PRE-LAB QUESTIONS

1. What is the general summary equation for photosynthesis?
2. Which method will we use to measure the rate of photosynthesis in this lab (generally)?
3. What is the spongy mesophyll typically infused with? What does this cause leaves to do in water?
4. What would you predict about the density of leaves (leaf disks) if the gases are removed from the spongy mesophyll and replaced with water?
5. How will we measure the rate of photosynthesis in this lab (give more details than #2)?
6. Write a testable hypothesis for your lab.
7. List your materials for this lab.
8. If I wanted to mix 1000ml of .2% bicarbonate solution, how much baking soda would I need to add? Show work.
9. The soap dilution we will be using is 5ml of soap for 250ml of water. What percentage is that? Show work.

Materials:
- Baking Soda (sodium biocarbonate)
- Liquid soap (approximately 5ml soap in 250 ml water)
- 2 10ml plastic syringes
- Living Leaves (spinach)
- Hole Punch/ Straw
- 2 clear plastic cups
- Timer
- Permanent Marker
- Pipette
- Distilled Water
- Light Source

Procedure:

1. Label your two plastic cups with a permanent marker. One should be labeled “With CO$_2$” and the other “Without CO$_2$” (This first step may already be done for you).
2. In a large beaker, obtain 250 ml of the .2% bicarbonate solution for your experiment. This will serve as a course of carbon dioxide for the leaf disks while they are in solution.
3. Pour the bicarbonate solution into the cup labeled “With CO$_2$”. Fill the other cup with water (tap works). Throughout the rest of the procedure you will be preparing material for both cups, so do everything for both cups simultaneously.
4. Using a pipette, add one drop of a dilute 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.
5. 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.

6. Draw the gases out of the spongy mesophyll tissue and infiltrate the leaves with the sodium bicarbonate solution by performing the following steps:
   a. Using a permanent marker, clearly label the syringes as “With CO₂” and “Without CO₂” (This may already be done for you)
   b. Remove the piston or plunger from both syringes. Place the 10 leaf disks into each syringe barrel.
   c. 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%).
   d. Using the appropriate syringe, pull a small volume (5 cc or ml) 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 e.
   e. 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 picture on right). 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.

7. Pour the disks and the solution from the syringe into the appropriate clear plastic cup.

8. Prepare a timer. 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.

9. Record data in a table.


**Post-Lab Questions**

1. Graph the results.
2. 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 leaf disks are floating (the median or ET$_{50}$, the Estimated Time it takes 50% of the disks to float) is a reliable and repeatable point of reference for this procedure. Calculate the ET$_{50}$.
3. What was the purpose of the sodium bicarbonate?
4. How does the buoyancy of the leaf disks in this lab help us measure the rate of photosynthesis? Give details.
5. Which part of photosynthesis is responsible for creating the gas product?
6. The purpose of this process is not to produce gas. What is the reason for the process in #5?
7. What factors can affect the rate of photosynthesis?
8. Design an experiment to test another variable that might affect the rate of photosynthesis. You can choose one of the following or come up with a question/problem of your own.
   - Environmental factors (why do you think they would affect it? How do you predict they would affect it?)
   - Features or variables of the plant leaves (why do you think they would affect it? How do you predict they would affect it?)
   - Methods/procedures followed (if the outcomes change, does it mean the net rate of photosynthesis has changed? Why do you think that?)
9. Write up a basic summary/hypothesis/experimental question to test and submit it to the teacher for approval.
10. Once your hypothesis has been approved, begin writing your formal lab report.
11. After you complete your experiment, you will present your findings to the class via Prezi or Powerpoint Presentation.