**ShoeBox Investigation**: Photosynthesis

AP Biology

**Essential Question**: How do you design an experiment to quantify the effect of an environmental variable (ex. pH, temperature, light intensity, age of leaves, etc.) on the rate of photosynthesis.

**Background:**

Photosynthesis is an anabolic process used by all photoautotrophs to capture light energy and convert it to the chemical energy in carbohydrates. It can be measured in a variety of ways. Although numerous intermediary reactions are involved, the overall photosynthetic reaction is simple: carbon dioxide combines with the hydrogen from water producing a carbohydrate– the six-carbon sugar glucose –and oxygen gas.

**6CO2 + 6H2O + sunlight → C6H12O6 + 6 O2**

The photosynthetic production of oxygen and knowledge of leaf anatomy will allow for the construction of a simple 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 would you happen if all the air is forced out of the air spaces in the leaf? What will the leaf do then? If basic requirements for photosynthesis are supplied, the oxygen the leaf produces will form gas bubbles and the leaf would re-float. In essence, this is the experimental method, however, small disks cut from leaves will be used instead of whole leaves to perform the floating leaf disk assay (FLDA). This assay of photosynthesis may be used to answer many questions, including: How do changes in light intensity, wavelength, or CO2 concentration affect the rate of photosynthesis?

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

**Materials:**

| * Sodium bicarbonate (Baking soda) * Liquid Soap * Plastic syringe (10 cc or larger)—remove any needle! * Leaf material * Hole punch (Thick Plastic Straw) | * Plastic cups * Timer * Group specific materials as needed * Light source |
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**Safety and Housekeeping:**

The primary safety issues in this lab have to do with solutions **NEAR** electric lights. Take **CAUTION** to observe proper care with solutions near lights. If you will be working in close proximity to exposed lightbulbs, make sure to wear **EYE PROTECTION** in the form of safety glasses. Moreover, some high-intensity light sources get extremely **HOT**. If you are using these, be **ADVISE** not to drip water on them (shatter hazard) or to lean against a light (burn hazard).

**Procedure**

| FLDAprocedure.png | 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 with the bicarbonate solution 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 beaker that has been filled with 100 mL of bicarbonate solution. Set up two separate beakers—one for your experimental group, and one for your control group. |
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9. Place the beakers 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 beakers. 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.

The procedure outlined above is a generalized procedure that all groups performing this lab will follow. Your group will be given an experimental condition to test using the same procedure as above. The conditions available are:

* **Amount of CO2:**varying concentrations of bicarbonate solution will be available
* **Wavelength of light:**  colored cellophane paper will be available
* **pH:** various pH solutions will be available
* **Source of CO2:**  different sources of carbon-containing solutions will be available
* **Light intensity:** use screens to shade the beaker containing your experiment

**REPORTING RESULTS:**

You and your lab group will produce a poster in class the following period that details the following things:

* **The experimental question your group sought to answer by performing the lab.**
* **What you predicted would occur, and why.**
* **Graphical representation of your results.**
* **A brief explanation and discussion of your results.**

You will only have **45 minutes** to produce your poster, so you must work together to produce a poster that efficiently and effectively communicates the above points to others who may not have performed your experiment. We will then have a gallery walk as a class, during which time your classmates and teacher will view your work and leave commentary on it for your review.