

The Floating Leaf Disk Assay for Investigating Photosynthesis

Background:

Photosynthesis fuels ecosystems and replenishes the Earth's atmosphere with oxygen. Like all enzyme-driven reactions, the rate of photosynthesis can be measured by either the disappearance of substrate or the accumulation of product (or by-products).

The general summary equation for photosynthesis is



What could you measure to determine the rate of photosynthesis?

- Production of O₂ (How many moles of O₂ are produced for one mole of sugar synthesized?)
- Consumption of CO₂ (How many moles of CO₂ are consumed for every mole of sugar synthesized?)

In this investigation, you will use a system that measures the accumulation of oxygen.

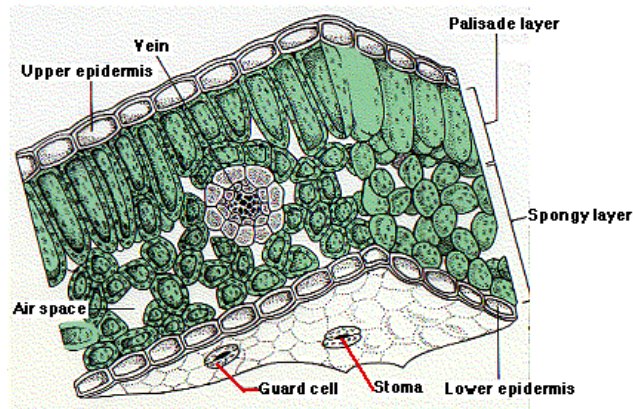


Figure 1. Leaf Anatomy

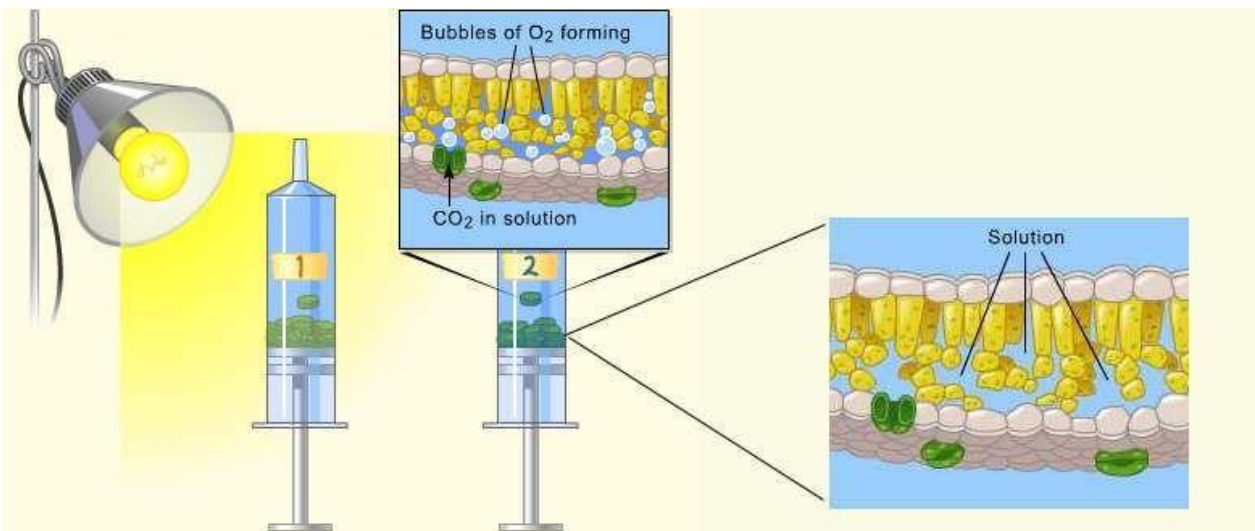
Because the spongy mesophyll layer of leaves (shown in Figure 1) is normally infused with gases (O₂ and CO₂), leaves — or disks cut from leaves — normally float in water. What would you predict about the density of the leaf disk if the gases are drawn from the spongy mesophyll layer by using a vacuum and replaced with water? How will that affect whether or not the leaf floats? If the leaf disk is placed in a solution with an alternate source of carbon dioxide in the form of bicarbonate ions, then photosynthesis can occur in a sunken leaf disk. As photosynthesis proceeds, oxygen accumulates in the air spaces of the spongy mesophyll, and the leaf disk will once again become buoyant and rise in a column of water. Therefore, the rate of photosynthesis can be indirectly measured by the rate of rise of the leaf disks. However, there's more going on in the leaf than that! You must also remember that cellular respiration is taking place at the same time as photosynthesis in plant leaves. (Remember that plant cells have mitochondria, too!) What else could be going on that might affect this process? Aerobic respiration will consume oxygen that has accumulated in spongy mesophyll. Consequently, the two processes counter each other with respect to the accumulation of oxygen in the air spaces of the spongy mesophyll. So now you have a more robust measurement tool — the buoyancy of the leaf disks is actually an indirect measurement of the net rate of photosynthesis occurring in the leaf tissue.

Learning Objectives

- To design and conduct an experiment to explore the effect of certain factors, including different environmental variables, on the rate of cellular photosynthesis
- To connect and apply concepts, including the relationship between cell structure and function (chloroplasts); strategies for capture, storage, and use of free energy; diffusion of gases across cell membranes; and the physical laws pertaining to the properties and behaviors of gases

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.



Materials:

- Sodium bicarbonate (Baking soda)
- Liquid Soap
- Plastic syringe (10 cc or larger)
- Leaf material
- Hole punch
- Plastic cups
- Timer
- Light source
- Optional: Buffer Solutions Colored Cellophane or filters
- Leaf material of different ages
- Variegated leaf material
- Clear Nail polish



Procedure:

1. Prepare 300 ml of bicarbonate solution for each trial.

- a. 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.)



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



- c. 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.
- d. 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. Avoid major veins.

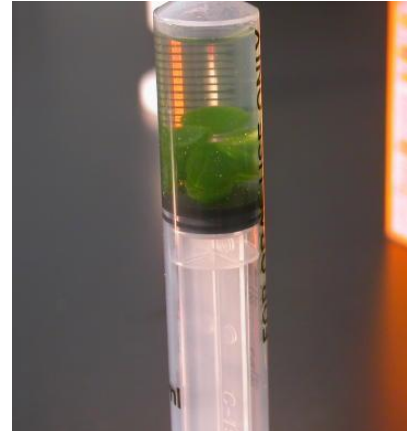


2. Infiltrate the leaf disks with sodium bicarbonate solution.

- a. Remove the piston or plunger and place the leaf disks into the syringe barrel. 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%).



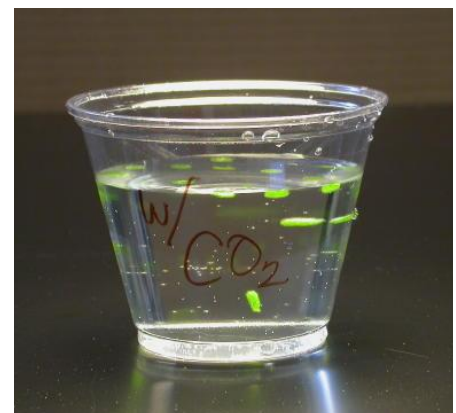
- b. Pull a small volume of sodium bicarbonate solution into the syringe. Tap the syringe to suspend the leaf disks in the solution.
- c. Holding a finger over the syringe-opening, draw back on the plunger to create a vacuum. Hold this vacuum for about 10 seconds. While holding the vacuum, swirl the leaf disks to suspend them in the solution. Let off the vacuum. The bicarbonate solution will infiltrate the air spaces in the leaf causing the disks to sink. 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.



- d. 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.

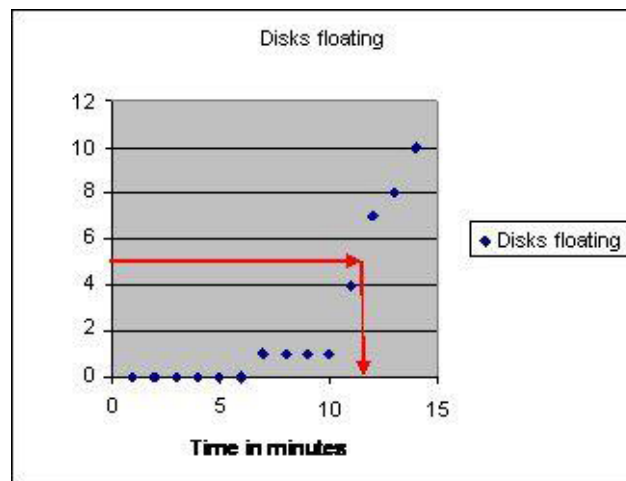


- e. For a control, infiltrate leaf disks with a solution of only water with a drop of soap--no bicarbonate. Place under the light source and start the timer. At the end of each minute, record the number of floating disks. Then swirl the disks to dislodge any that are stuck against the sides of the cups. Continue until all of the disks are floating.

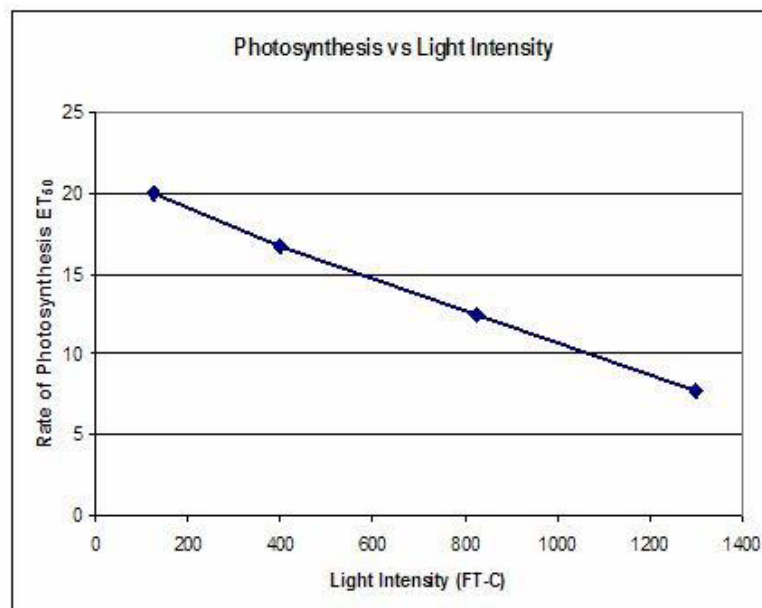


Data Collection and Analysis

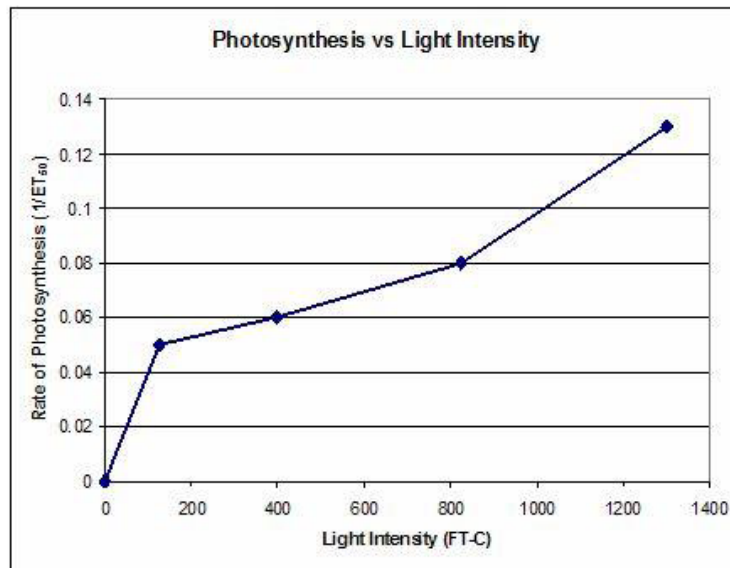
- Data will be collected every minute until all leaf disks are floating (this time will be a maximum of 25 minutes)
- After data is recorded for each minute, swirl the disks in the cup gently to remove any disks stuck to the sides of the cup
- At the conclusion of the lab, determine the point at which 50% of the leaf disks are floating (the median). This is the point of reference for this procedure.
 - o Example: By extrapolating from the graph, the 50% floating point is about 11.5 minutes. Using the 50% point provides a greater degree of reliability and repeatability for this procedure.
 - o As Steucek, et. al. (1985) described this term is referred to as the ET_{50} .



- o The problem with ET_{50} is that it goes down as the rate of photosynthesis goes up--it is an inverse relationship and creates the following type of graph (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 ET_{50} term can be modified by taking the inverse or $1/ET_{50}$. This creates a graph like this (data from Steucek, et al. 1985.):



Designing and Conducting Your Investigation

What factors affect the rate of photosynthesis in living plants?

1. Using the floating disk technique, you will design an experiment to test a variable that might affect the rate of photosynthesis. Some ideas include the following, but don't limit yourself to just these:
 - a. What environmental variables might affect the net rate of photosynthesis? Why do you think they would affect it? How do you predict they would affect it?
 - b. What features or variables of the plant leaves might affect the net rate of photosynthesis? How and why?
 - c. Could the way you perform the procedure affect the outcome? If the outcome changes, does it mean the net rate of photosynthesis has changed? Why do you think that?

Note: If you are truly stumped, your instructor can give you some guidance. Keep in mind that leaves with hairy surfaces should be avoided. Ivy and spinach work well, but many others do as well. Differences between plants may be one of the ideas that you want to investigate.

2. Use the rubric to determine the components that need to be in your lab notebooks.

Additional Guidelines

1. Consider combining variables as a way to describe differences between different plants. For instance, if you investigate how light intensity affects the rate of photosynthesis, you might generate a “photosynthesis light response curve”—the rate of photosynthesis at different light intensities. The shape of this curve may change for different plants or plants in different light environments. The “light response curve” is a form of measurement itself. How do you think a light response curve (the first variable) for a shade-grown leaf compares to that of a sun-grown leaf? In this situation, sun versus shade is the second variable. Comparing light response curves is a standard research technique in plant physiological ecology.
2. When you compare the ET_{50} across treatments, you will discover that there is an inverse relationship between ET_{50} and the rate of photosynthesis — ET_{50} goes down as rate of photosynthesis goes up, which plots a graph with a negative slope. This creates a seemingly backward graph when plotting your ET_{50} data across treatments. To correct this representation and make a graph that shows increasing rates of photosynthesis with a positive slope, the ET_{50} term can be modified by taking its inverse, or $1/ET_{50}$. This creates a more traditional direct relationship graph.