Photosynthesis/Respiration in Leaf Disks

Ruthanne B. Pitkin

Biology Department
Shippensburg University
Shippensburg, PA 17257
717-477-1752
Fax: 717-477-4064
rbpitk@ship.edu

Ruthanne B Pitkin graduated from the University of Massachusetts *cum laude* in Zoology, received a M.S. in Biological Oceanography from the University of Washington, and a Five-College Ph.D. in Zoology granted by the University of Massachusetts. She teaches introductory biology courses to majors and non-majors, animal physiology, neurobiology, and undergraduate and graduate seminars.

Background Information

I use this lab in a two-hour introductory majors' course, Principles of Biology II, which covers cell and molecular biology, genetics, and evolution. Over the years I have discovered that students come to this class "knowing" that plants have chloroplasts for photosynthesis, and animals have mitochondria for cellular respiration. My goal is to have the students understand that plants also have mitochondria and use cellular respiration as well as photosynthesis. This lab uses plants to illustrate both respiration and photosynthesis. The students also learn to estimate the photosynthetic and respiratory rates by creating scatterplot graphs with Excel and by calculating the rates after a visual inspection of the data.

The students prepare a lab plan before they come to class by writing the objectives, null hypotheses, predictions, and a brief overview of the procedures in their lab notebooks. In class, I go over the infiltration procedure and emphasize safety concerns to start the lab. You need to remind the students that the leaf disks can get caught in the surface tension of the bicarbonate solution so they need to "poke" the disks, especially during the demonstration of respiration.

I do help the students with how to plot data and calculate s. They have a difficult time

understanding that to calculate a rate you need to take the reciprocal of the ET50, so I spend time reminding them that the rate a car travels is in miles/hr, heart rate is in beats/minute, and osmotic rate they calculated previously was in g/min.

The students are required to record, plot, and analyze data, and then answer the questions in the lab manual in their lab notebooks. Some instructors require a formal IMRAD lab report from their students. The rubric that I use to grade this report is attached. The students are also expected to compare their photosynthesis rate with the ET50'2 one published by Steucek and Hill (1985).

We use English Ivy from my yard even in March or Swiss chard or lettuce from the grocery store and get good results. Make sure that you have 150-watt light bulbs so that reaction occurs in a reasonable time.

For extensions of this lab, you can try different light bulbs (i.e., red) for the lamps, put the lamps at different heights to simulate different light intensities, or put filters between the light and the beaker to see the effects of different colored light on photosynthesis and respiration.

Student Handout

Objectives
1. To investigate the role of light in photosynthesis.
2. To investigate respiration in plants.
3. To calculate and compare the effective time for 50% of the leaf disks to float.

Introduction

Photosynthesis is a series of reactions in which green plants and cyanobacteria convert the energy of sunlight into chemical bond energy of organic molecules such as monosaccharides. Cellular respiration is a series of reactions in eukaryotic cells that converts the energy in the bonds of organic molecules such as glucose into ATP molecules that are used to power many cellular activities. These complex reactions can be summarized as follows:

$$CO_2 + H_2O \overset{\text{Photosynthesis} \rightarrow}{\underset{\leftarrow \text{Respiration}}{\rightleftharpoons}} (CH_2O)_n + O_2$$
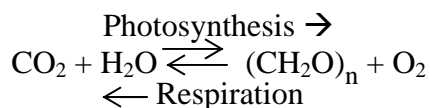
Basic Experimental Design

There are complex biochemical techniques to study photosynthesis and cellular respiration. However, we can follow the rate of photosynthesis by simply estimating the rate of oxygen production in disks of leaf tissue and the rate of respiration by estimating the amount of oxygen used during cellular respiration. To estimate the changes in the amount of oxygen in the intracellular spaces of leaf disks, first the leaf disks are vacuum infiltrated, i.e., the intracellular air in the leaf disks is vacuumed off, and the spaces are infiltrated with liquid. As photosynthesis proceeds, the oxygen gas produced will displace the liquid from the intracellular space, and the specific gravity will decrease, and the disks will float. The time required for the disks to float is inversely related to the rate of photosynthesis. The source of the carbon dioxide in this process is the bicarbonate solution.

Respiration occurs in all cells all the time, but we can only measure it in plants that are not

photosynthesizing. You will need to experimentally stop photosynthesis in your leaf disks to measure respiration. Think about the role of oxygen in respiration. How will cellular respiration influence the floating leaf disc assay for photosynthesis? How can you demonstrate that cellular respiration is occurring? What is the role of oxygen in cellular respiration?

You will plot the percent leaf disks floating versus time to be able to calculate the rates of respiration and photosynthesis. Remember that a rate is a change in a variable over time. The time required for 50% of the leaf disks to float (effective time = ET_{50}) is a number that will account for "early floaters" and "late floaters." To estimate the rates, you take the reciprocal of a change over time. For example: if the time for respiration is 10 minutes, then the estimated rate of respiration is $1/ET_{50resp}. = 1/10$ min. $= 0.10$ min^{-1}

Investigations

Investigation #1: Is photosynthesis light dependent?
Include in your lab notebook a NULL HYPOTHESIS, PREDICTION, and PROCEDURE.

Pour approximately 100mL of infiltration solution into a 250mL flask. Cut 20-22 leaf disks with a paper punch and put directly into the flask. Do not include the large veins. Note that the disks float. Why?

Infiltrate the disks by vacuuming the gas from the flask using the aspirators at the sinks. Make sure that the tape covers the hole and turn on the cold water. When the solution begins to "boil", break the vacuum suddenly by peeling back the tape. Swirl the flask to see if the disks sink. Why do they sink? Repeat the vacuum and breaking the vacuum until the leaf disks sink. Store disks in the dark until you are ready to start the experiment.

Put approximately 30mL of bicarbonate solution in the bottoms of two glass Petri dishes. Transfer half of the leaf disks that sank into each dish. If you put a piece of cheesecloth over the Erlenmeyer flask and then pour out the infiltration solution, you will have the disks trapped on the cheesecloth. After your transfer the disks to the Petri dishes, discard any that did not sink. Cover one dish with aluminum foil. This is your control. For your experimental disks, place the cover of the Petri dish facing upwards on top of the Petri dish with solution and disks, place a 600mL beaker with tap water on top of the cover, and position the goose-necked lamp so that the light shines through the beaker onto the leaf disks. Observe (at the height of the Petri dish) the disks in <u>both</u> Petri dishes at specific time intervals (1 to 2 minutes), and record the number of disks floating. Poke with blunt probe to see if caught in surface tension of the water. You must decide your criteria for floating, i.e., the disks are on their side or floating on the surface of the bicarbonate solution.

Data
Calculate the % leaf disks floating (%floating = # of floating disks/total # of leaf disks X 100) and plot them as a function of time for both the disks exposed to light and those kept in the dark. You can use Excel Scatterplot option that allows you to connect the data points.

From your graph, determine the time required for 50% of the leaf disks to float or the $ET_{50light}$, the effective time for 50% of the disks to float. This time $(1/ET_{50})$ in minutes^{-1} can be used as a rough estimate to compare rates of photosynthesis. Do not assume that this is a linear relationship. See the figure in Steucek and Hill (1985).

Can you support or reject your hypothesis? Explain. What is the source of carbon dioxide for the

photosynthesis in the leaf disks?

Investigation #2: Do plants respire?

Include in your lab notebook a NULL HYPOTHESIS, PREDICTION, AND PROCEDURE. (Hint: you can continue with the disks from investigation #1 that are already floating. Think about what happens if photosynthesis is not occurring, but cellular respiration continues.)

Data

Plot % disks floating (not % disks sinking) as a continuation of the line from the graph of the data from investigation #1. Label "in light" and "in dark" on your figure.

Determine the ET_{50resp} on your figure. Remember this is the time for 50% to sink after the leaf disks were transferred to the dark conditions. Because respiration occurs in both the light and dark, the rate of photosynthesis is the sum of the rate in the light plus the respiration rate.

$$1/ET_{50light} + 1/ET_{50resp} = 1/ET_{50ps}$$

Rearranging, you get the following:

$$1/ET_{50light} = 1/ET_{50ps} - 1/ET_{50resp}$$

What is the rate of photosynthesis for your experiment?
Can you support or reject your hypothesis?

Adapted from:

Juliao, F. and H.C. Butcher IV. 1989. Further improvements to the Steucek and Hill assay of photosynthesis. Am. Bio. Teacher 51(3): 174-176.

Steucek, G.L. and R.J. Hill. 1985. Photosynthesis I: an assay utilizing leaf disks. Am. Bio. Teacher 47(2): 96-99.

Materials List

Plants: fresh leaves from rapidly growing plants (no geraniums or succulents); Swiss chard, spinach, beans (English ivy or Swiss chard work well)

Per pair of student:
- Paper punch
- 2 glass Petri dishes
- 600mL beaker
- Goose-necked lamp with 150 watt light bulb
- Aluminum foil
- 250mL Erlenmeyer flask with cheesecloth and rubber band
- Small plastic graduated cylinder
- Blunt probe
- Stop watches

At each sink:
- Aspirators with #8 two-holed stoppers with tape over one hole at each sink
  (Carolina ER-71-1974 Nalgene Polypropylene Vacuum Filter Pump attaches to your sink faucet and only cost about $9 each. No need for pump.)

For each bench of 4 students:
- 250 mL infiltration solution in a bottle or flask

- 250 mL bicarbonate solution in a bottle or flask

How to make solutions:

- 0.1 M citric acid: dissolve 19.2 g of anhydrous citric acid in water and bring up to 1 liter
- 0.2 M Na_2HPO_4: dissolve 28.4 g of anhydrous dibasic sodium phosphate in water and bring up to 1 liter
- pH 6.8 Buffer: add 182 mL of 0.1 M citric acid to 618 mL of 0.2 M Na_2HPO_4 and 200 mL of distilled water
- Infiltration solution: add 10 drops of Tween-80 to 1000 mL of buffer
- Bicarbonate solution: add 2 g of $NaHCO_3$ to 1000 mL of buffer (no Tween)

Addendum

I have included a figure and sample calculations for the convenience of the instructors. These are from data collected by students.
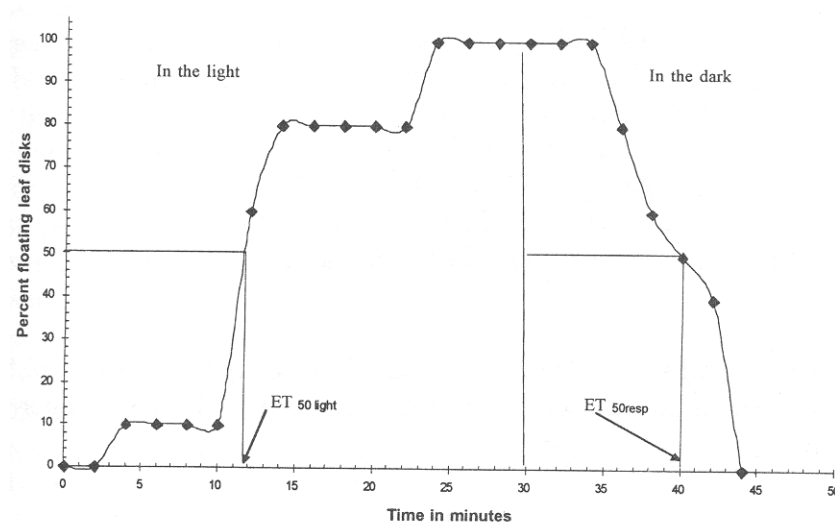

Figure 1. Percent floating Ivy leaf disks incubated in bicarbonate solution in the light and transferred to the dark at 30 minutes

Example of Calculations

Respiration Rate

ET_{50resp} = 40 min - 30 minutes = 10 minutes

$1/ ET_{50resp}$ = 1/10min = 0.10 min^{-1}

Photosynthesis Rate

$ET_{50light}$ = 11.5 min

$1/ ET_{50light}$ = 1/11.5 min = 0.09 min^{-1}

$1/ET\ 50\ ps$ = $1/ ET_{50light}$ + / ET_{50resp} = 0.10 min^{-1} + 0.09 min^{-1} = 0.19 min^{-1}

Photosynthesis rate is 2.1 x $10^{-1}min^{-1}$