Baker's Dozen Lab 5: Photosynthesis

Objectives

Before doing this lab you should understand the process of photosynthesis, and the relationship between light intensity/wavelength and photosynthetic rate.

After doing this lab you should be able to describe a technique to determine photosynthetic rates and explain why the rate of photosynthesis varies under different environmental conditions.

Introduction

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:

$$12H_20 + 6CO_2 + \text{light energy} \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O$$

What could you measure to determine the rate of photosynthesis?

- **Production of O_2:** How many moles of O_2 are produced for one mole of sugar synthesized?
- **Consumption of CO_2:** How many moles of CO_2 are consumed for every mole of sugar synthesized?

In this system, you will use a system that measures the accumulation of oxygen.

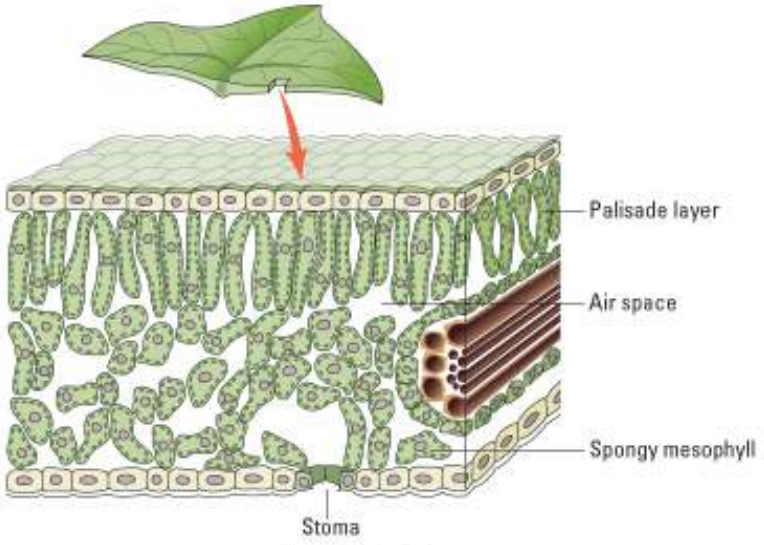


Figure 1. Leaf Anatomy

Because the spongy mesophyll layer of leaves (shown in Figure 1) is normally infused with gases (O_2 and CO_2), 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 they are replaced by water? How will that affect whether or not the leaf disks float? If the leaf disk is placed in a solution with an alternate source of CO_2 in the form of bicarbonate ions (HCO_3^-), 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 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 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 that is occurring in the leaf tissue.

Materials

baking soda ($NaHCO_3$)	timer	plastic cups
liquid soap	ruler	hole punch/straw
10 mL syringes	pipette	light source
living leaves		

Procedure 5A:

1. Pour baking soda into one clear plastic cup to a depth of about 3 cm. Label this cup "With CO_2."
2. Fill the other clear plastic cup with tap water. Label this cup "Without CO_2."
3. Using a pipette, add one drop of dilute liquid soap solution to the solutions in each cup. ***It is critical to avoid suds.*** If either solution generates suds, then dilute it with more baking soda or tap water. 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 straw, cut about 15 uniform leaf disks for each cup. Avoid major leaf veins.
5. Remove the plunger from both syringes. Place the 15 leaf disks into each barrel.
6. Replace the plunger, being careful not to crush the leaf disks. Push in the plunger until only a small volume of air and leaf disks remain in the barrel (less than 1 mL).
7. Pull 5 mL of solution from the "With CO_2" cup into one syringe and the same volume of solution from the "Without CO_2" cup into the other syringe.
8. Tap each syringe to suspend the leaf disks in each solution. Make sure that with the plunger inverted, the disks are suspended in solution. Make sure no air remains by pushing the plunger in to release any excess air after tapping it to get the air up to the open end of the syringe.
9. Create a vacuum in the syringe by holding a finger over the narrow opening of the syringe while drawing back the plunger. Hold this vacuum for about 5 seconds. Release the vacuum by letting the plunger spring back. The solution should infiltrate the air spaces in the leaf disk, causing them to sink in the syringe. You may need to repeat the procedure two to three times in order to get at least 10 disks to sink. If you can't get 10 disks to sink after three tries, add a few more drops of soap to the solution in the cup and replace the liquid in the syringe.

Figure 6a. Creating a Vacuum in the Plunger

Figure 6b. Sinking Leaf Disks

10. Pour the disks that have sunk (hopefully 10 in each, if not 10, at least make sure to start with the same number of sunk disks) and solution from the syringe back into the appropriate clear plastic cup.
11. Place both cups under the light source and start the timer. At the end of each minute, record the number of floating disks in the data table below.
12. Every minute or two, swirl the disks to dislodge any that have stuck to the side of the cups. Continue timing and recording data until **all of the disks are floating in one of the cups.**

DATA TABLE: Floating Disks

Minute	With CO_2	Without CO_2
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		

Identify the independent variable in this experiment: _____

Identify the dependent variable in this experiment: _____

Procedure 5B: Investigating Other Variables

Once you have mastered the floating disk technique, you can design your own experiment to test another variable that might affect the rate of photosynthesis. Some ideas are:

- What environmental factors might affect the net rate of photosynthesis? How or why would they affect it?
- What features or variables of the plant leaves might affect the net rate of photosynthesis? How and why?

Identify the variable you will test. _____

Write a hypothesis concerning your variable: _____

_____

Briefly describe the changes you will make to the procedure already used in order to test your chosen variable.

_____

_____

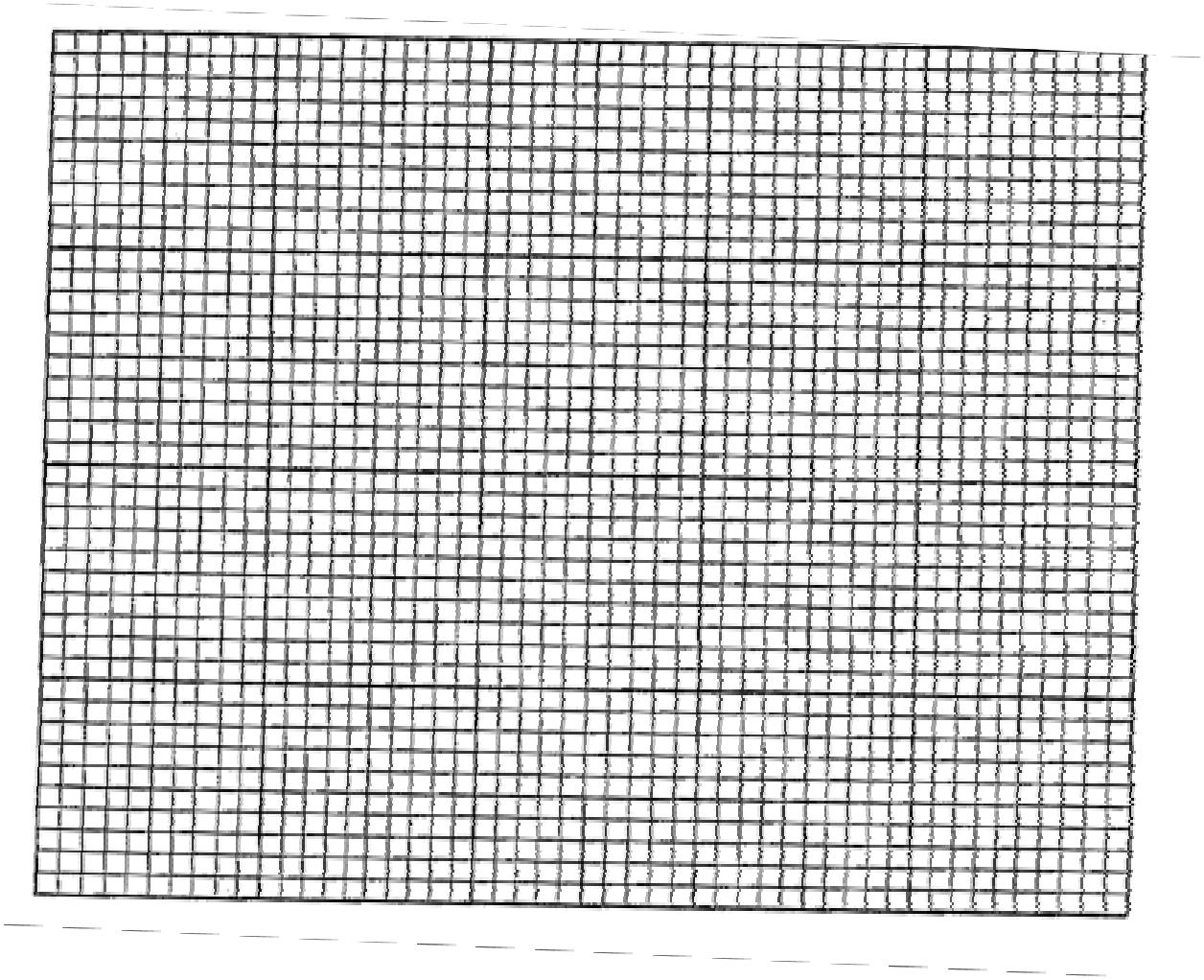
_____

_____

_____

Make a data table on a separate paper and attach it to this packet.

Graph your data from 5B on the graph below.



Write a conclusion that addresses whether your hypothesis was supported or not. Be sure to include quantifiable data in your discussion. Include a one to two sentence summary of what factors affect the net rate of photosynthesis and how they affect it.