Photosynthesis

What factors affect the rate of photosynthesis in living leaves?
The Floating Disk Technique

Because the spongy mesophyll layer of leaves is normally infused with gases (O$_2$ and CO$_2$), leaves — or disks cut from leaves — normally float in water.

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. 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.
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 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 process is shown in Figure 3.
Step 1 Prepare 300 mL of 0.2% bicarbonate solution for each experiment. The bicarbonate will serve as a source of carbon dioxide for the leaf disks while they are in the solution.

Step 2 Pour the bicarbonate solution into a clear plastic cup to a depth of about 3 cm. Label this cup “With CO₂.” Fill a second cup with only water to be used as a control group. Label this cup “Without CO₂.” 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 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:

a. Remove the piston or plunger from both syringes. Place the 10 leaf disks into each syringe barrel.

b. 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 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 CO₂” cup, and disks infiltrated with the water go in the “Without CO₂” 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.

Figure 7a. Cup Under Light Source

Figure 7b. Disks Floating in Cup with 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 ET₅₀, the Estimated Time it takes 50% of the disks to float) is a reliable and repeatable point of reference for this procedure.

Step 9 Record or report findings.
The following method of data collection is suggested for students, although others work as well. In this case, the disks floating are counted at the end of each time interval. The median is chosen over the mean as the summary statistic. For most student work, the median will generally provide a better estimate of the central tendency of the data because, on occasion, a disk fails to rise or takes a very long time to do so. Consequently, for this sample, the median time for five disks to rise is somewhere between 11 and 12 minutes. A term coined by G. L Steucek and R. J Hill (1985) for this relationship is $ET_{50}^*$, the estimated time for 50% of the disks to rise. That is, rate is a change in a variable over time. The time required for 50% of the leaf disks to float is represented as Effective Time $= ET_{50}^*$. 

Figure 1. Disks Floating
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, as shown in Figure 8a. 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, as shown in Figure 8b.
Figure 2 is a sample graph of a photosynthesis light response curve utilizing the $ET_{50}$ concept.

![Graph: Photosynthesis vs. Light Intensity]

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

Note that the shape of this curve is not the expected curve that rises and levels off. This is because the times to float are the inverse of the rate of photosynthesis. Taking the reciprocal of $ET_{50}$, $1/ET_{50}$ allows the graphic presentation to more closely express the physical phenomenon, as shown in Figure 3.
This procedure is particularly useful for comparing photosynthetic rates between C4 and C3 plants. This procedure is also very useful for exploring the connection between photosynthesis and cellular respiration. Once the infiltrated disks have floated because of photosynthesis, the rate of cellular respiration can be determined by placing the systems in a dark environment. If the disks are still swirled after each minute, the process of cellular respiration will consume the oxygen bubbles in the mesophyll spaces, causing the disks to sink again. This phenomenon is illustrated in Figure 4.
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**Figure 4. Disks Floating**

(In this case, the cup with floating disks was placed under a cardboard box with no light at the 14-minute mark. Note that the slope of the sinking rate is less than that of the floating rate.)
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