

PHOTOSYNTHETIC GAS EXCHANGE

EXERCISE 17 PHOTOSYNTHETIC GAS EXCHANGE, CO₂ RESPONSE

I. INTRODUCTION

To this point in our lab we have measured O₂ evolution and electron transport rates of photosynthesis. However, the reason plants absorb solar radiation is to convert inorganic carbon in the form of CO₂ into organic molecules (sugars). This is termed carbon fixation since the carbon in CO₂ from the atmosphere is “fixed” into molecules the plant can later use. The enzyme that fixes CO₂ in the Calvin cycle of photosynthesis is Ribulose biphosphate carboxylase oxygenase (Rubisco). As the name suggests this enzyme can fix both CO₂ (carboxylase) and O₂ (oxygenase). Carbon dioxide and oxygen compete for the active site of Rubisco and, at atmospheric CO₂ and O₂ concentrations, a portion of the substrate (ribulose biphosphate – RuBP) is oxygenated. As with any enzyme an increase in a substrate (CO₂) should increase the rate of reaction that enzyme (Rubisco) catalyzes. In addition, an increase in CO₂ should reduce the amount of photorespiration since O₂ and CO₂ are competing for the same active site on Rubisco.

The process of photosynthesis involves the exchange of gases between the leaf and the environment. In higher plants, this exchange primarily occurs through the stomata. Carbon dioxide is taken up by the leaf, water vapor is lost and O₂ is evolved. Therefore, if a leaf were to be enclosed in a chamber, air passing through the chamber would become depleted in CO₂ and enriched in water vapor and O₂. By measuring the rate at which these gases are exchanged between the leaf and its environment, we can determine the rate of photosynthesis and transpiration. The exchange of CO₂ between a leaf and the atmosphere is most often estimated using an infra-red gas analyzer (IRGA), which measures the concentration of CO₂ in a gas entering a leaf chamber, and the concentration of CO₂ in the same gas after it leaves the chamber. Measurement of this CO₂ differential, and measurement of the flow rate of gas through the chamber and leaf area allow calculation of photosynthetic CO₂ fixation rate. The differential between the concentration of CO₂ entering and exiting the leaf chamber (cuvette) would change with different flow rates and the amount of photosynthetic tissue inside the chamber. Should conditions be unfavorable for CO₂ fixation, the method can also estimate the CO₂ evolved from the leaf in the dark (dark respiration) or in the light (photorespiration).

In this experiment you will demonstrate that CO₂ is required for photosynthesis, and that the rate of photosynthesis increases with CO₂ concentration in the atmosphere until a CO₂ saturation point is reached. At that point, photosynthetic rate is limited by the ability of the leaf to process the CO₂ that is delivered to it. Limitation may be caused by insufficient light energy to drive the maximum rate of photosynthesis, or by the rate at which enzymes catalyze the steps in photosynthetic CO₂ metabolism (enzyme kinetics, i.e., V_{max}). At very low concentrations of CO₂ the rate of CO₂ fixation in photosynthesis approaches the rate of CO₂ production in photorespiration. When these two opposing fluxes of CO₂ balance, the plant is at the CO₂ compensation point. You will also estimate the CO₂ compensation point in this experiment.

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II. METHODS AND MATERIALS

- (1) The instruments will be set up and calibrated prior to the lab by your instructor.
- (2) Attach the ambient air line to the inlet of the pump, and adjust the flow to 500 ml min^{-1} . The outlet of the flow meter should be attached to the inlet of the humidity/temperature sensor and the outlet of the humidity sensor is attached to the water trap (ice bath), which is attached to the drying column. Click on the **COLLECT** icon to start measurements. If the IRGA was calibrated correctly, the stable CO_2 concentration shown on the LCD display will match that shown numerically on the computer screen underneath the graph.
- (3) Your experiment should take approximately 30 minutes to complete. If the time axis on the computer display shows a maximum value different from 30 mins, adjust this by clicking on the maximum value displayed and typing in an appropriate value.
- (4) Record the ambient CO_2 concentration. **This is your “reference CO_2 ” concentration.**
- (5) To observe CO_2 consumption, or evolution from the leaf, you will need to set the display so that the y axis of the graph has a range of approximately 130 ppm CO_2 , (30 ppm above and 100 ppm below the reference CO_2 value). For example, if the reference $\text{CO}_2 = 330$ ppm then the upper and lower limits should be 360 and 230 ppm respectively. At any time if the y axis requires adjustment, click on the current maximum and minimum values on the y axis and enter new values. Press Enter. Alternatively, you may select ‘Autoscale’ from the VIEW menu.
- (6) Seal a leaf from the plant provided inside the leaf chamber, turn on the LCD light source to the maximum output and detach the outlet of the flow meter from the humidity sensor and attach it to one of the tee-pieces on the leaf chamber (chamber inlet). Attach the other tee-piece (chamber outlet) to the humidity sensor
- (7) Observe the decline in the CO_2 concentration of the gas leaving the chamber as photosynthesis consumes the CO_2 delivered to the leaf in the reference gas. Wait until a steady state value of CO_2 is observed. This may take several minutes, especially if the leaf was not in high light (lab bench) previously. Typically there will be a rapid decline in CO_2 followed by a more gradual decline as the leaf responds to the light level by opening its stomata. Once the leaf has opened its stomata subsequent measurements reach steady state rapidly.
- (8) Record the CO_2 concentration and humidity when steady state conditions have been attained. **This is your “analysis” CO_2 concentrations** at that reference CO_2 concentration. Also record the light level, humidity, temperature and flow rate.

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- (9) Detach the ambient air line from the inlet of the pump and attach the bag with the highest concentration of CO₂ to the inlet. The outlet of the flow meter should be attached to the inlet of the humidity/temperature sensor and the outlet of the humidity sensor should remain attached to the water trap (ice bath).
- (10) Record the CO₂ concentration in the gas bag as your **new reference CO₂ concentration**. As described in point 5 above, adjust the y axis of the CO₂ display so that the range encompasses values 30 ppm above, and 100 ppm below, the new reference value.
- (11) Repeat procedures 6 through 10 above for all the gas bags provided. At low photosynthetic rates (low CO₂), it is often helpful to reduce the flow rate in order to enhance the differential between reference and sample CO₂ measurements. **Any change in flow rate must be noted** since it affects all your calculations. The final CO₂ concentration is zero and is obtained by sending the flow through a column filled with soda-lime which absorbs all the CO₂ in the air. The procedures are identical to those above only instead of a gas bag, laboratory air is sent through the soda-lime column.
- (12) If the leaf you were using completely filled the leaf chamber, the area enclosed would be 9 cm². If the leaf did not completely fill the chamber, you will need to estimate the leaf area enclosed. To do this remove the LCD light source and place the acetate grid on the surface of the chamber so that it covers the leaf. Count the number interstices completely enclosed in 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 by 4 giving the leaf area in cm².

III. CALCULATION OF CO₂ EXCHANGE RATE

Measurements of photosynthetic and respiratory rates in leaves are usually expressed as rates of CO₂ exchange per unit time per unit leaf area. The units most commonly used are μmole CO₂ m⁻² s⁻¹ (1 μmole = 10⁻⁶ mole). To express your data in these units use the following calculations:

- Calculate the difference between the CO₂ concentration in the reference and analysis gases. For example, if an experiment was conducted in air of 360 ppm CO₂, at a flow rate of 500 mL min⁻¹, the depletion of CO₂ due to leaf uptake in photosynthesis at high light may result in an analysis gas CO₂ concentration of 320 ppm. The difference between the reference and analysis gas streams (*d*CO₂) in this example would be 40 ppm (360 - 320 ppm).
- Convert the *d*CO₂ value from ppm into μmole per liter thus:

$$d\text{CO}_2 / \{22.413 \cdot ([T+C]/T)\}$$

where C is the temperature in °C and T is the absolute temperature at 0 °C (= 273 °K)

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At a temperature of 20 °C and a $d\text{CO}_2$ of 40 ppm, the $d\text{CO}_2$ would be equivalent to 1.66 $\mu\text{mole CO}_2$ per liter.

- Multiply this number ($d\text{CO}_2$ in $\mu\text{mole L}^{-1}$) by the flow rate (in L s^{-1}) used in your experiment to obtain a CO_2 exchange rate per second. A 500 ml min^{-1} flow rate is equivalent to 0.0083 L s^{-1} . So the CO_2 exchange rate in our example would be $0.014 \mu\text{mole s}^{-1}$.

$$0.014 \mu\text{mole s}^{-1} = 500 \text{ ml/min} \cdot 1 \text{ L} / 1000 \text{ ml} \cdot 1 \text{ min} / 60 \text{ s} \cdot 1.66 \mu\text{mole CO}_2 / \text{L}$$

- Express your CO_2 exchange rate on a leaf area basis by dividing the CO_2 exchange rate per second by the leaf area in m^2 . If the leaf completely filled the chamber, the area used in the calculation would be 9 cm^2 , equivalent to 0.0009 m^2 ($10,000 \text{ cm}^2 \text{ m}^{-2}$). The photosynthetic rate in our example would therefore be $0.014 \mu\text{mole s}^{-1} / 0.0009 \text{ m}^2 = 15.6 \mu\text{mole m}^{-2} \text{ s}^{-1}$, which is reasonable for a C_3 species under ambient conditions.

Calculate the photosynthetic rate for each CO_2 concentration tested.

If you failed to record any of the essential data for your calculations during the experiment, you may retrieve the data from your saved file using the following procedure:

- Open the file containing your data. Your data will appear on the screen exactly as it appeared when you saved it at the end of the experiment.
- Select 'Analyze' from the menu at the top of the screen by clicking and holding with the mouse. Select 'Examine' and then release the mouse button. A vertical line will appear on each of your graphs, which can be moved along the data points on the graph by moving the mouse. Boxes will also appear on each graph showing data values and time values for each run displayed. As you move the vertical line on a graph, the numerical display in the box will change to show you the exact data values and time value at the point on each graph where the line is situated. If the box obscures any part of the trace, click on the box and hold, then drag with the mouse to place the box in a convenient location. Record the appropriate data from your experiment by copying the values.

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IV. RESULTS AND DISCUSSION

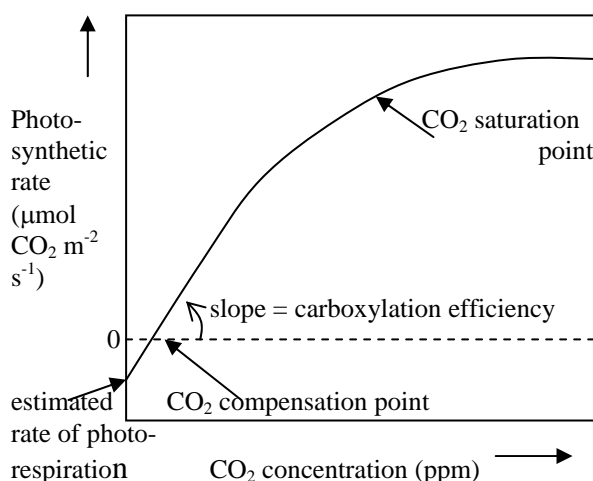
Table 17.1 Estimates of photosynthetic rates under varying CO₂ concentrations

Leaf Area = _____ cm²

CO ₂ concentration (ppm)	Flow rate (ml min ⁻¹)	dCO ₂ (ppm)	Photosynthetic Rate (μmol CO ₂ m ⁻² s ⁻¹)

When you have calculated rates of photosynthesis at each CO₂ concentration used in your experiment, present your data as a graph with photosynthesis on the y axis and CO₂ concentration on the x axis. Use the graph paper attached to the Discussion questions.

A photosynthetic CO₂ response curve for a generalized leaf is shown in Figure 17.1. Note that at low CO₂ concentrations, photosynthesis increases almost linearly as CO₂ concentration is increased. This is because at these concentrations the rate of photosynthesis is limited by the availability of CO₂ for the carboxylation reaction by Rubisco. At higher CO₂ concentrations there is less of an increase in photosynthetic rate per unit increase in CO₂, and eventually photosynthesis reaches CO₂ saturation at the highest CO₂ concentration used in this experiment. Under these conditions, the carboxylation reactions of photosynthesis are maximized, and photosynthetic



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rate is limited by the supply of photons to the light reactions (and thus RuBP regeneration), or by the turnover rate of the photosynthetic enzymes.

The photosynthetic CO₂ response curve of a particular plant is influenced by many factors, and a study of components of the curve can tell us a great deal about the physiology of the plant. Important aspects of the CO₂ response curve include:

The CO₂ compensation point. Extrapolate the linear portion of the CO₂ response curve to intercept the x axis at the point where the photosynthetic rate is zero. The CO₂ concentration at this point is called the CO₂ compensation point, and it represents the CO₂ concentration at which CO₂ consumption in photosynthesis is balanced by CO₂ production in photorespiration.

The rate of photorespiration. If the linear portion of the CO₂ response curve is extrapolated to intercept the y axis at zero CO₂ concentration, the negative rate of photosynthesis at this point gives an estimate of the photorespiration rate.

Carboxylation efficiency. Carboxylation efficiency may be defined as the increase in photosynthetic rate achieved per unit increase in CO₂ at the site of CO₂ fixation (C_i = intercellular CO₂ concentration). If in your experiment, you did not measure C_i, but only the CO₂ concentration in the external atmosphere, a qualitative measurement of carboxylation efficiency may still be made by calculating the initial slope of the CO₂ response curve.

The CO₂ saturation point. The CO₂ concentration beyond which the CO₂ response curve plateaus is called the CO₂ saturation point of photosynthesis. At this point increases in CO₂ concentration do not cause increases in photosynthetic rate, so other factors other than the supply of CO₂ must be limiting the photosynthetic process such as:

- i. The supply of light to the leaf providing the ATP and NADPH needed in the Calvin cycle for RuBP regeneration.
- ii. The amount, and turn-over rate, of enzymes involved in the Calvin cycle of photosynthesis (more investment in Rubisco can increase the CO₂ saturation point).

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V. ASSIGNMENT AND DISCUSSION QUESTIONS

NAME _____

- A. (4 pts) Graph showing relationship of carbon assimilation (Y axis) to various CO₂ concentrations (X axis).
- B. (2 pts) Explain why the CO₂ response of photosynthesis makes sense. From this data what would be the predicted effect of rising atmospheric CO₂ concentrations on plant productivity.
- C. (2 pts) Why is it important to measure the leaf area enclosed in the cuvette and the flow rate in order to obtain accurate estimate of photosynthesis?
- D. (2pts) In your opinion, what are the advantages/disadvantages for each of the 3 methods (O₂ evolution, chlorophyll fluorescence and gas exchange) we used to measure photosynthesis in this class.

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Place graph paper here