

Name:

Photosynthesis Inquiry

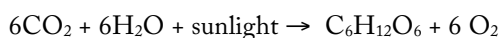
Problem:

Design an experiment to quantify the effect of an environmental variable (ex. pH, temperature, light intensity, age of leaves, etc.) on the rate of photosynthesis.

Background:

Photosynthesis is an anabolic process used by all photoautotrophs to capture light energy and convert it to the chemical energy in carbohydrates. It can be measured in a variety of ways.

Although numerous intermediary reactions are involved, the overall photosynthetic reaction is simple: carbon dioxide combines with the hydrogen from water producing a carbohydrate—the six carbon sugar glucose—and oxygen gas.



The photosynthetic production of oxygen and knowledge of leaf anatomy will allow for the construction of a simple system that can be used to experimentally investigate many of the photosynthetic variables. Many extracellular spaces exist within plant leaves that are normally filled with air for purposes of gas exchange. This is why leaves will float on the surface of bodies of water. But would you happen if all the air is forced out of the air spaces in the leaf? What will the leaf do then?

If basic requirements for photosynthesis are supplied, the oxygen the leaf produces will form gas bubbles and the leaf would re-float. In essence this is the experimental method, however, small disks cut from leaves will be used instead of whole leaves to perform the floating leaf disk assay (FLDA). This assay of photosynthesis may be used to answer many questions, including: How do changes in light intensity, wavelength, or CO₂ concentration affect the rate of photosynthesis?

One problem in measuring a rate of photosynthesis is that there is a competing process occurring at the same time, cellular respiration, a process that uses oxygen. The FLDA actually measures the rate of photosynthetic oxygen production minus the rate of respiratory oxygen use during the same period. The FLDA measures the net rate of photosynthesis, the energetic “profit” made by the plant. Actual photosynthetic activity is greater than this and is called the gross rate of photosynthesis. If cellular respiration can be measured separately, a simple calculation can determine gross photosynthesis.

Materials:

- Sodium bicarbonate (Baking soda)
- Liquid Soap
- Plastic syringe (10 cc or larger)—remove any needle!
- Leaf material
- Hole punch
- Plastic cups
- Timer
- Group specific materials as needed
- Light source

The Floating Leaf Disk Assay for Photosynthetic Activity:

For this experiment, you will use the “Floating Leaf Disk Assay” for photosynthetic activity. A detailed protocol for this procedure is attached. Essentially, the assay follows the following steps:

1. Small disk sections of leaf tissue are punched from a leaf.

2. The disks are infiltrated with a bicarbonate solution. The infiltration serves two major purposes:
 - a. It increases the density of the leaf disks so that they sink
 - b. It supplies the disks with a carbon source (the bicarbonate ion) for the purpose of photosynthesis.
3. After infiltration, the disks are placed at the bottom of a container (we will use petri dishes) of the bicarbonate solution.
4. When exposed to light, the disks will produce Oxygen gas, decreasing their density to the point that they will float to the top of the container.
5. The time it takes for disks to float is directly related to the rate of photosynthetic activity taking place in the leaf disks.

Procedural Constraints & Suggestions:

The Need for A Control:

You wouldn't want to run an experiment without (at least one control). That would be super silly.

Determination of the rate of Photosynthesis AND the Rate of Respiration:

The rate of respiration needs to be determined in order to calculate the rate of gross photosynthesis

Steps to complete before beginning your experiment:

- Develop a detailed protocol & clear experimental plan for your experiment.
- Develop a data table for your experiment.
- Determine any calculations that will be necessary for your data.
- Think about how you will graph your results (at least one graph is required for this lab).

After reviewing your protocol and discussing any safety hazards, your instructor must approve your protocol before you can conduct your experiment.

Analysis Questions (to be answered in your report following your conclusion):

1. Why was the soap needed in the protocol?
2. Explain the specific purpose of the Bicarbonate Ions in the leaf disk photosynthesis (in other words, what molecule is Bicarbonate standing in for).
3. What is the source of electrons for the leaf disk photosynthesis?
4. Propose two other ways of measuring the rate of photosynthesis in a photoautotroph (any organism you want).
5. What relationship do you see between the PS ET-50 and time? What relationship do you observe between the RS ET-50 and time?
6. Why is it important to use the average rate of photosynthesis (or respiration)?
7. What factors had the largest effect on photosynthetic rate (intergroup collaboration alert!)? Why do you think this?
8. What is the importance of establishing a control for this experiment?
9. Why must we consider respiration when performing this activity?
10. Why is it important to study photosynthetic rate of plants?

The Floating Leaf Disk Assay for Investigating Photosynthesis

Brad Williamson



Figure 1: Materials needed for assay

Introduction:

Trying to find a good, quantitative procedure that students can use for exploring photosynthesis is a challenge. The standard procedures such as counting oxygen bubbles generated by an elodea stem tend to not be “student” proof or reliable. This is a particular problem if your laboratory instruction emphasizes student-generated questions. Over the years, I have found the floating leaf disk assay technique to be reliable and understandable to students. Once the students are familiar with the technique they can readily design experiments to answer their own questions about photosynthesis.

The biology behind the procedure:

Leaf disks float, normally. When the air spaces are infiltrated with solution the overall density of the leaf disk increases and the disk sinks. The infiltration solution includes a small amount of Sodium bicarbonate. Bicarbonate ion serves as the carbon source for photosynthesis. As photosynthesis proceeds oxygen is released into the interior of the leaf which changes the buoyancy--causing the disks to rise. Since cellular respiration is taking place at the same time, consuming oxygen, the rate that the disks rise is an indirect measurement of the net rate of photosynthesis.

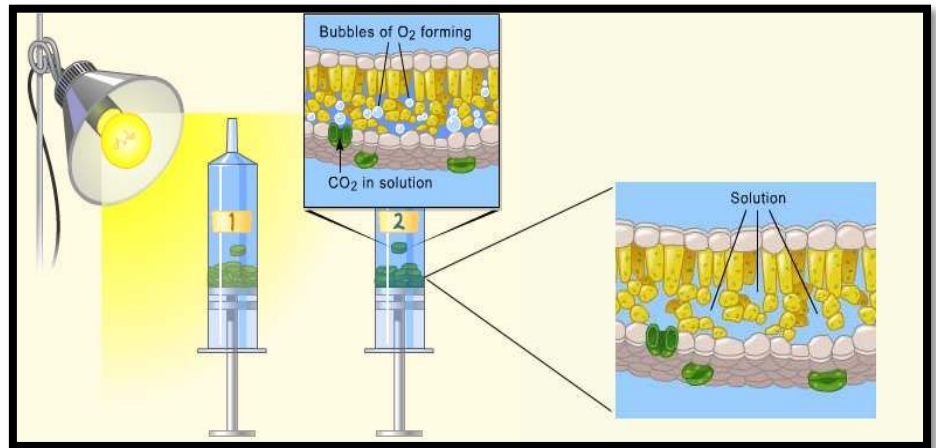


Figure 2: Diagram of the process in a syringe

Procedure:

1. The bicarbonate serves as an alternate dissolved source of carbon dioxide for photosynthesis. Prepare a 0.2% solution. (This is not very much it is only about 1/8 of a teaspoon of baking soda in 300 ml of water.)
2. Add 1 drop of dilute liquid soap to this solution. The soap wets the hydrophobic surface of the leaf allowing the solution to be drawn into the leaf. It's difficult to quantify this since liquid soaps



Figure 2: Preparation of 0.2% bicarbonate solution

vary in concentration. Avoid suds. If your solution generates suds then dilute it with more bicarbonate solution.

3. Cut 10 or more uniform leaf disks for each trial. Single hole punches work well for this but stout plastic straws will work as well.
4. Choice of the leaf material is perhaps the most critical aspect of this procedure. The leaf surface should be smooth and not too thick. Avoid plants with hairy leaves. Ivy, fresh spinach, Wisconsin Fast Plant cotyledons--all work well. Ivy seems to provide very consistent results. Many different plant leaves work for this lab. My classes have found that in the spring, Pokeweed may be the best choice. Avoid major veins.
5. Infiltrate the leaf disks with sodium bicarbonate solution.
 - a. Remove the piston or plunger and place the leaf disks into the syringe barrel.
 - b. Replace the plunger being careful not to crush the leaf disks. Push on the plunger until only a small volume of air and leaf disk remain in the barrel (< 10%).
 - c. Pull a small volume of sodium bicarbonate solution into the syringe. Tap the syringe to suspend the leaf disks in the solution
 - d. Holding a finger over the syringe-opening, draw back on the plunger to create a vacuum. Hold this vacuum for about 10 seconds. While holding the vacuum, swirl the leaf disks to suspend them in the solution.
 - e. Let off the vacuum. The bicarbonate solution will infiltrate the air spaces in the leaf causing the disks to sink.
 - f. You will probably have to repeat this procedure 2-3 times in order to get the disks to sink. If you have difficulty getting your disks to sink after about 3 evacuations, it is usually because there is not enough soap in the solution. Add a few more drops of soap.
6. Pour the disks and solution into a clear plastic cup. Add bicarbonate solution to a depth of about 3 centimeters. Use the same depth for each trial. Shallower depths work just as well.
7. For a control infiltrate leaf disks with a solution of only water with a drop of soap--no bicarbonate.
8. Place under the light source and start the timer. At the end of each minute, record the number of floating disks. Then swirl the disks to dislodge any that are stuck against the sides of the cups. Continue until all of the disks are floating.



Figure 3: Don't forget your soap!

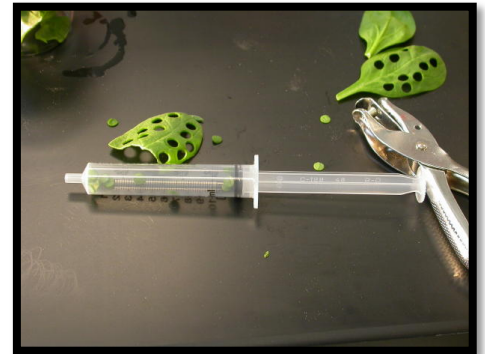


Figure 4: Make sure you choose your leaves, and punch locations wisely!

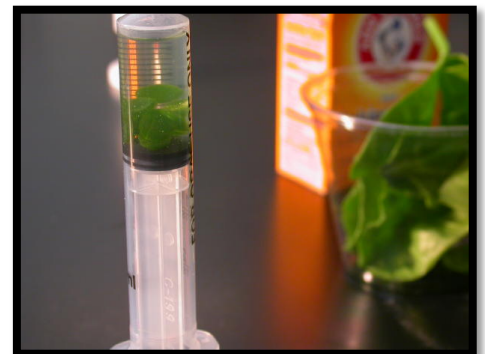


Figure 5: Infiltrated leaf disks. Note the sinkage!



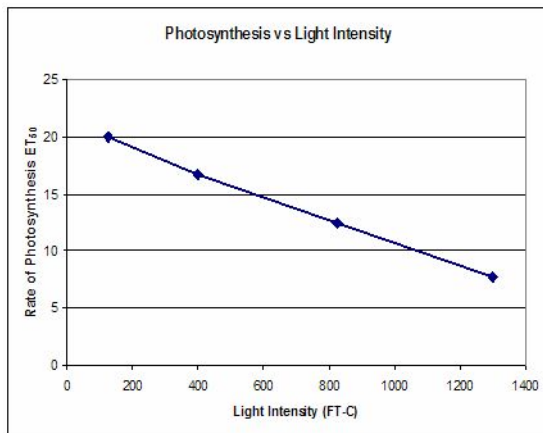
Figure 6: Floating Disks!

Notes on Data Collection and Analysis

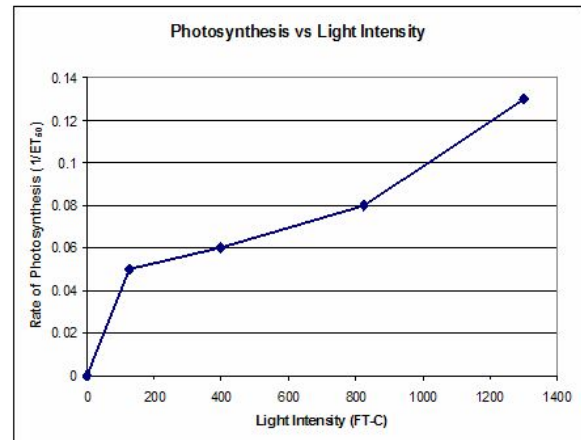
The point at which 50% of the leaf disks are floating (the median) is the point of reference for this procedure. Using the 50% point provides a greater degree of reliability and repeatability for this procedure. As Steucek, et. al. (1985) described this term is referred to as the ET50.

The problem with ET50 is that it goes down as the rate of photosynthesis goes up--it is an inverse relationship and creates the following type of graph (Graph 1: data from Steucek, et al. 1985.):

To correct for this representation of the data and present a graph that shows increasing rates of photosynthesis with a positive slope the ET50 term can be modified by taking the inverse or $1/ET_{50}$. This creates a graph like this (Graph 2: data from Steucek, et al. 1985.):



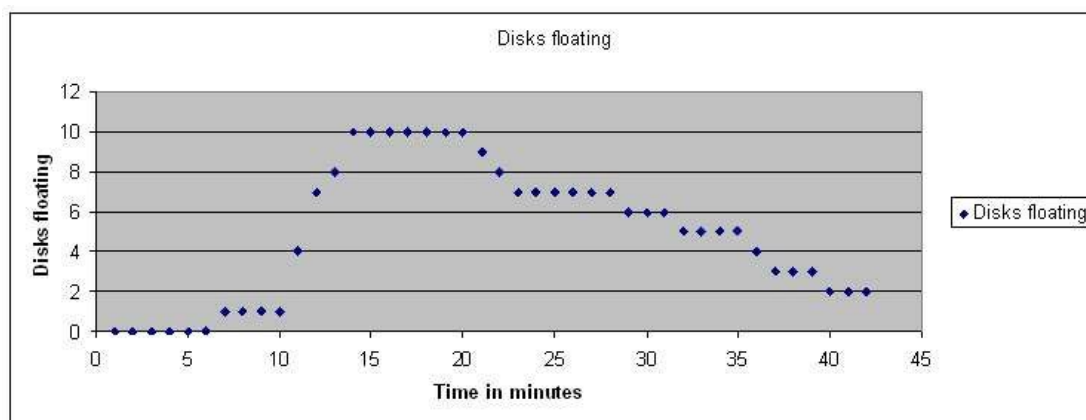
Graph 1: ET50 Data vs. Light Intensity



Graph 2: $1/ET_{50}$ Data vs. Light Intensity

Extension:

In this graph, the light was turned off at 14 minutes and the cups with their floating disks (grape ivy) were placed in the dark.



Graph 3: Disks floating vs. Time (light turned off at 14 minutes)

Every minute, I removed the dark cover and counted how many were still floating. Then I stirred the disks. Note that after a while the disks begin to sink. Why? Cellular respiration removes the oxygen from the cell spaces. The rate that the disks sink is an indirect measure of the rate of cellular respiration. Can you think of a way to how you might measure the gross rate of photosynthesis with this technique?

Print and Web Resources:

Wickliff, J.L. and Chasson, R.M. 1964. Measurement of photosynthesis in plant tissues using bicarbonate solutions. *Bioscience*, 14: 32-33.

Steucek, Guy L. Robert J. Hill and Class/Summer 1982. 1985. Photosynthesis I: An Assay Utilizing Leaf Disks. *The American Biology Teacher*, 47(2):96-99.

Tatina, Robert E. 1986. Improvements to the Steucek and Hill Assay of Photosynthesis. *The American Biology Teacher*, 48(6): 364-366.

Juliao, Fernando and Henry C. Butcher IV. 1989. Further Improvements to the Steucek and Hill Assay of Photosynthesis. *The American Biology Teacher*, 51(3): 174-176.

Armstrong, Joseph E. 1995. Investigation of Photosynthesis using the Floating Leaf Disk Assay. <http://www.bio.ilstu.edu/Armstrong/biolab/cellbio/psynex1.htm>

Rukes, Kari L. and Timothy J. Mulkey. 1994. Measurement on the Effects of Light Quality and Other Factors on the Rate of Photosynthesis. *Bioscene*, 20(3): 7-11. http://www.acube.org/volume_20/v20-3p7-11.pdf

Greenler, John. 1990. Exploring Photosynthesis with Fast Plants. *WisconsinFast Plant Notes*, 4(1): 4-5. http://www.fastplants.org/pdf/activities/exploring_photosynthesis.pdf

BioPi listserv archives. <http://listserv.ksu.edu/archives/biopi-1.html>

Enter the "Leaf Disks" for a search to review a thread on the technique. Dan Mott attached a copy of his lab using this technique to one of his postings.

Richard, David S. Measure of Photosynthetic Rate In Spinach Leaf Disks <http://www.susqu.edu/FacStaff/r/richard/photosynthlab.html>