

PHOTOSYNTHESIS, O₂ EVOLUTION

EXERCISE 15 PHOTOSYNTHETIC OXYGEN EVOLUTION, ACTION SPECTRA

I. INTRODUCTION

Almost all of the organisms that occur in the biosphere are ultimately dependent upon the process of photosynthesis as their source of utilizable energy. This energy is derived by the ability of green plants to convert light energy into usable chemical energy in the form of molecules of glucose. The overall equation for this process can be written as follows:



In reality, the overall reaction requires the involvement of many different substrates, enzymes, and cofactors in a sequence of complex interactions. In simplifying the process, photosynthesis is subdivided into the light reactions and the dark reactions. The light reactions involve absorption of quanta of light and generation of ATP and NADPH. A byproduct of this light-energy conversion is oxygen liberated from the splitting of H₂O. The dark reactions then utilize ATP and NADPH for the conversion of CO₂ into sugars.

Photosynthesis in intact plants is responsive to changes in the environment. Variations in light intensity cause changes in the photosynthetic rate of plants, and changes in temperature also affect this process, which is a composite of many chemical reactions. Likewise, the concentration of available CO₂ plays a role in regulating photosynthesis. These as well as other environmental factors affecting photosynthesis can be quantitatively evaluated by measuring the amount of CO₂ fixed into organic compounds or by the amount of O₂ liberated.

The objectives of this exercise are as follows:

- A. An introduction to the principles and applications of techniques that measure O₂ evolution.
- B. Determination of the rates of photosynthesis as oxygen evolved from a leaf.
- C. Generate a simplified action spectra for photosynthesis by measuring O₂ evolution under different wavelengths of light.

A. Principle techniques for measuring O₂ evolution

Measurements of oxygen evolution in photosynthesis can be made by manometric, O₂ electrode or gas analysis methods. The original methods

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(Warburg in the 1920's) to study photosynthesis utilized the manometric method. In these methods, living material is allowed to photosynthesize in a closed vessel and pressure changes within the vessel are measured by sensitive manometers in which the rise or fall of a liquid in a capillary indicates, respectively, decreases or increases in pressure. Increase in pressure within the vessel is due to the evolution of oxygen by the living system, while a decrease is due to oxygen utilization. For algal systems and detached leaves an O₂ electrode has been used to measure the light reactions of photosynthesis.

We will be using an oxygen sensor attached to a closed leaf chamber. Thus we can measure photosynthesis on intact leaves. The sensor does not measure the rate of photosynthesis directly, but monitors the changes in O₂ concentration of the atmosphere within the leaf chamber. Using data acquisition software, you will record these changes in O₂ concentration, and will calculate the rate of change of O₂ as a measurement of photosynthesis.

B. The Oxygen Sensor: Principle of Operation

The leaf chamber that you will be using in your experiments has an O₂ sensor incorporated into its upper surface. This sensor is a galvanic cell (a lead-oxygen battery) consisting of a lead anode, an O₂ cathode and an acid electrolyte. O₂ diffusing through a Teflon FEP membrane is reduced electrochemically at the electrode. A resistor and a thermistor (for temperature compensation) are connected between the anode and the cathode, so that the battery is always discharged. Current flowing through the resistor and thermistor is proportional to the partial pressure of O₂ (pO₂) in contact with the membrane (i.e. the partial pressure of O₂ in the leaf chamber), and by measuring the current flowing through the resistor and thermistor, the pO₂ can be determined.

C. Aim of the Experiment

The purpose of this experiment is to demonstrate that photosynthesis is affected not only by the amount of light that impinges on a leaf, but also by the quality of that light. The range of wavelengths that the human eye sees (390 to 760 nm) is also what drives photosynthesis, but there are differences between the degrees to which equal amounts of light at each wavelength are capable of stimulating photosynthesis. The leaf has several different types of photosynthetic pigments that absorb light energy, and these have absorption peaks in different parts of the spectrum (Exercise 14). This experiment will show how particular wave-lengths stimulate photosynthesis and how an **action spectra** (the effectiveness of any particular wavelength at driving a metabolic process) is produced. Photo-biologists use action spectra to determine what pigments are likely associated with a particular physiological process.

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II. MATERIALS AND METHODS

- (1) The equipment will be set up and calibrated ahead of time by your instructor. Ensure that the light is off before starting.
- (2) The computer screen should display percent O₂ plotted against time. Click on “Experiment” in the main menu and then “Sampling”. Reset the experiment length to 90 mins, and check that the sampling rate is 60 samples per minute. Click on OK.
- (3) Ensure that the leaf chamber gaskets have a **very thin** coating of vacuum grease. If any grease is visible it will be unnecessary to apply more. With the light off, place a leaf from the plant provided inside the chamber and seal it. Make sure no part of the leaf is shaded by the O₂ sensor or the gas inlet and outlet ports. It does not matter if the leaf is too large to be fully sealed within the chamber, and the “excess” may protrude out of the chamber without influencing your results. When closing the chamber turn the thumb-screws finger tight only. **Note: You MUST place the 200 mL beaker of cold water on top of the leaf chamber before turning on the light. Replace the water in the beaker every fifteen minutes if your experiment involves continuous illumination.**
- (4) Using a drinking straw, inflate a plastic gas bag with your breath being careful not to put pressure on the seams by over-inflating the bag. Seal the bag with the luer lok plug provided. Depending on your metabolic condition, your exhaled breath should contain between 16 and 18% O₂ and 3 to 5% CO₂.
- (5) Click on the “Collect” button. The button will change to a “Stop” button, and data will begin to appear on the graph on the screen, and as numerals on the bottom of the screen. The initial O₂ concentration should be close to 20.7% O₂.
- (6) Remove the luer-lok plug from the tubing on the bag, and attach this tubing to one of the gas ports on the upper surface of the leaf chamber. Press the bag *gently* so that your breath is flushed through the chamber. After approximately ten seconds of flushing, remove the bag from the inlet port, seal the bag and seal both ports of the leaf chamber with the luer-lok plugs provided. Observe the decline in the O₂ reading on the computer screen until this reaches a stable value (approximately 4 minutes).
- (7) Your experiment should take about 80 minutes to complete. If, however, your experiment goes over time, click on “Data”, and then “Store Latest Run” before restarting the experiment. Your initial data will appear as a faint line in the background, and the most recent data will appear in bold. You may choose which data to view and analyse using the “Show Run” and “Hide Run” command under the “Data “ menu.

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- (8) Illuminate the leaf by sliding the dimmer control to maximum output. Observe the changes in the O₂ concentration within the chamber. If the leaf has been maintained in near darkness (e.g. room light) prior to the experiment, there will be little change in the O₂ reading during the first 3 - 5 min of illumination. This corresponds to the "induction period" of photosynthesis during which photosynthetic metabolites are synthesized until they reach the critical pool sizes required for photosynthesis to occur. Once this has been achieved, the partial pressure of O₂ in the chamber will increase as O₂ is released in photosynthesis. After the photosynthetic induction period, the pO₂ in the chamber will rise slowly at first and then will increase linearly.
- (9) Observe the linear increase in O₂ concentration for 10 min then switch off the light. Attach the gas bag to a chamber inlet port and flush the chamber for 10 seconds by pressing gently on the bag. Re-seal the chamber and the gas bag with the luer lock plugs and wait until the O₂ concentration of the chamber has declined to a new steady level.
- (10) Remove the 200 mL beaker water filter from the leaf chamber and refill it with cold water. Place the blue filter on the chamber so that it shades the entire leaf, and then place the beaker of water on top of it. Switch on the light again. Your instructor will reduce the light level on the dimmer switch to an appropriate light level. Action spectra need to be performed under light limiting conditions since with high enough irradiance any wavelength may saturate photosynthesis even those wavelengths that chlorophyll absorbs less strongly.
- (11) Measure the increase in pO₂ of the chamber for 15 min, then turn off the light.
- (12) Remove the 200 mL beaker water filter and blue filter from the surface of the leaf chamber, and refill the beaker with cold water.
- (13) Flush the chamber for 10 seconds with breath from the gas bag, and then place the green filter above the leaf. Place the 200 mL beaker above the filter and then turn on the light. The light level should be the same as for the blue filter. These filters are designed to give the same total amount of light (mols photons m⁻² s⁻¹) with the same white light input, but just at different wavelengths.
- (14) Observe the increase in O₂ concentration in the chamber for 15 minutes and then turn off the light.
- (15) Remove the 200 mL beaker and the green filter. Place the red filter on the leaf chamber and put the beaker filled with cold water back on to the chamber. Flush the chamber with breath for 10 seconds and then seal both the chamber and the gas bag. Turn on the light (same level) and observe the increase in chamber O₂ concentration for 15 minutes.

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- (16) Stop the experiment by clicking on the "Stop" button, and then save your data by clicking on "File" in the menu, and selecting "Save as....". Give your data an appropriate file name, and save it to disk, or in the subdirectory allocated for it by your laboratory instructor.
- (17) Remove the 200 mL beaker from the chamber, detach the leaf from the plant, and detach the leaf chamber from its mounting bracket. Be careful not to touch any hot surface of the lamp or its fittings while doing this. Place the acetate grid on the surface of the chamber so that it covers the leaf. Count the number of interstices completely enclosed by the area of the leaf. 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 equal to the area of the leaf in cm².

III. RESULTS AND DATA ANALYSIS

The O₂ sensor measures only the partial pressure of O₂ present in the leaf chamber, it does not measure the rate at which this O₂ is produced. The rate of a process, such as photosynthesis, is expressed as the increase in product per unit time. To measure the rate of photosynthesis in your experiment, you will need to measure the increase in pO₂ within the cuvette as a function of time. This is achieved by measuring the gradient of the O₂ response which, when the x axis of your graphs is presented in min, will give a rate in %O₂ per min. The procedure for analyzing your data is as follows:

- (1) Open the file containing your data.
- (2) Select "Analyze" from the menu at the top of the screen and then "Examine". A vertical line will appear on your graphs, which can be moved along the data points on the graphs by moving the mouse. Note that as you move the vertical line, the numerical display in the box on the screen will change to show you the exact O₂ concentration, irradiance and time value at the point on each graph where the line is situated.
- (3) Move the vertical line to the point at which you exposed the leaf to the highest light intensity and measure the steady (i.e. linear phase) photosynthetic rate. Move the cursor on the screen to the point on your O₂ data where you wish to start the measurement, 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. A box will be drawn around the selected part of the data during this procedure.
- (4) Select "Analyze" from the menu and then "Linear Fit". A linear regression will be made of the data that you have selected and an equation will appear in a box on the screen. This will provide values for b₀ (the intercept of the regression line on the y axis), and m (the gradient of the line). Make a note of these values

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- (5) After noting your data, click on the symbol in the top right hand corner of the data box. The box will disappear allowing you to analyze more data in a similar way.
- (6) Repeat the data analysis procedure for measurements made in blue, green and red light. Record the m values in Table 15.1. Express photosynthetic rates for all parts of the experiment in units of $\mu\text{mol O}_2 \text{ m}^{-2} \text{ s}^{-1}$ as described in the "Calculations" section, and also enter them in Table 15.1.

Calculations

Each m value from each regression that you performed represents the rate of increase of O₂ concentration in the chamber with time. As such, each of these m values are rates of photosynthesis expressed as %O₂ per min. However, photosynthesis is usually expressed in terms of $\mu\text{moles of O}_2$ evolved per unit leaf area per unit time i.e. in units of $\mu\text{mol O}_2 \text{ m}^{-2} \text{ s}^{-1}$. To make this conversion the following procedure is required.

Let us assume that the slope value (m) was 0.05 i.e. the O₂ concentration of the chamber increased by 0.05 %O₂ min⁻¹.

0.05 %O₂ is equivalent to 10,000 x 0.05 part per million (500 ppm) O₂ which, in turn, is equivalent to 500 μL of O₂ per L of gas in the chamber ($\mu\text{L L}^{-1} = \text{ppm}$).

At standard temperature ($273 \text{ }^\circ\text{K} = 0 \text{ }^\circ\text{C}$) and pressure (1 atmosphere) 1 μmole of any gas occupies 22.413 μL , so if we assume the temperature T of the laboratory is 20 $^\circ\text{C}$, 500 μL of O₂ contains:

$500 / [(273 + 20)/273] \times 22.413$ $\mu\text{moles of O}_2$, or $500 / (1.07 \times 22.413)$ or $500 / 24.05$, which equals 20.8 $\mu\text{moles of O}_2$. Since the units input are 500 $\mu\text{L O}_2 \text{ L}^{-1} \text{ min}^{-1}$ (microliters of oxygen produced per liter of chamber volume per minute) the value becomes 20.8 $\mu\text{moles of O}_2 \text{ L}^{-1} \text{ min}^{-1}$.

To obtain photosynthetic rate we must now multiply this value by the volume of the chamber expressed in litres. The chamber is designed so that when closed it has a fixed internal volume of 0.047 L. Therefore, in our example, photosynthetic rate would be $0.047 \text{ L} \times 20.8 \mu\text{moles of O}_2 \text{ L}^{-1} \text{ min}^{-1} = 0.978 \mu\text{moles of O}_2 \text{ min}^{-1}$. To express this rate on a leaf area basis per second (more common unit for time in photosynthesis studies) it is necessary to divide the value by the area of the leaf (in m²) that you obtained using the acetate grid and by 60 (60 seconds per minute).

If the leaf area inside the chamber was 20 cm² and there are 10,000 cm² per m², then the leaf area is $20 \text{ cm}^2 \times (1 \text{ m}^2/10,000 \text{ cm}^2) = 0.002 \text{ m}^2$. The photosynthetic rate would be $0.978 \mu\text{moles of O}_2 \text{ min}^{-1} / 0.002 \text{ m}^2 / 60 \text{ s} = 8.14 \mu\text{moles of O}_2 \text{ m}^{-2} \text{ s}^{-1}$.

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Now let's simplify these calculations! If we assume the only two variables that you will measure are the slope (**m** in %O₂ min⁻¹) and the leaf area (**LA** in cm²) and room temperature is 20 °C, then the equations simplify to,

$$\frac{m \times 10,000 \times 0.047}{\frac{(273 + 20)}{273} \times 22.413 \times \frac{LA}{10,000} \times 60} = \frac{m}{LA} \times 3,257$$

Thus, in our example, if $m = 0.05 \text{ \%O}_2 \text{ min}^{-1}$ and the leaf area is 20 cm² then,

$$\frac{0.05}{20} \times 3,257 = 8.14 \cdot \mu\text{mol} \cdot \text{O}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$$

Table 15.1. Effect of different wavelengths of light on oxygen evolution

Leaf Area (cm²) = _____

Color of Light	m (%O ₂ min ⁻¹)	Photosynthetic Rate (μmoles of O ₂ m ⁻² s ⁻¹ .)
White		
Blue		
Green		
Red		

Create a bar chart of the three colored light treatments. Place the colors on the X axis in an order that corresponds to the differences in their wavelengths (short to long λ).

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IV. ASSIGNMENT AND DISCUSSION QUESTIONS

NAME _____

- A. (4pts) Bar chart showing relationship of O₂ evolved (Y axis) to the color (wavelength) of light.
- B. (3pts) How did the photosynthetic rate differ in each light treatment? Relate your results to the absorption spectra of photosynthetic pigments (Exercise 14).
- C. (2pts) The filters reduced the amount of light impinging on the leaf to a level that was insufficient to saturate photosynthesis. Why was this important? What other quality of the filters, with respect to light absorption, is necessary for this experiment to work?
- D. (2pts) After illuminating the leaf, it took some time before the O₂ concentration of the chamber increased. Why didn't photosynthetic O₂ evolution begin the moment that light was supplied?
- E. (1pts) Is O₂ evolution directly related to CO₂ fixation in a 1:1 ratio? Explain.

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