

Photosynthesis

In the next three weeks, you will explore photosynthesis, the series of chemical reactions by which plants and other organisms extract energy from sunlight and convert it into carbohydrates such as glucose. Photosynthesis is thus the complement to respiration.

Objectives

1. You should learn the fundamental principles of photosynthesis. These include the functions and activities of the light-dependent and light-independent reactions of photosynthesis, and some of the ecological and physiological factors that affect the rate of photosynthesis.
2. You will learn how data logging sensors and computer software can be used to perform real-time measurements of photosynthesis.
3. You will further develop your ability to design investigations and present and interpret scientific results.

Synopsis of activities

Week 1: You will learn how to perform real-time data logging to measure oxygen evolution by leaves. You will learn how to calculate photosynthetic rates.

Week 2: Each group will study the effect of irradiance (light level) on the rate of photosynthesis and prepare a photosynthesis “light response curve.”

Week 3: You will be expected to come to class with your results calculated and graphed. The class will discuss and interpret results.

The lab report

You will prepare a lab report for this lab exercise. The lab report will include the Introduction, Procedures, Results, Discussion and Literature Cited sections. Further guidelines concerning the lab report are presented at the end of this lab.

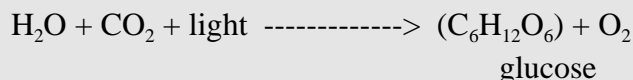
Photosynthesis Fundamentals

PHOTOSYNTHESIS is the process by which certain organisms obtain energy from sunlight, and carbon from carbon dioxide (CO₂) in the atmosphere. Plants use this energy and carbon in making the sugar glucose. Because of this capability, photosynthetic organisms are said to be AUTOTROPHIC ("auto" = self, "trophe" = food; make their own food).

The advent of photosynthesis possibly had a greater impact on life on Earth than any other evolutionary innovation. Photosynthetic organisms provide the food that supports all animal life, either directly or indirectly. Animals are therefore said to be HETEROTROPHIC ("hetero" = other; i.e. obtain food from others). However, the release of OXYGEN gas (O₂), an unwanted byproduct of photosynthesis, possibly had even greater repercussions. Over a period of a few billion years, oxygen released by photosynthetic organisms increased the oxygen in the Earth's atmosphere from essentially zero to its present day 21% oxygen content. The presence of oxygen made possible cellular respiration, the oxygen-requiring process of digesting glucose. All higher organisms, both plant and animal, depend upon cellular respiration, and thus could not have evolved if oxygen-releasing photosynthesis did not occur.

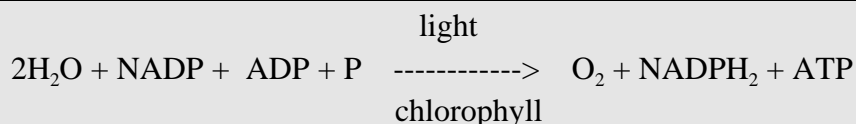
During photosynthesis, light is used to convert CO₂ and H₂O into glucose (C₆H₁₂O₆); glucose molecules can later be bonded together to form starch.

Overall reaction of photosynthesis:



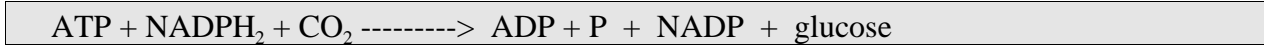
The process of photosynthesis actually occurs in two stages, called the light-dependent reactions and light-independent reactions (or 'carbon fixation'). During the **light-dependent reactions**, energy from sunlight is trapped and used to make: (a) ATP (Adenosine Triphosphate) by adding a phosphate onto ADP (Adenosine Diphosphate) and (b) NADPH₂ by transferring electrons (and hydrogens) to NADP. During the light-dependent reactions, water molecules (H₂O) are broken apart (releasing electrons and hydrogen ions) and the oxygen is released as a gas. The energy stored in ATP and the electrons stored in NADPH₂ are used for the process of carbon fixation.

The overall light-dependent reaction:



You should remember that the function of the light reaction is to collect energy from sunlight and to store it in ATP and NADPH₂, and that oxygen is merely given off as an unwanted byproduct of the reaction.

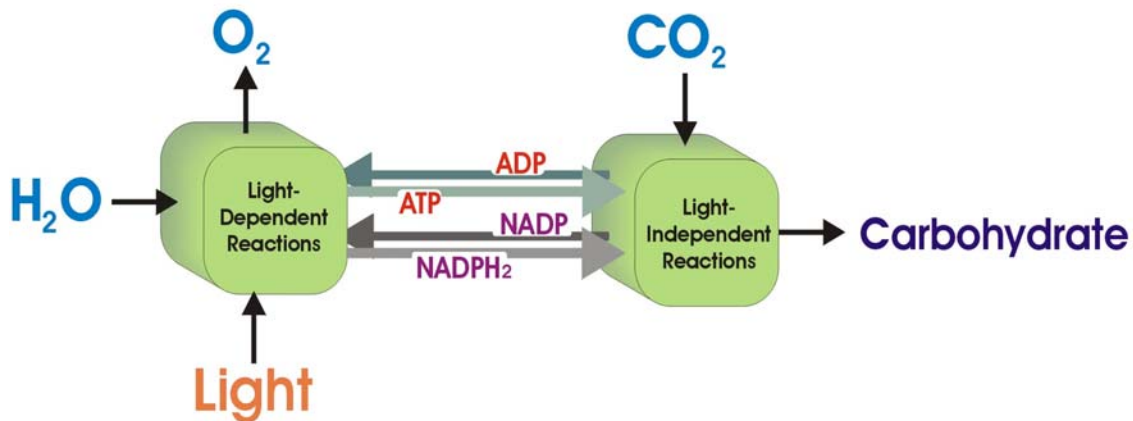
Carbon fixation occurs in the second process of photosynthesis, the **light-independent reaction**. This is the process through which CO_2 is converted into carbohydrates such as glucose and starch. Although light is not used directly by this process, energy (from ATP) and electrons (from NADPH_2) stored by the light-dependent reactions are required. Notice that light is not used directly by this process. The overall light-independent reaction is as follows:



Glucose that is produced during photosynthesis later can be broken down to provide energy and raw materials. It is broken down by the same process of cellular respiration that occurs in yeast and animal cells.

The light-dependent and light-independent reactions are interdependent –one cannot occur without the other within the plant (see Figure 1). Thus, despite the common misconception, the light-independent reactions will not occur in the dark because they must use ATP and NADPH_2 provided by the light-dependent reactions.

Figure 1. Interdependence of the light-dependent and light-independent reactions.



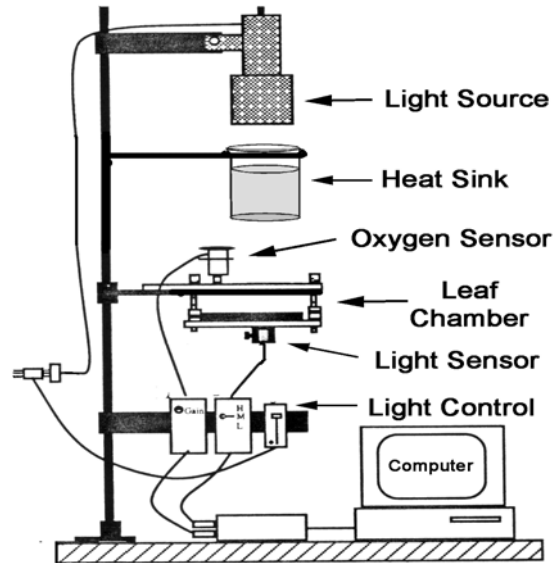
Measuring photosynthesis

How can the rate of photosynthesis be measured? To measure the rate of any process a biologist has two choices: either measure the disappearance of the reactants (in this case H_2O or CO_2) or the formation of the products (O_2 and glucose). For photosynthesis, it is not feasible to measure H_2O utilization, since the change is infinitesimal in proportion to the total amount of water in the cell. One could measure the quantity of glucose or starch produced, but this requires destruction of the leaf to extract these substances, which precludes taking multiple measurements of the same leaf over time. When continuous measurements of photosynthesis in a functioning system are desired, measurements of O_2 or CO_2 are usually performed. Since these gases are released into the surrounding atmosphere, monitoring their release or uptake from the leaf provides a measure of the rate of the photosynthesis process.

For most plants under normal conditions the amount of CO_2 in air (0.035%) “limits” the rate of photosynthesis. This means that availability of CO_2 is the proverbial “bottleneck” that slows the entire process, and therefore, increasing CO_2 concentration will increase the rate of photosynthesis. Thus, for this exercise we will use air that is “enriched” in CO_2 to allow for faster rates of photosynthesis. CO_2 enriched air will be obtained as human breath exhaled into a plastic bag.

Needless to say, obtaining rapid, accurate, quantitative measurements of these colorless, odorless gases requires rather sophisticated analytical equipment. In this investigation, you will measure photosynthesis using an apparatus (Figure 2) that allows measurement of oxygen. The system operates with a leaf sealed into an transparent, airtight chamber. Light shines onto the leaf from above, and the relative light intensity is measured with a sensor below the leaf. An oxygen sensor coupled to the chamber takes continuous measurements of the oxygen concentration. The light and oxygen sensors send data to a computer with software that records continuous, real-time, measurements of O_2 levels. Notice the presence of a beaker of water that serves as a 'heat sink' to absorb heat from the light source.

Figure 2. The photosynthesis apparatus.



Week 1 Procedures

The use of the photosynthesis apparatus will be demonstrated during the class period. An overview of the basic steps are described below.

WARNING: *The oxygen sensor is delicate and expensive!! Always remove the plug from the gas ports on the leaf chamber before separating the two halves of the chamber. Failure to do so will cause damage to the O_2 sensor! *****

A. Before beginning the experiment

1. Fill the gas bag with air.

1. Using a clean straw, inflate the plastic bag with breath from a group member. Be sure to open the pinch clamp before attempting to inflate the bag, and then pinch the clamp closed afterwards.

2. Connect the gas bag hose to the rear gas port of the leaf chamber.

2. Insert a leaf into the leaf chamber

1. To open the leaf chamber, first remove the stopper from the gas port, and then loosen the 4 thumb-screws on the upper plate of the leaf chamber to afford approximately 5 mm of space between the gaskets of the upper and lower plates. If necessary, gently pull downward on the lower plate to separate the gaskets.

2. Slip a leaf between the gaskets. It is OK for part of the leaf to extend outside the chamber.

3. *Gently* tighten the 4 thumb-screws to seal the upper and lower gaskets around the leaf petiole and any protruding leaf surfaces.

4. Lay the light shade over the leaf chamber.

5. Plug the open gas ports.

3. Position the heat sink

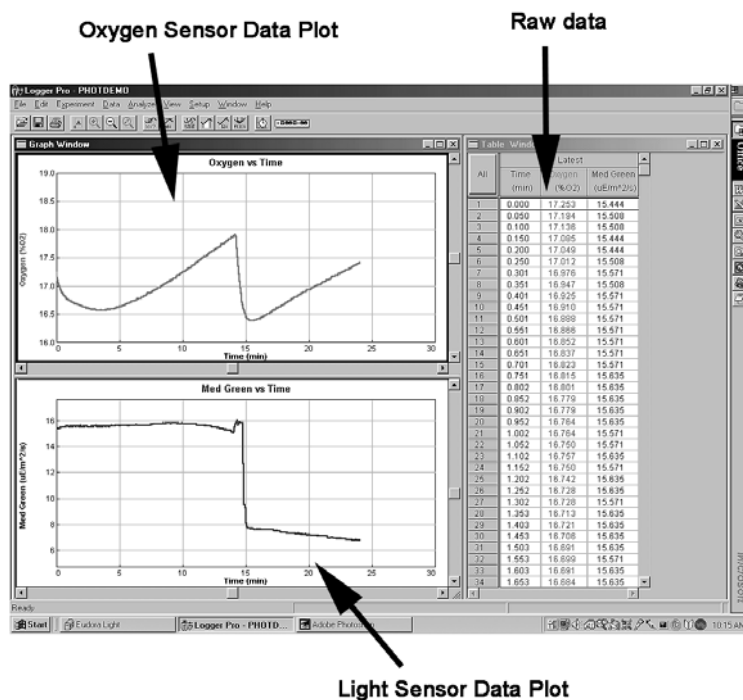
1. Fill the 400 ml beaker 3/4 full with cold water and slip it into the ring of the ring-stand.

2. Slide the ring stand so that the beaker lies between the light source and the leaf chamber. *Be careful not to splash water onto the leaf chamber.*

Figure 3. The LoggerPro window.

4. Start LoggerPro and load the photosynthesis experiment

Turn on the computer. After Windows loads, double-click “Photosyn.mbl” icon to start software. Make sure that serial-interface box and light source are plugged in. The LoggerPro Data collection window (with sample data) is shown in Figure 3.



B. Collecting Data

1. Start collecting data.

1. Begin collecting data by clicking the “Collect” button on the tool bar. The logger will begin to collect data at a rate of 20 samples per minute. Collect data for approximately 2 minutes and record the ambient (normal) %O₂ of the air on the results page.

2. Flush the leaf chamber with air from the gas bag and measure photosynthesis in the dark.

1. Unplug the front gas port on the leaf chamber.

2. Open the hose clamp, and apply gentle pressure to the bag to flush the chamber with air containing high-CO₂. After approximately 15 seconds, replug the open port of the leaf chamber and seal the bag by closing the hose clamp. You can leave the bag attached to the leaf chamber. The data recorder will show the %O₂ level dropping as you flush the chamber. *Why do we flush the chamber with air enriched in CO₂?* Record the %O₂ level of the exhaled gas on the results page.

3. Monitor O₂ levels of your leaf in the dark for 10 minutes.

3. Make 5 measurements of photosynthesis with the light on.

1. Reflush the chamber as described above.

2. Remove the light shade.

3. Slide the light dimmer control to maximum output. The log of light level should show an immediate increase. Record the light level in Table 1 on the results page.

4. Allow photosynthesis to proceed until the level of O₂ increases linearly (at a constant rate) for approximately 10 minutes, then shut off the light.

5. Open the gas port on the leaf chamber. Reflush the chamber as described above.

6. Repeating steps 3 and 4 to take four more measurements of photosynthesis.

7. After the fifth measurement, Click the ‘Stop’ button on the LoggerPro toolbar, turn off the light, disconnect the gas bag from the leaf chamber, and remove the plug from the other gas port.

4. Save the Data

Click “File” and then “Save as...”. Navigate to the directory provided during lab and then save the data under the file name “W1_Grp1”, “W1_Grp2”, or “W1_Grp3”, etc; where the group number corresponds to the number posted on your computer.

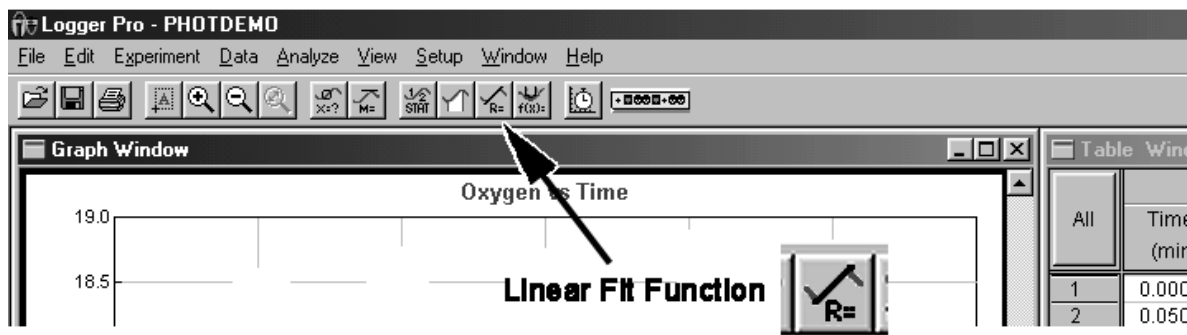
C. Working with the data.

1. Determine the slope of the lines on the O₂ release graph.

The rate of photosynthesis is calculated from the slope of the O₂ evolution lines. LoggerPro can calculate the slope of the line for you:

1. Using the mouse, place the cursor near the beginning of the region of the line to be analyzed.
2. Hold the left mouse button down, and drag the cursor horizontally across the line; the beginning and ending points of the line being analyzed will be bounded by 2 vertical lines. When you are satisfied with the position of the endpoint, release the mouse button.
3. To calculate the slope of a line, click on the “Linear Fit” command button on the Toolbar (Figure 4).

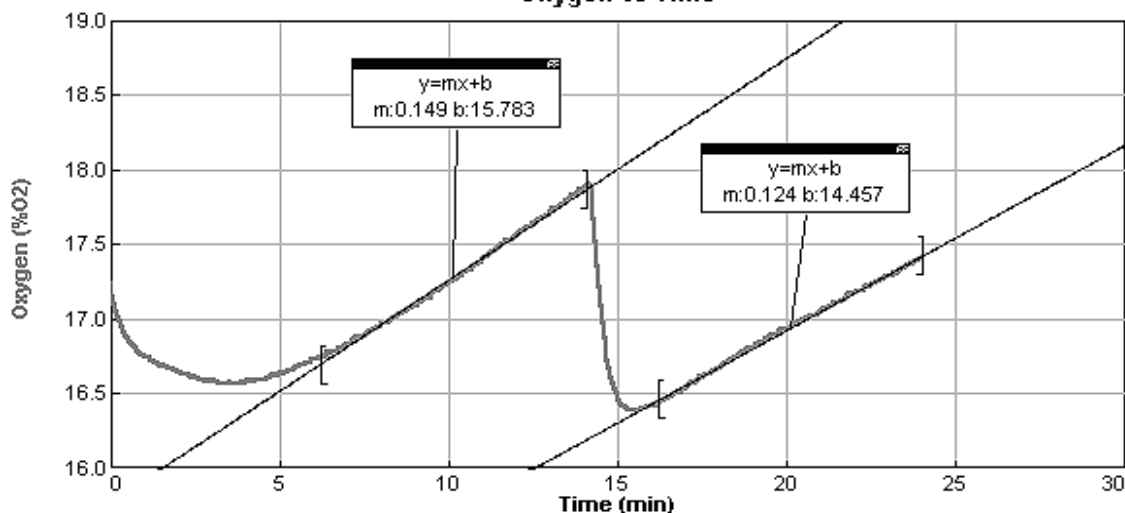
Figure 4. Linear fit button on toolbar.



A regression line will be drawn through the selected data and a box will appear showing the equation of a line, $y = mx + b$ (see Figure 5). The constants for the line representing the selected data are also given; ‘m’ is the slope of the line, and ‘b’ is the y-intercept. The equation box can be repositioned by dragging it where desired.

4. Repeat the process for each photosynthesis measurement, and record the slopes on the results page.

Figure 5. Oxygen data with trendlines.
Oxygen vs Time



2. Calculate the rate of photosynthesis in units of “ $\mu\text{mole O}_2 / \text{min}/\text{m}^2$.”

The slope of the line generated by LoggerPro has units of “ $\% \text{O}_2 / \text{min}$.” To convert this to units of $\mu\text{mole O}_2 / \text{min}/\text{m}^2$ you must first convert “ $\% \text{O}_2 / \text{min}$ ” to “ $\mu\text{mole O}_2 / \text{min}$ ” (plant physiologists prefer absolute units to relative units). You must also factor in the surface area of the leaf that you studied, because even if their actual rates of photosynthesis were the same, bigger leaves would release more O_2 per minute than smaller leaves.

1. Converting “ $\% \text{O}_2 / \text{min}$ ” to “ $\mu\text{mole O}_2 / \text{min}$ ”

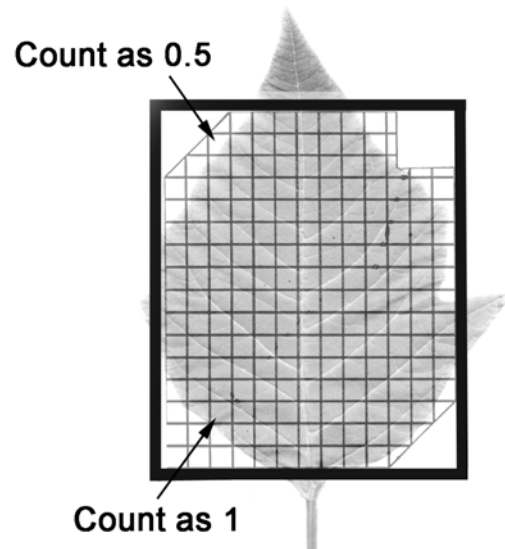
We will not discuss in detail all of the factors involved in converting “ $\% \text{O}_2 / \text{min}$ ” to “ $\mu\text{mole O}_2 / \text{min}$ ”; however, it does involve converting the volume of O_2 in the leaf chamber to a molecular quantity while factoring in the temperature. For our purposes, it is adequate to make the conversion by merely multiplying the slope of the line by 19.3. For example, using the slope of the first line in Figure 4, we would get:

$$0.149 \% \text{O}_2 / \text{min} \times 19.3 = 2.87 \mu\text{mole O}_2 / \text{min}$$

2. Calculate the area of your leaf in the leaf chamber.

The area of the leaf within the leaf chamber can be determined using the acetate grid. Carefully move the water heat sink away from the photosynthesis apparatus, and be careful not to touch the lamp which may be quite hot. Lay the grid on the top surface of the leaf chamber, and count the number of squares lying fully over the area of the leaf. Count the number of squares that lie partially over the area of the leaf (i.e., along the leaf edge) and multiply this value by 0.5. Sum the values and divide by 4 to obtain the area in cm². Convert this to m² by dividing by 10,000.

Figure 6. Determining the leaf area.



Thus, for the leaf shown in Figure 6:

$$\begin{aligned} \# \text{ of squares lying fully over leaf} &= \dots\dots\dots \\ \dots 142 \\ \# \text{ of squares lying partially over leaf} &= 30 \times 0.5 = 15 \\ \text{Sum} &= 157 \\ 157 \div 4 &= 39.3 \text{ cm}^2 \\ 39.3 \div 10,000 &= 0.0039 \text{ m}^2 \end{aligned}$$

3. Calculate the photosynthesis rate.

Now the rate of photosynthesis can be calculated by dividing the rate of O₂ release by the leaf area. Thus, for the preceding example:

$$2.87 \text{ } \mu\text{mole O}_2/\text{min} \div 0.0039 \text{ m}^2 = 735 \text{ } \mu\text{mole O}_2/\text{min}/\text{m}^2$$

Names: _____

Results for the Photosynthesis Exercise Week 1

1. What is the %O₂ in ambient air? _____
2. What is the %O₂ in the exhaled air? _____
3. What is CO₂ content of the exhaled air? Hint: the difference between the %O₂ in ambient air and that in the exhaled air represents the %CO₂.
4. The CO₂ concentration in ambient air is approximately 0.035%. How much higher is the CO₂ concentration in the exhaled air than in the ambient air?
5. Atmospheric air contains 20.7% O₂, and approximately 0.035% CO₂. Which gas comprises most of the remaining 79%?
6. Calculate the average rate of photosynthesis in your leaf under full light conditions. Complete Table 1.

Table 1. Photosynthesis rate data.

replication #	slope (%O ₂ /min)	irradiance (μE/m ² /sec)
1		
2		
3		
4		
5		
Average slope		

What was the area of your leaf within the leaf chamber? (Show how this was calculated)

Calculate the average rate of photosynthesis for the average slope in units of “μmole O₂/min/m²”. (show calculations)

7. What is the function of the light-dependent reactions of photosynthesis? What is the byproduct of this reaction?

8. What is the function of the light-independent reactions of photosynthesis?

9. Since no oxygen was released when photosynthesis was measured in darkness, we can conclude that the light-dependent reactions did not occur. What about the light-independent reactions; do these occur in the dark? Explain why or why not.

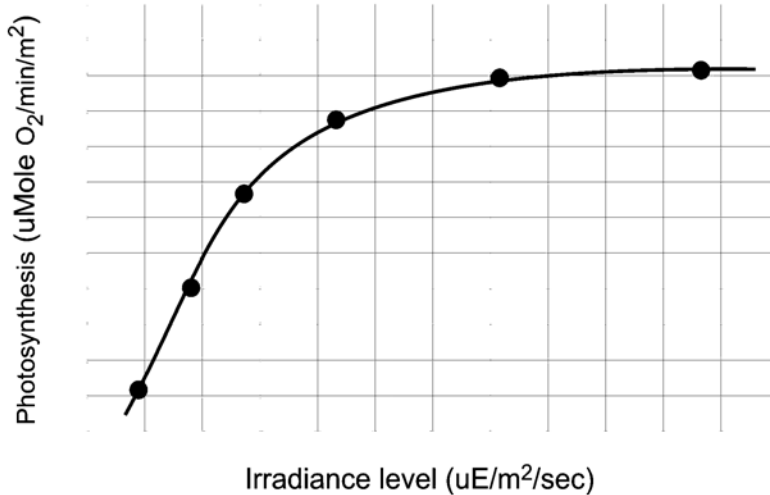
10. Why is exhaled air used in this experiment instead of ambient air?

11. List three reasons (other than mere human error) why calculated photosynthesis rates may differ for the different groups even though all of the leaves were from the same species?

Photosynthesis Week 2

During Week 2 of the photosynthesis section, you will prepare a **Photosynthesis Light Response Curve** for a leaf. A light response curve is a second-order graph of photosynthesis rates measured at different light levels (irradiances). To create this curve you will follow the same basic procedures that you learned last week. You will prepare a full lab report for this lab exercise, and further information about the lab report is provided at the end of this lab.

Figure 7. Idealized photosynthesis light response curve.

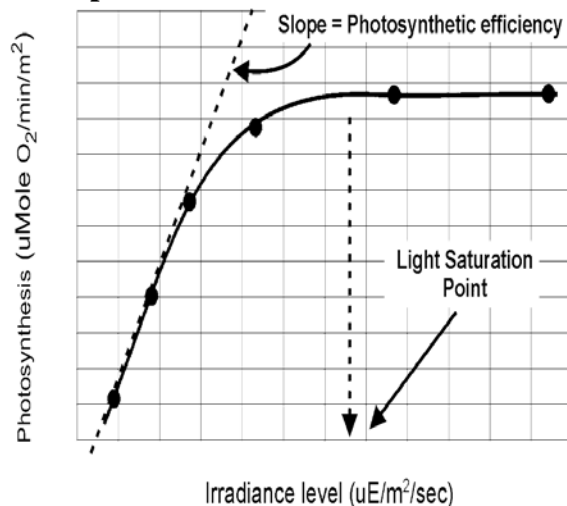


The Photosynthesis Light Response Curve

A photosynthesis light response curve is a graph showing the relationship between irradiance level and the rate of photosynthesis (Figure 7). Different plants (even different leaves on the same plant) show differences in the details of their light response curves.

The response curve can be divided into two phases (Figure 8). Notice that under low-light levels, the rate of photosynthesis increases linearly as the irradiance level is increased. The slope of this part of the curve is called the “photosynthetic efficiency” value of the leaf. Notice that the rate of photosynthesis eventually levels off; above a certain irradiance value, called the “light saturation point”, the rate of photosynthesis no longer increases.

Figure 8. Some characteristics of the response curve.



The photosynthetic efficiency and light

saturation point of a leaf reveal characteristics of the underlying photosynthetic processes. Photosynthetic efficiency is a measure of the capacity of the light-dependent reactions; leaves that can efficiently absorb light and pass that energy into ATP and NADPH₂ will have higher photosynthetic efficiencies (steeper slopes) in their response curves. The value of the light saturation point is a measure of the capacity of the light-independent reactions, and indicates the maximum rate at which the leaf can fix CO₂ into carbohydrate. Above this value, the light provides more energy than the light-independent reactions can use – the process is ‘saturated’, like a wet sponge that can absorb no more water. Under natural conditions, the rate of CO₂ fixation is limited by the availability of CO₂ in the air. However, you will recall from last week’s exercise that the CO₂ concentration of exhaled air is much higher than in ambient air, which allows for a higher rate of CO₂ fixation and a higher light saturation value.

Procedures for week 2

This week you will measure the rate of photosynthesis at a series of different light levels and then plot rate vs irradiance to generate a light response curve. From this curve you will determine the photosynthetic efficiency and light saturation point of the leaf.

1. Measure O₂ evolution at different light levels

The essential steps of the procedure you will follow are essentially identical to those used last week. Only significant changes are indicated below.

1. Measure photosynthetic rate with the light source set at maximum output following the procedures described for week 1.
2. Record data until a linear rate of O₂ evolution has been observed for 8 - 10 minutes. While the data is being logged, record the irradiance level in Table 2, and then calculate irradiance levels representing 75%, 50%, 25%, 12%, 6%, and 3% of the full output. Record these values in the appropriate places in Table 2. These will be the light output settings that you will use in the subsequent runs.
3. Follow steps 1 - 3 under ‘B. Collecting Data’ in last week’s lab procedure. Do not click the ‘Stop’ button until all the measurements have been taken.

2. Save the Data

Click “File” and then “Save as...”. Navigate to the directory provided during lab and then save the data under the file name “W2_Grp1”, “W2_Grp2”, or “W2_Grp3”, etc; where the group number corresponds to the number posted on your computer. If you fail to save the data correctly you will need to repeat the experiment. To share the data, make copies of the file onto thumb drives and/or email the file among group members.

3. Determine the area of the leaf.

Follow the procedures described last week to determine the area of the leaf inside of the

leaf chamber.

Results for Week 2

Table 2. Results of photosynthesis measurements.

Relative irradiance	Irradiance ($\mu\text{E}/\text{m}^2/\text{sec}$)	Slope ($\% \text{O}_2/\text{min}$)	Photosynthesis Rate ($\mu\text{mole O}_2/\text{m}^2/\text{min}$)
100 %			
75 %			
50 %			
25 %			
12 %			
6 %			
3 %			

Leaf area: _____ (show data and calculations)

Graphing your photosynthesis light response curve

This will be done in the computer lab.

1. Open Microsoft Excel.
2. Create a table with 2 columns, 'Irradiance' and 'Photosynthesis Rate'
3. Enter the data for each irradiance level in the rows beneath the headings
4. Plot the data in a 'scatter graph with lines' format.
5. Format your graph (titles, units, etc), add trendline through linear portion, and print it.
6. Save your file to a 3 1/2" disk or thumb drive.

Photosynthetic efficiency: _____

To determine the photosynthetic efficiency, use the slope of the line and divide by 60 (this converts the photosynthetic rate units from minutes to seconds). Write equation of trendline and show calculation:

Light saturation point: _____ (briefly explain how this was determined)

The Lab Report

You are expected to apply lab writing skills learned last semester, and follow the lab report guidelines that were provided (a copy of these are available on the Home Page for this course). Some general instructions for the report are provided below. The **Photosynthesis Web Study Guide** should be consulted for a further discussion of the interpretation of the photosynthesis light response curve.

1. Your lab report will be on the second week's investigation, and should include Introduction, Procedures, Results, Discussion and Literature Cited sections.

Introduction section: In the section you should fully describe the basic principles of photosynthesis. There will not be a hypothesis section for this report.

Procedure section: Besides describing the basic procedures, be sure to explain how the leaf area, photosynthetic rates, light saturation point and photosynthetic efficiency were calculated. Be sure to identify the type of plant from which the leaf was obtained.

Results section: Tables and Figures must be properly formatted as described in the lab report guidelines and should include:

1. A graph showing the O₂ evolution curves for your leaf at the different light intensities. This should be copied and pasted directly from LoggerPro into a Word document, and then a suitable title should be added.
2. A Table presenting data as shown in Table 2 above.
3. Another graph (with a trendline through linear portion) showing the photosynthesis light response curve.

The Description of Data section should include a general description of the data curves and trends shown in the figures, and present the light saturation point and photosynthetic efficiency values.

Discussion: This lab report should include only "Explanation of Results" and "Future Experiments" sections. In the Explanation of Results section you will be expected to explain the basic principles of the photosynthesis light response curve. Some other topics you should discuss include why the metabolic capacity of light-dependent reactions influences the photosynthetic efficiency. Does your leaf show a light saturation point? If not, why? Why does the metabolic capacity of the light-independent reaction influence the light saturation point? What are some of the factors that affect the characteristics of a light response curve? The **Photosynthesis Web Study Guide** should be consulted for a further discussion of some of these factors. Finally, for a future experiment state a question about photosynthesis that this system could be used to answer, and briefly describe an experimental design that could be used.

Literature Cited: You should cite the Web Resource Page for information drawn from it. Any other sources should be cited and referenced following the format described in the Lab Report Guidelines.