and then the next, etc. The electrons ultimately end up reducing a compound called nicotinamide adenine dinucleotide phosphate (NADP⁺). Since the addition of an electron to the one proton that confers the + charge on this compound produces a hydrogen atom, the reduced compound = NADPH. Energy from light-driven electron flow is also captured to drive the synthesis of adenosine triphosphate (ATP) by a membrane-dependent mechanism called chemiosmosis.

In the “dark reactions” of photosynthesis, electrons from NADPH and energy from ATP breakdown are used, respectively, to reduce other small organic compounds (metabolites) and form covalent bonds between their carbons. The carbon atoms of these metabolites come originally from carbon dioxide (CO₂) that plants capture out of “thin air”; air is only 0.0365% CO₂. The products of the dark reactions are simple 6-carbon sugars like glucose and fructose. Glucose and fructose can be covalently joined to form sucrose, common table sugar.

Although it is a gross oversimplification, most textbooks reduce photosynthesis to the following chemical equation:



Note that photosynthesis consumes CO_2 , but it liberates O_2 ; thus, changes in concentrations of either gas over time can be used to measure rates of photosynthesis. Today, we will measure rates of O_2 liberation from illuminated leaves.

Where does the O_2 liberated in the light reactions come from? If the electrons ejected from chlorophyll were not replaced, photosynthesis would eventually stop because the light reactions would cease. The electrons lost initially from chlorophyll are replaced with electrons from water. During the light reactions, water is “split” - $2 \text{H}_2\text{O} \rightarrow 4 \text{H}^+ + 4 \text{e}^- + \text{O}_2$. The electrons from water are used to reduce NADP^+ ; the protons are used in chemiosmotic ATP synthesis; and the O_2 is liberated into the air. All aerobic organisms, including you, me, and the plants, require O_2 for our cellular respiration. Thanks to photosynthesis, the concentration of O_2 in air is about 20.7%.

Measure the “light reactions” of photosynthesis:

Demonstrate to yourself that leaves produce O_2 at rates that are in direct proportion to the intensity of light to which they are exposed. At some point your leaf may reach a “light saturation point” above which more light will not increase the rate any further. At that point, photosynthesis is limited either by the ability of the light to convert the light energy it absorbs to chemical energy or by the supply of some other factor required for photosynthesis, such as CO_2 . You should also determine whether altering the wavelength (color) of light has an effect on the rate of water splitting.

Step 1 - Read: Visit <http://www.grinnell.edu/courses/bio/qubitmanual/> and read the “Overview” and “General Setup” sections of the exercise entitled “Measurement of Photosynthesis using O_2 sensor.”

Step 2 - Do: Do the following:

Set up the instruments:

- 1) Plug the USB cable from the Vernier LabPro into the USB port nearest to you on the left side of the iBook.
- 2) Turn on the multi-outlet power strip.
- 3) Plug in the iBook; turn it on. (NOTE: *The USB cable from the LabPro must be plugged in before the iBook is turned on; otherwise, the computer will not “see” that the LabPro is plugged into the USB port.*)
- 4) Cancel or quit any start up programs (e.g. Norton Antivirus).

- 5) When the desktop screen appears, launch Logger Pro by double-clicking on the "Logger Pro alias" icon on the desktop. If you get this message - "Cannot find the preferred experiment folder. Using default experiment folder." - hit OK.
- 6) Pull down the "Setup" menu and choose "Sensors."
- 7) Click on the Icon for CH1; Use the pull down menus that pop up to set channel 1 to "O₂ Gas Sensor"; set calibration to "O2gas20."
- 8) Click on the Icon for CH2; set channel 2 to "Light Sensor"; calibration = "W_300qu"; hit OK.
- 9) Pull down the "View" menu and select "Graph Layout"; select "Two panes"; hit OK.
- 10) On the upper graph, click on "Mixed labels"; leave the Oxygen box checked; uncheck the "Med White" box and any other boxes; click OK.
- 11) On the lower graph, click on "Mixed labels"; leave the "Med White" box checked; uncheck the Oxygen box and any other boxes; click OK - the upper graph will display oxygen, the lower one, light intensity.
- 12) Pull down the Setup menu and choose "Data Collection"; choose "Sampling"; set the "Time Units" to minutes; set the "Experiment Length" to 120 minutes; set the "Sampling Speed" to 6 samples per minute; click OK.
- 13) Click on the "Y" axis of the top graph; set the "Y-axis scale" to a minimum of 16 and a maximum of 22; hit OK.

(NOTE: If your data go off scale, these values can be changed during data collection by this same procedure without stopping the sampling; the arrows at the top and bottom of the Y axis label can also be used to "slide" the scale if your data go off scale. As an alternative you can try "Autoscale")

- 14) Click on the "Y" axis of the bottom graph; set the "Y-axis scale" to a minimum of 0 and a maximum of 300
- 15) You are ready to collect data.

Experimental:

- 1) With the light off, seal a leaf inside the chamber so that no part of the leaf is shaded. Adjust the position of the leaf so that the top leaf chamber is not completely sealed off from the bottom leaf chamber. It does not matter if the leaf is too large to be fully sealed within the chamber. The excess may protrude out of

the chamber without affecting your results. When you seal the chamber, turn the thumb-screws finger tight only; don't crush the leaf!

- 2) Place a beaker with 200 mL of water on top of the chamber so that it covers the major part of the leaf area. This serves as a heat filter; the water should be changed every 10-15 minutes (have a fresh beaker ready) to prevent heating (burning) the leaf. Position the light above the beaker so that its bottom edge is 11 cm from the top of the leaf chamber.
- 3) Click on the "Collect" button at the top of the screen. The button will change to a "Stop" button and data will begin to appear on the two graphs on the screen and as numerals on the bottom of the screen. The initial O_2 should be around 20.7%; if it is more than 1% different, use a screwdriver to adjust the gain on the amplifier box to calibrate it - you can watch the concentration change on the bottom of the screen.
- 4) Using the tubing provided, flush the chamber gently, but completely, with your breath; quickly plug the top and bottom chamber ports. Depending on your metabolic condition, the O_2 concentration should drop to between 16 and 18%. Wait until this value stabilizes, then turn on the light to full intensity and record the irradiance, reading its value at the bottom of the screen.
- 5) There will be little change in the O_2 reading for the first 5-15 minutes of illumination. This corresponds to the "induction period" for photosynthesis during which photosynthetic metabolites are synthesized until they reach critical pool sizes required for photosynthesis to occur. It also takes some time for stomata to open. Once this has been achieved, the partial pressure of O_2 (pO_2) in the chamber will increase as O_2 is released by photosynthetic water splitting. After the photosynthetic induction period, the pO_2 will increase slowly at first and then will increase linearly.
- 6) After 5-10 minutes of linear data collection, turn the light off. Breathe into the chamber again and wait for the O_2 level to stabilize at or near the original baseline. Then turn on the light and adjust it to 80% of the initial maximum value; record another 5-10 minutes of data.
- 7) Repeat step 6 at 60, 40, 20, 10, and 0 % of the initial light intensity.
- 8) To alter the wavelength (color) of light, flush the chamber again with your breath. Start an experiment at full intensity of white light, then alter the wavelength at maximum intensity by placing different colored filters over the leaf chamber, under the beaker of water. Allow each reading to proceed until a uniform rate is established, usually about 5 minutes. Between filters, turn off the light and change the water.

- 9) After you have made all of your measurements, click on "Stop" and SAVE YOUR FILE using "Save as..." in the File menu. Give your data an appropriate name and save it to the desktop or your own data folder.
- 10) Remove the beaker of water, turn off the light. Place the acetate grid on the surface of the chamber so that it covers the leaf and count the number of interstices completely enclosed by the leaf area. Any interstices falling exactly on the leaf margin should be given a value of 0.5. Sum the results and divide the total by 4. The value you obtain is the leaf area in cm^2 .

Step 3 - Analyze data:

The O_2 sensor measures only the partial pressure of O_2 (pO_2) in the chamber; it does not measure the *rate* at which O_2 is produced. To measure the rate of photosynthesis in your experiment, you will need to measure the increase in pO_2 as a function of time. This can be measured with LoggerPro as % O_2 produced per minute.

- 1) Open the data file from your experiment.
- 2) Open the "Analyze" menu and select "Examine." A vertical line will appear on your graphs. You can move the line along the data points on the graph by moving the mouse. As you move the vertical line, the numerical display in the box on the screen will change to show you the exact O_2 concentration, time, and/or light intensity at the point on the graph where the line is situated.
- 3) To measure photosynthetic rate at any given light intensity, move the vertical line to the point on your O_2 data where you wish to start the measurement of the slope of the line. Click on the mouse button and hold it down. Move the mouse over the part of the data you wish to analyze, and then release the mouse button, capturing the portion to be analyzed inside the box that appears.
- 4) Select "Linear Fit" from the Analyze menu. In the command box on the screen you will see the equation for a straight line, $y = mx + b$, along with values for m and b . The value for m , the slope of the line, is the rate of O_2 production in units of % O_2 produced per minute. Record this value. Close the box on the screen by clicking in the upper right hand corner.
- 5) Measure photosynthetic rate at the next light intensity by moving the vertical line to the linear part of the next data set. Select the next area of data to be analyzed by clicking and dragging with the mouse. Again, select Linear Fit from the Analyze menu - record the value in the data table.
- 6) Repeat the procedure for all of the light intensities and wavelengths that you tested. You now have all of the data you need to do your calculations.

Step 4 - Calculate:

Each m (slope) is the rate of increase in O_2 concentration in the chamber with time in units of % O_2 produced per minute. However, these are not standard international units, nor would they enable scientists to compare your rates to those observed for different plants or different leaves of the same plant because your data have not been "normalized" to leaf area or mass. By convention, plant scientists have agreed to express rates of photosynthesis as the number of micromoles (μmol) of O_2 produced per square meter of leaf area each second ($\mu\text{mol m}^{-2} \text{s}^{-1}$). To convert, do the following:

Each 1% O_2 in the chamber = 10,000 parts per million (ppm) = 10,000 microliters (μL) per liter of gas in the chamber. Therefore, multiplying m , % O_2 /minute, by 10,000 $\mu\text{L}/\text{liter}/\text{percent}$ = the number of μL of O_2 produced per liter of air, per minute (**A**).

At standard temperature and pressure (STP), 1 micromole (μmol) of any gas occupies 22.413 microliters (μL) of volume. At the temperature (T in $^{\circ}\text{C}$) of the lab (= 23°C), dividing the number of μL of O_2 produced per liter per minute, (**A**), by $\{(273 + T)/273\} \times 22.413 \mu\text{L}/\mu\text{mole } O_2$ = the number of μmoles of O_2 per liter produced/min (**B**).

Multiplying (**B**) by the volume of the leaf chamber, which is 0.009 liters (L), yields a volume-independent rate in $\mu\text{mol } O_2$ produced per minute = (**C**).

Dividing **C** by the leaf area in square meters (NOT SQUARE CENTIMETERS - 1 meter = 100 centimeters) gives the $\mu\text{mol } O_2$ produced per minute per square meter of leaf area = (**D**).

Finally, dividing **D** by 60 seconds per minute gives **E**, the photosynthetic rate in μmoles of O_2 produced per square meter of leaf area each second. The abbreviated units are $\mu\text{mol}/\text{m}^2/\text{s}$ or, preferably, $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Do these conversions for all of your slopes.

Step 5 - Report:

Write report - organize it as follows:

On the first one or two pages, neatly show all of your calculations from **Step 4**, canceling units; turn in a copy of your data graphs with the slopes annotated.

Provide a *computer-generated* graph of your light intensity data with photosynthetic rates in $\mu\text{mol m}^{-2} \text{s}^{-1}$ on the Y axis (ordinate) and light intensity ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$) on the X axis (abscissa). Label both axes clearly and correctly with proper units.

Present your wavelength data in a table similar to the one below.

Answer the questions below.

Color of Filter	$m = \% \text{O}_2 \text{ min}^{-1}$	Photosynthetic Rate ($\mu\text{mol m}^{-2} \text{ s}^{-1}$)
None		
Red		
Blue		
Green		

Questions:

- 1) Was there a light intensity beyond which rates of oxygen production did not increase? If so, this is the light saturation point for photosynthesis. If not, why do you think your leaves did not reach the light saturation point? Describe how you would design an experiment to test your hypothesis.
- 2) When you used colored light filters, was the intensity of light coming through each different filter the same? If not, how has this affected your conclusion? If the intensities were the same, does this mean that the amount of *energy* coming through each filter was the same? Why or why not? In light of your answer(s), describe how you would re-design your experiment to address these questions.