

IB BIOLOGY

Name _____ Period _____ Date _____

Reference: Adapted from "AP BIOLOGY INVESTIGATIVE LABS: AN INQUIRY-BASED APPROACH" © 2012 THE COLLEGE BOARD, ADVANCED PLACEMENT PROGRAM

Learning Objectives

- 2.9.U4 Oxygen is produced in photosynthesis from the photolysis of water.
- 2.9.U5 Energy is needed to produce carbohydrates and other carbon compounds from carbon dioxide.
- 2.9.U6 Temperature, light intensity and carbon dioxide concentration are possible limiting factors on the rate of photosynthesis.
- 2.9.S2 Design of experiments to investigate the effect of limiting factors on photosynthesis.

Photosynthesis Lab: Floating Leaf Disc Assay

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: $2 \text{H}_2\text{O} + \text{CO}_2 + \text{light} \rightarrow \text{carbohydrate (CH}_2\text{O)} + \text{O}_2 + \text{H}_2\text{O}$
What two things 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. Because the spongy mesophyll layer of leaves 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.

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.

In this 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
- Liquid soap
- 2 plastic syringes without needle
- Spinach leaves
- Hole punch
- 2 clear plastic cups
- Timer
- Light source

Pre-Lab Questions

1. Why is baking soda added to only one of the solutions?
2. What is the purpose of the detergent?
3. Explain how this lab is an indirect measure of the net rate of photosynthesis.

Procedure

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.
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.
3. Using a pipette, add one drop of liquid soap 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.
4. Using a hole punch, cut 10 or more uniform leaf disks for each cup. Avoid major leaf veins.
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 remains 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. 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.
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.
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.
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.
9. Record your observations in a data table.

Post-Lab

Discuss the results of your experiment; include an explanation for the changes that occurred within the leaf tissue that allowed the leaf disks to rise to the surface.

Designing and Conducting Your Investigation

What factors affect the rate of photosynthesis in living plants?

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? (*i.e. light intensity, temperature, pH*)
- What features or variables of the plant leaves might affect the net rate of photosynthesis? How and why? (*i.e. leaf color, type of plant*)
- 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? (*i.e. size of leaf disc, soap amount*)