Investigating Photosynthesis Using A Floating Leaf Disk Assay

Introduction
Photosynthesis is the metabolic process used by all autotrophs to capture light energy and convert it to the chemical energy of carbohydrates. But how can we measure the rate at which this process occurs?

Although numerous intermediary reactions are involved, the overall photosynthetic reaction is simple: Carbon dioxide combines with the hydrogen from water yielding a carbohydrate—the 6-carbon sugar glucose—and oxygen gas.

The photosynthetic production of oxygen and our knowledge of leaf anatomy allow us to construct a 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 what would happen if all the air is forced out of the air spaces in the leaf? What will the leaf do then?

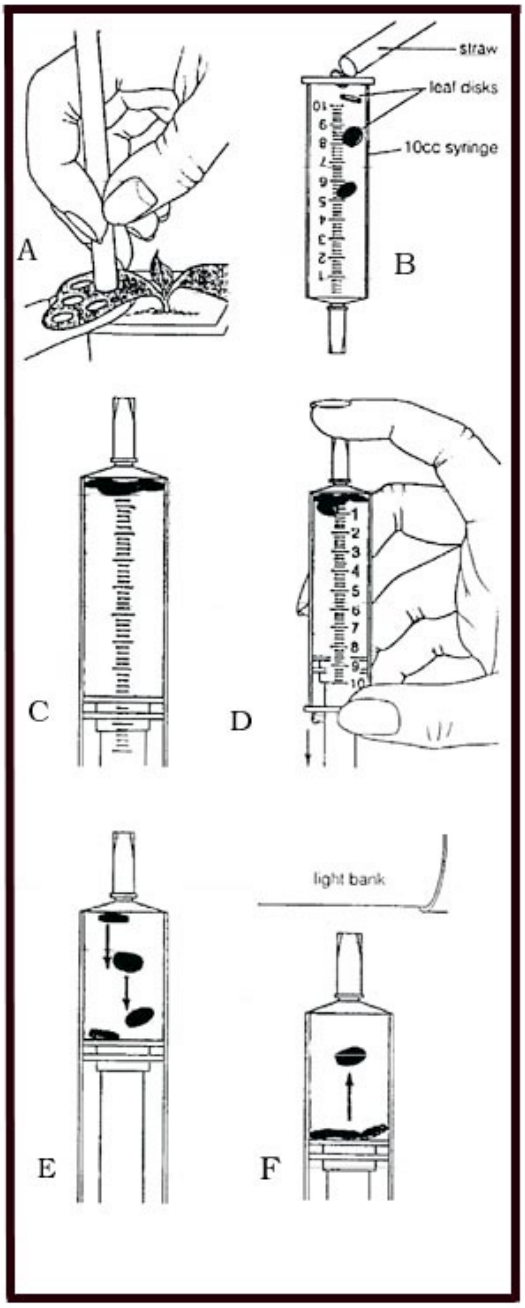
If we supply the leaf with the necessary requirements for photosynthesis, the oxygen the leaf produces will form gas bubbles and the leaf would re-float. In essence this is our experimental method, however, you will use small disks cut from leaves rather than a whole leaf to perform the floating leaf disk assay (FLDA). This assay of photosynthesis may be used to answer many questions, including: What factors affect the rate of photosynthesis? How do changes in light intensity, wavelength, CO_2 concentration, plant adaptations, respiration, and chlorophyll content change the rate of photosynthesis?

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

Materials:

0.2% sodium bicarbonate solution
liquid soap
10 cc plastic syringe
Fresh spinach leaves
Thick plastic straw
Plastic cups
Timer
Light source

PROCEDURE



1. Using a punch made from a small diameter soda straw, cut 10 leaf disks from fresh spinach leaves by supporting the leaf with your index finger while pressing and using a twisting motion of the straw as shown in part A of the diagram at left. Do **not** cut into the vein of the leaf.

2. Remove the plunger from a clean 10-ml syringe. Blow the 10 disks into the body of the syringe. Be sure the leaf disks are near the tip of the syringe as you re-insert the plunger so as not to damage the disks as shown in part B.

3. Insert the tip of the syringe into a beaker of 0.2% sodium bicarbonate solution and draw about 8 ml of solution into the syringe. The leaf disks should be floating at this time. *See diagram C.*

4. Hold the syringe tip upward and expel the air by depressing the plunger carefully. Don't squash the disks.

5. Seal the tip of the syringe using the index finger of your left hand. Pull back on the plunger, creating a partial vacuum within the syringe. If you have a good seal, it should be hard to pull on the plunger and you should see bubbles coming from the edge of the leaf disks. This is how you will fill the air spaces of the leaf disks so that they will sink. *See diagram D.*

6. Simultaneously, release your index finger and the plunger. Some of the leaf disks should start to sink. Tap the side of the tube to dislodge bubbles on the edges of the disks. *See diagram E.*

7. Repeat steps 5 and 6 until all disks sink. Do not overdo these steps!! You have been successful if the disks sink to the bottom. Don't repeat "just to be sure" as it is possible to damage the cells of the leaves.
   a. For a **control group**, repeat steps 1-7 with a solution of only water with a drop of soap—no bicarbonate added.

8. Pour the disks and solution into a clear plastic cup that has been filled with 100 mL of bicarbonate solution. Set up two separate cups—one for your experimental group, and one for your control group.

9. 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. The time required for a leaf disk to float is an index of the net rate of photosynthesis in that leaf disk. However, since some leaf disks will be "early floaters" and others will be "late floaters", this variable can be reduced in significance by plotting the percentage of leaf disks floating as a function of time. The time required for 50 percent of the leaf disks to float is called the **photosynthetic effective time**, shortened to PS ET-50, sort of an average rate. Use graph paper to plot the percent disks floating as a function of time and determine the PS ET-50 for each experimental treatment and control you use. PS ET-50s can be easily compared.

10. Turn off the light and record the number of disks still floating each minute. The time the disks take to sink in the dark is an index of the rate of respiration (RS). Since some of the leaf disks will be "early sinkers" and others will be "late sinkers", once again this variable will be dealt with by plotting the percentage of leaf disks floating as a function of time,

and finding the time required for 50 percent of the leaf disks to sink. This is called the RS ET-50, or the **respiratory effective time** for 50 percent of the leaf disks to sink. Use graph paper to plot the percent of disks floating as a function of time.

Choose an experimental condition from the list below. You will repeat this experiment for the variable that you choose. Remember to establish a control condition for your experiment!

Amount of CO_2
Light Intensity
Wavelength of light
Type of pigment available
Source of CO_2

LAB REPORT:

You will need to collect data in an appropriately constructed data chart/charts. You will also need to plot graphs based on the data you collect. You should also write up an analysis and conclusion about the activity you have performed which addresses the following:

- What relationship do you see between the PS ET-50 and time? What relationship do you observe between the RS ET-50 and time?
- Why is it important to use the average rate of photosynthesis (or respiration)?
- What factors had the largest effect on photosynthetic rate? Why do you think this?
- What is the importance of establishing a control for this experiment?
- Why must we consider respiration when performing this activity?
- Why is it important to study photosynthetic rate of plants?

As always, include an error analysis and suggestions for improvements. Don't forget to consult expert sources of information when writing your report!

References:

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

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http://www.elbiology.com/labtools/Leafdisk.html