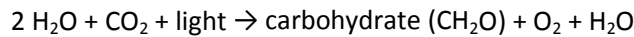


## Photosynthesis Lab

### 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

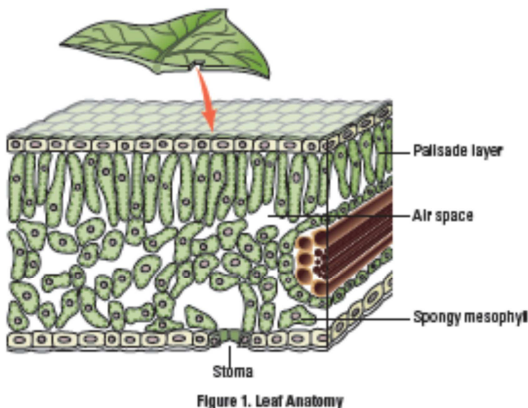


*What could you measure to determine the rate of photosynthesis?*

- Production of O<sub>2</sub> (How many moles of O<sub>2</sub> are produced for one mole of sugar synthesized?) or
- Consumption of CO<sub>2</sub> (How many moles of CO<sub>2</sub> are consumed for every mole of sugar synthesized?)

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

Because the spongy mesophyll layer of leaves (shown in Figure 1) is normally infused with gases (O<sub>2</sub> and CO<sub>2</sub>), 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.

### Variables Affecting Rate of Photosynthesis

Environmental Variables	Plant or Leaf Variables	Method Variables <small>(These variables may not affect photosynthesis but are still important to investigate.)</small>
<ul style="list-style-type: none"> <li>• Light intensity (brightness)</li> <li>• Light color (How can you explain that plants are green and that chlorophyll does not absorb green light?)</li> <li>• Temperature</li> <li>• Bicarbonate concentration (CO<sub>2</sub> source)</li> <li>• Direction of incoming light</li> <li>• pH of solution</li> </ul>	<ul style="list-style-type: none"> <li>• Leaf color (chlorophyll amount)</li> <li>• Leaf size</li> <li>• Stomata density</li> <li>• Stomata distribution</li> <li>• Light-starved leaves vs. leaves kept in bright light</li> <li>• Type of plant</li> <li>• Leaf age</li> <li>• Leaf variegation</li> <li>• Role of respiration in plants along with photosynthesis —measuring gross photosynthesis</li> </ul>	<ul style="list-style-type: none"> <li>• Size of leaf disk</li> <li>• Depth of bicarbonate solution</li> <li>• Methods of cutting disks</li> <li>• Leaf disk overlap</li> <li>• Soap amount</li> <li>• How many times can the procedure be repeated with the same disks?</li> <li>• How long can the disks remain sunk in the solution — can they be stored overnight?</li> <li>• Method of collecting data</li> </ul>

## Procedure

In this part of the lab, you will learn how the floating leaf disk technique can measure the rate of photosynthesis by testing a variable that you know affects photosynthesis. Later, you will apply this technique to test a variable that you choose. It is important for you to develop a few skills during this part of the investigation in order to carry out your own investigation. For the floating disk technique, the most challenging skill is getting the disks to sink. Don't just watch someone do this; make sure you can get the disks to sink as well.

## Materials

- Baking soda (sodium bicarbonate)
- Liquid soap (approximately 5 mL of dishwashing soap in 250 mL of water)
- 2 plastic syringes without needle (10 mL or larger)
- Living leaves (spinach, ivy, etc.)
- Hole punch
- 2 clear plastic cups
- Timer
- Light source

When immersed in water, oxygen bubbles are usually trapped in the air spaces of the spongy mesophyll in the plant leaf. By creating a vacuum in this experimental procedure, the air bubbles can be drawn out of the spongy mesophyll, and the space is refilled by the surrounding solution. This allows the leaf disks to sink in the experimental solution. If the solution has bicarbonate ions and enough light, the leaf disk will begin to produce sugars and oxygen through the process of

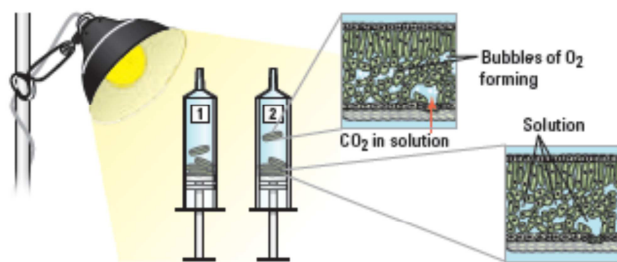


Figure 3. Photosynthesis at Work

photosynthesis. Oxygen collects in the leaf as photosynthesis progresses, causing the leaf disks to float again. The length of time it takes for leaf disks to float again is a measure of the net rate of photosynthesis. This is shown in Figure 3.

*Question: If the leaf disks are treated in a way you know increases the net rate of photosynthesis, should they start to float faster or slower? Why?*

**Step 1** Read the procedures.

**Step 2** Pour 0.2% bicarbonate solution into a clear plastic cup to a depth of about 3 cm. Label this cup "With CO<sub>2</sub>." Fill a second cup with only water to be used as a control group. Label this cup "Without CO<sub>2</sub>." Throughout the rest of the procedure you will be preparing material for both cups, so do everything for both cups simultaneously.

**Step 3** Using a pipette, add one drop of a dilute liquid soap solution (5 ml soap in 250ml of water) to the solution in each cup. It is critical to avoid suds. If either solution generates suds, then dilute it with more bicarbonate (or water) solution. The soap acts as a surfactant or "wetting agent" — it wets the hydrophobic surface of the leaf, allowing the solution to be drawn into the leaf and enabling the leaf disks to sink in the fluid.

**Step 4** Using a hole punch, cut 10 or more uniform leaf disks for each cup. Avoid major leaf veins. (The choice of plant material is perhaps the most critical aspect of this procedure. The leaf surface should be smooth and not too thick.)

**Step 5** Draw the gases out of the spongy mesophyll tissue and infiltrate the leaves with the sodium bicarbonate solution by performing the following steps:

- Remove the piston or plunger from both syringes. Place the 10 leaf disks into each syringe barrel.
- Replace the plunger, but be careful not to crush the leaf disks. Push in the plunger until only a small volume of air and leaf disk remain in the barrel (<10%).

c. Pull a small volume (5 cc) of sodium bicarbonate plus soap solution from your prepared cup into one syringe and a small volume of water plus soap into the other syringe. Tap each syringe to suspend the leaf disks in the solution. Make sure that, with the plunger inverted, the disks are suspended in the solution. Make sure no air remains. Move the plunger to get rid of air from the plunger before you attempt Step d.

d. You now want to create a vacuum in the plunger to draw the air out of the leaf tissue. This is the most difficult step to master. Once you learn to do this, you will be able to complete the entire exercise successfully. Create the vacuum by holding a finger over the narrow syringe opening while drawing back the plunger (see Figure 6a). Hold this vacuum for about 10 seconds.

While holding the vacuum, swirl the leaf disks to suspend them in the solution. Now release the vacuum by letting the plunger spring back. The solution will infiltrate the air spaces in the leaf disk, causing the leaf disks to sink in the syringe. If the plunger does not spring back, you did not have a good vacuum, and you may need a different



Figure 6a. Creating a Vacuum in the Plunger



Figure 6b. Sinking Leaf Disks

syringe. You may have to repeat this procedure two to three times in order to get the disks to sink. (If you have any difficulty getting your disks to sink after three tries, it is usually because there is not enough soap in the solution. Try adding a few more drops of soap to the cup and replacing the liquid in the syringe.) Placing the disks under vacuum more than three times can damage the disks.

**Step 6** Pour the disks and the solution from the syringe into the appropriate clear plastic cup. Disks infiltrated with the bicarbonate solution go in the “With CO<sub>2</sub>” cup, and disks infiltrated with the water go in the “Without CO<sub>2</sub>” cup.

**Step 7** Place both cups 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 stuck against the side of the cups. Continue until all of the disks are floating in the cup with the bicarbonate solution.

**Step 8** To make comparisons between experiments, a standard point of reference is needed. Repeated testing of this procedure has shown that the point at which 50% of the leaf disks are floating (the median or ET<sub>50</sub>, the **Estimated Time it takes 50% of the disks to float**) is a reliable and repeatable point of reference for this procedure because, on occasion, a disk fails to rise or takes a very long time to do so.

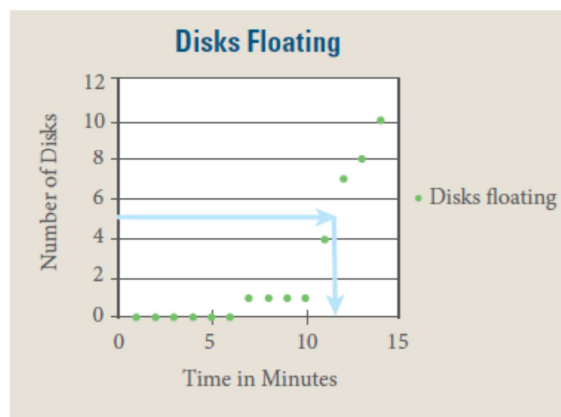


Figure 1. Disks Floating

**Step 9** Record findings.

## Designing and Conducting Your Investigation

*What factors affect the rate of photosynthesis in living plants?*

1. Once you have mastered the floating disk technique, you will design an experiment to test another variable that might affect the rate of photosynthesis. Some ideas include the following, but don't limit yourself to just these:

- 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?
- What features or variables of the plant leaves might affect the net rate of photosynthesis? How and why?
- 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 your results to prepare a lab report and be prepared to briefly present your findings.

### 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). Because the times to float are the inverse of the rate of photosynthesis, the graphic *appears* to indicate a drop in rate (Fig. 2). To make the data visibly represent the trend, the  $ET_{50}$  term can be modified by taking its inverse, or  $1/ET_{50}$  which allows the graphic presentation to more closely express what is happening, as shown in Figure 3.

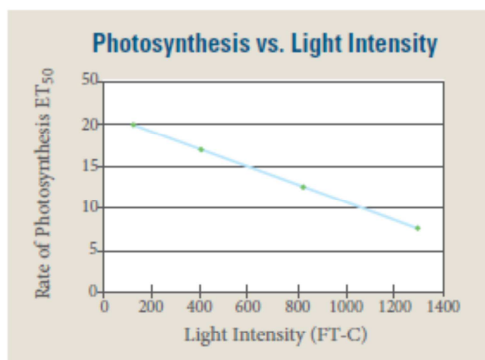


Figure 2. Photosynthesis vs. Light Intensity [Source: Steucek and Hill, 1985]

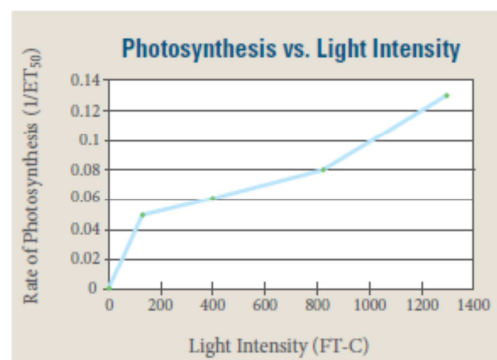


Figure 3. Photosynthesis vs. Light Intensity ( $1/ET_{50}$ ) [Source: Steucek and Hill, 1985]

3. Don't forget to include other appropriate data analyses as you prepare and study your discussion and conclusions. In particular for this investigation, where possible, you should somehow indicate the variability in your data. The  $ET_{50}$  measurement is calculated from the median. To indicate the spread of your data, you could use error bars around the  $ET_{50}$  point that express that variation.