**AP Biology Name\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Ms. Ellis**

**Photosynthesis Lab[[1]](#footnote-1)**

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:

2H2O + CO2 + light 🡪 carbohydrate (CH2O) + O2 + H2O

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

* production of O2 (How many moles of O2 are produced for one mole of sugar synthesized?) or
* 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 gas.

Because the spongy mesophyll layer of leaves (shown in Figure 1) is normally infused with gases (including O2 and CO2), leaves—or disks cut from leaves—normally float in water. What would you predict about the density of a leaf disk if the gases were

Figure 1 from p. S62 in lab manual

drawn out of the spongy mesophyll layer using a vacuum or negative pressure and were replaced by water? Will that affect whether or not the leaf floats? How?

If a 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 spaces of the spongy mesophyll, and the leaf disk will once again become buoyant and will 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!) Aerobic cellular respiration will consume oxygen that has accumulated in the 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, in fact, you have a more robust measurement tool than you may have first thought—the change in buoyancy of the leaf disks is actually an indirect measurement of the *net* rate of photosynthesis occurring in the leaf tissue.

Note: carbon dioxide is much, much more soluble in water than oxygen is at normal temperatures, so any carbon dioxide produced by cellular respiration is going to dissolve in the water instead of forming bubbles that would contribute to leaf buoyancy, or it will be used in photosynthesis. Thus, buoyancy is a good measure of net photosynthesis.

Learning objectives

* To design and conduct an experiment to explore the effects 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

Before coming to lab

1. To study photosynthesis, review the properties of light and how it interacts with matter.
2. Read through the procedure for Part A, and write it out in your own words or pictures in your lab notebook. Put it in a format you will be able to follow easily. You should also watch the following YouTube video. It demonstrates the proper technique for drawing gases out of leaf disks, one of the more challenging aspects of this lab. The url is <http://www.youtube.com/watch?v=vw8baZO89oc>. If you watch this video, you may not need to include as much detail in your procedure because you will have seen the correct way to do the technique.
3. Leave a space for your hypothesis.
4. Set up a data table in which you can record your data from Part A.

**Part A: Floating Disk Assay**

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 be able to carry out your own investigation properly. For the floating disk technique, the most challenging skill is getting the disks to sink. You’ve already watched a YouTube video about this, but each member of the group should also attempt the technique to be sure that everyone understands what’s involved. Later, for consistency, you may decide to have one member of the group do it for all of the samples, but take time now to have everyone get a chance to experience the technique.

Remember the overall concept of the assay. When immersed in water, oxygen-rich air bubbles are usually trapped in the air spaces of the spongy mesophyll layer in the plant leaf. Be creating negative pressure in this experimental procedure, the air bubbles can be drawn out of the spongy mesophyll, and the space is re-filled by the surrounding solution. This allows the leaf disks to sink in the experimental solution. If the solution has bicarbonate ions and the disks are exposed to enough light, the leaf disks 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.

Procedure

1. As a group, discuss the experimental protocol and develop both a null hypothesis and an experimental hypothesis. Record your hypotheses in your lab notebook. You will be comparing leaf disks in plain water to leaf disks in 0.2% sodium bicarbonate (in water). The bicarbonate will serve as a source of carbon dioxide for the leaf disks while they are in the solution. (Note: a 0.2% solution means that there is 0.2 g of solid (NaHCO3, in this case) in every 100 mL of solution.)
2. Pour 0.2% sodium bicarbonate solution into one 150 mL beaker to about the 70 mL line. Label this beaker “with CO2”.
3. Fill a second 150 mL beaker to about the 70 mL line with water. Label this beaker “without CO2”.
4. Throughout the rest of the procedure you will be preparing materials for both beakers, so do everything for both beakers simultaneously.
5. Using a transfer pipet, add one drop of soap solution to the liquid in each beaker and stir it into solution using a stirring rod. It is critical to avoid suds. (The soap solution was prepared by adding approximately 5 mL of dishwashing soap to 250 mL of dH2O.) If either solution generates suds, then dilute it with more bicarbonate or water solution. The role of the soap is to act as a surfactant or “wetting agent”—it wets the hydrophobic surface of the leaf, allowing the solution to be drawn into the leaf and thus enabling the leaf disks to sink in the fluid. It also reduces the surface tension of the air bubbles inside the leaf, which makes it easier for them to be pulled out.
6. Using a hole punch, cut 10 or more uniform leaf disks for each beaker. 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.) It’s also best to use the disks when they are freshly cut. Dry disks don’t perform well in this assay.
7. Draw the gases out of the spongy mesophyll tissue and infiltrate the leaves with the sodium bicarbonate solution by performing the following steps:
   1. Remove the piston or plunger from both syringes.
   2. Place 10 leaf disks in each syringe barrel.
   3. Replace the plunger, but be careful not to crush the leaf disks. Push in the plunger until only a small volume of air (less than 10% of the total volume) and the leaf disks remain in the barrel.
   4. Pull a small volume (around 5 mL) of sodium bicarbonate plus soap solution from your prepared beaker into one syringe and a small volume of water plus soap solution into the other syringe. (Labeling the syringes might be a good idea.)
   5. 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 syringe before you attempt the next step.
   6. You now want to create negative pressure in the syringe to draw the air out of the leaf tissue. As previously stated, this is the most difficult step to master. Once you learn how to do this, you will be able to complete the entire exercise successfully. Create the negative pressure by holding a finger or thumb over the narrow syringe opening while drawing back the plunger. Hold this for about 10 seconds. While holding, swirl the leaf disks to suspend them in the solution.
   7. Release it by letting the plunger spring back. The solution will infiltrate the air spaces in the leaf disks, causing them to sink in the syringe. If the plunger does not spring back, you did not have good negative pressure, and you may need a different syringe.
   8. You may need to repeat this procedure two to three times to get the disks to sink. If they do not sink after three tries, there is probably not enough soap in the solution. Pour the liquid from the syringe back into the beaker, add another drop of soap to the solution, stir, and try again with new leaf disks. Never use leaf disks that have been through more than three attempts to have their air drawn out. They will likely be damaged and partially collapsed, so they will not give useful results.
8. Pour the disks and the solution from the syringe into the appropriate beaker. Make sure the correct sample ends up in the correct beaker!
9. Place both beakers under identical light sources and start the timer. We will use LED lights that produce little heat. You may want to use the light meters to be sure that the two samples are getting the same amount of light.
10. Swirl the beakers periodically to dislodge any disks that may be stuck against the sides of the beakers.
11. 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. Thus, for each beaker you should record the time at which five disks were first floating.

Analysis of Results

Make a bar graph depicting your results. Use proper graph format. Then evaluate your hypotheses based on your results.

You should also take the time to make note of any relevant observations and any difficulties you encountered. Write about how you think they affected your experiment and what you will need to consider in the next part of the lab.

**Part B: Designing and Conducting Your Investigation**

The main question you should consider for this section of the lab is, “What factors affect the rate of photosynthesis in living plants?” Now that you have mastered the floating disk technique, you will design an experiment to test another variable (besides presence of bicarbonate) that might affect the rate of photosynthesis. Here are some questions that your group can consider in developing your experimental plan, but don’t feel that you have to limit yourself to these ideas.

* What environmental variables might affect the net rate of photosynthesis? Why do you think they would affect it? How do you predict they will 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 that mean the net rate of photosynthesis has changed? Why do you think that?

Keep in mind that leaves with hairy surfaces should be avoided when using this procedure. Ivy and spinach work well, but so do many other leaves. Differences between plants may be one of the ideas that you want to investigate.

1. Develop a hypothesis and a procedure within your group.
2. Get your teacher’s approval to perform your experiment.
3. Write out your procedure in your lab notebook in enough detail that someone else could replicate it. You do not need to re-write the basic procedure in detail since that’s already in your lab notebook. Just explain the changes that you will be making to the basic procedure.
4. Prepare a data table before you begin your experiment.
5. Perform your experiment with duplicates (at least). Record all of your data and observations in your lab notebook

Data Analysis

When graphing your data, use error bars around the ET50 points in order to indicate your data spread. Different experiments will call for different ways of displaying the data graphically, so your group should work together to decide how to best communicate what you learned from your experiment.

Consider combining variables as a way to describe the 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.

Your teacher will let you know if you will be producing a lab report, a mini-poster, or doing an oral presentation of your work.

1. Adapted from the College Board AP Biology Student Lab Manual, 2012 edition. [↑](#footnote-ref-1)