

## Measuring Gas Exchange during Photosynthesis

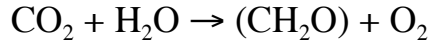
Russell Johnson

week #10 (08 November)

week #11 (15 November)

week #12 (29 November)

The basic reaction that occurs during photosynthesis can be summed up as:



A plant physiologist who wishes to measure the amount of photosynthesis that is taking place in a plant (or in isolated leaves, chloroplasts etc.) has a number of possible choices. The amount of new carbon incorporated into sugars can be measured, the amount of CO<sub>2</sub> used up can be measured, or the amount of O<sub>2</sub> produced can be measured. You will use a common method to measure the amount of O<sub>2</sub> that is given off by a photosynthetic tissue.

It is important to keep in mind that at the same time photosynthesis is occurring (to produce O<sub>2</sub>) respiration (both mitochondrial respiration and photorespiration) is also taking place and using up O<sub>2</sub>. As a result, the amount of O<sub>2</sub> production that you measure will represent net photosynthesis rather than total photosynthesis.

### Qubit photosynthesis system

The Qubit photosynthesis system uses an oxygen electrode to measure the concentration of oxygen that is present in a small chamber. By placing a leaf into the chamber and recording the rate at which the oxygen concentration increases, we can measure the rate of photosynthesis that is occurring.

Measure the rate of photosynthesis of a tobacco leaf using the following protocol:

1. Turn on the computer and open the Applications folder. Open the Logger Pro 3 folder, then open up the Experiments folder, and then inside that open the PH1LP Photosynthesis folder. Double click on PH1LP.cmb1 to start up the program. Choose **Page → Auto Arrange**. You should now have a data table on the left side of the screen, and two graphs on the right. The upper graph will display data from the O<sub>2</sub> sensor and the lower graph will display data collected from the light sensor. At the top left of the screen, the outputs of the two sensors will be displayed digitally.
2. The O<sub>2</sub> sensor should read 20.7% in normal air. If it does not read 20.7%, use a small screwdriver to adjust the screw on the O<sub>2</sub> amplifier box so that the display on the screen reads 20.7%. The oxygen sensor is now calibrated.
3. Set the switch on the light amplifier box to Medium.

4. Choose **Experiment** → **Calibrate** → **Light sensor** from the menus at the top of the computer screen. Select the calibration file that you want (eg. Med. White) then click on Calibrate Now. The light sensor is now calibrated.
5. With the light turned off, place a leaf (one that is still attached to a plant will probably work better) into the leaf chamber. Tighten the thumb screws to get a good seal.
6. Place a 200 mL beaker containing water on top of the leaf chamber. This will absorb the heat from the lamp and prevent your leaf from getting hot. The initial O<sub>2</sub> concentration should be close to 20.7% and the irradiance should be close to zero  $\mu\text{mol m}^{-2}\text{s}^{-1}$ .
7. Attach a hose to one of the gas ports on the leaf chamber and open up the other gas port. Fill up your lungs with air and then slowly exhale the air through the hose and into the leaf chamber. Seal both of the gas ports. The leaf chamber will now be filled with your exhaled air, which contains about 13-16% O<sub>2</sub> and 3-5% CO<sub>2</sub>.
8. Allow the O<sub>2</sub> reading on the computer screen to reach a stable value. This may take a few minutes.
9. When the O<sub>2</sub> reading has reached a steady value, turn on the light just long enough to make a note of the irradiance reading at the bottom of the computer screen, then turn it off again.
10. Adjust the range of values displayed on the axes of the upper O<sub>2</sub> graph so that the lowest value is about 0.5 units below that of your exhaled breath and the upper value is about 21%. To do this, click on the lowest value currently displayed on the graph. This value will become highlighted allowing you to type in a new number. Adjust the high O<sub>2</sub> value in the same manner.
11. Adjust the high value of the light response graph to a value 10 units above the value measured when you turned on the lamp. Adjust the time axis on both graphs to 20 minutes.
12. Now you are ready to make the actual measurements of the photosynthesis rate. Allow your leaf to be in the dark for a few minutes, then push the Green arrow start button to begin collecting data. Collect data in the dark for the first 4 minutes, then switch on the light and continue to collect data for the rest of the 20 minute period.
13. Use the **File** → **Save As** function to name your file and save it into the folder entitled Student data. Please make sure that you do NOT save any changes to the PH1LP.cmb1 file.
14. Determine the slope of the line, which will then give you the rate of photosynthesis (% O<sub>2</sub>/min). Use the mouse to select the relevant portion of your curve. Choose **Analyze** → **Linear fit** and the computer will tell you the slope.

*After illuminating the leaf, how long did it take before any increase in the O<sub>2</sub> concentration of the chamber was observed?*

*Why was there such a lag?*

*What was the rate of photosynthesis of your tobacco leaf in % O<sub>2</sub>/min?*

*Was it a constant rate?*

A more meaningful way to report the rate of photosynthesis is in  $\mu\text{mol O}_2 \text{ m}^{-2} \text{ min}^{-1}$ . That is, the actual amount of O<sub>2</sub> produced per unit of leaf area per unit time.

*Calculate the rate of photosynthesis in  $\mu\text{mol m}^{-2} \text{ min}^{-1}$ .*

The following information will be helpful:

$$1\% \text{ O}_2 = \frac{10,000 \mu\text{L O}_2}{\text{L gas}}$$

The volume of the chamber is 0.047 L

at STP 1  $\mu\text{mol}$  of a gas has a volume of 22.4  $\mu\text{L}$

You will also need to measure the area of the leaf that you used in the chamber.

## **Part II - Assessment of factors that influence photosynthesis**

Your mission for the next two lab periods (15 and 29 Nov) will be to design and carry out a series of experiments to test the effects of some factors that you think may have an influence on photosynthesis. The factors you test could be environmental factors, the presence of certain chemical compounds, or whatever you can think of that would be interesting to test. The experiments could be carried out using any whole plant or part of a plant that is able to fit into the leaf chamber of the Qubit system. Use whatever material is most appropriate for the experiments you want to do.

Write out a written (typed) research proposal outlining the experiments that you intend to do, any specific hypotheses that you will be testing, what controls you will be including, and any unusual equipment or supplies that you will need. Turn in this proposal by the end of lab on 8 November.

Do your experiments and *record your observations and results*. *In addition to the raw data that you collect, also include in your notebook some appropriate graphical representation of the data.*

On 6 December (the last day of lab) you and your lab partner will work together to present a 10 minute oral presentation on the results of your photosynthesis experiments. Computer projection facilities will be available for this presentation so that you may show figures and photographs to accompany your talk.

## **How to give a good presentation**

A short oral presentation at a scientific conference is a very common way for scientists to present their newest findings to other researchers. You will have a chance to give a presentation at a meeting of the “International Society for Photosynthesis Research and Training”, which will be held right here at Colby.

A good oral presentation (like a good paper) should explain your research in a manner that is clear, interesting, and scientifically correct. It is usually a good idea to divide your talk into sections such as:

Introduction

(Methods)

Results and Discussion

Conclusions

The Introduction should provide some general information about your experimental question and why it is important. You want to convince your audience right away that it will be worthwhile to keep paying attention.

Any explanation of Methods in your talk should be brief. Your talk is short and you don't want to use up much of your time on this.

The Results portion of your talk should be the longest and most important section. Here you will present and explain the results you obtained. You can discuss the significance of your findings and perhaps address how they compare to results other researchers have obtained.

The Conclusions can be a very brief wrap-up of what you have said in your talk.

All of the sections of your talk should be appropriately illustrated with clear slides that show what you are talking about. A picture can be worth a thousand words.

Timing is an important element to consider when preparing your presentation. If you go over the allotted 10 minutes, you will throw off the schedule of the conference and cause inconvenience for the other speakers. On the other hand, you want to fully utilize the time you are allotted so that you can explain your work as well as possible.