Name Period 1 / 2 / 4 / 5

Lesson 3.4 Leaf Disk Assay- Measuring the Rate of Photosynthesis

Engage

Photosynthesis fuels ecosystems and replenishes the Earth's atmosphere with oxygen.

1) What is the general summary equation for photosynthesis is

2) Name two examples of biotic things that undergo photosynthesis
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   -

Explore

To determine the rate of photosynthesis you will use a system that measures the accumulation of oxygen.

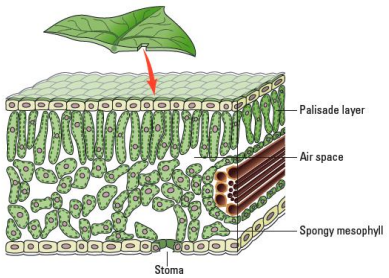


Figure 1. Leaf Anatomy


Figure 2. Materials

Because the spongy mesophyll layer of leaves (shown in Figure 1) is normally infused with gases (O_2 and CO_2), leaves — disks cut from leaves — normally float in water. 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.

Procedure--Be careful to keep your solutions away from the electrical cord of your light source and your light bulbs. Light bulbs could break so use caution when moving them. Light bulbs will also be hot during use so avoid contact. In this part of the lab, you will learn how the floating leaf disk technique can measure the rate of photosynthesis by testing the rate of photosynthesis in the dark, light, and with and without carbon.

You will need: • Baking soda (sodium bicarbonate) • Liquid soap • 2 plastic syringes without needle (10 mL or larger)• Living leaves (spinach, ivy, etc.) • Hole punch • 4 clear plastic cups • Timer • Light source

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. Your teacher will demonstrate the procedure for you to follow.

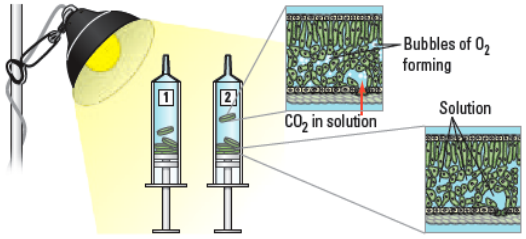


Figure 3. Photosynthesis at Work

Figure 4. Dilute Liquid Soap Solution Added to Cup

Figure 5. Leaf Disks

Step 1 Pour bicarbonate solution into two clear plastic cups to a depth of about 3 cm- 150ml for most cups. Label these cups “With CO2.” Fill two other cup with only water to be used as a control group. Label these cups “Without CO2.” Throughout the rest of the procedure you will be preparing material for both sets of cups, so do everything for both cups simultaneously.

Step 2 Using a pipette, add one drop of a dilute liquid soap solution to the solution in each cup. It is critical to avoid suds. The soap acts as a surfactant or “wetting agent” — it wets the waxy side of the leaf so that water will flow in.

Step 3 Using a hole punch, cut 10 or more uniform leaf disks for each cup. Avoid major leaf veins.

Step 4 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 and leaf disk remain 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 (see Figure 6a). 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 five to six more times in order to get the disks to sink. Placing the disks under vacuum more than three times can damage the disks.

Figure 6a. Creating a Vacuum in the Plunger

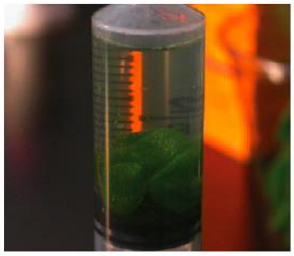


Figure 6b. Sinking Leaf Disks

Figure 7a. Cup Under Light Source

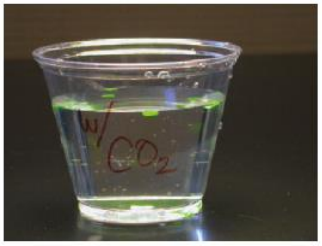


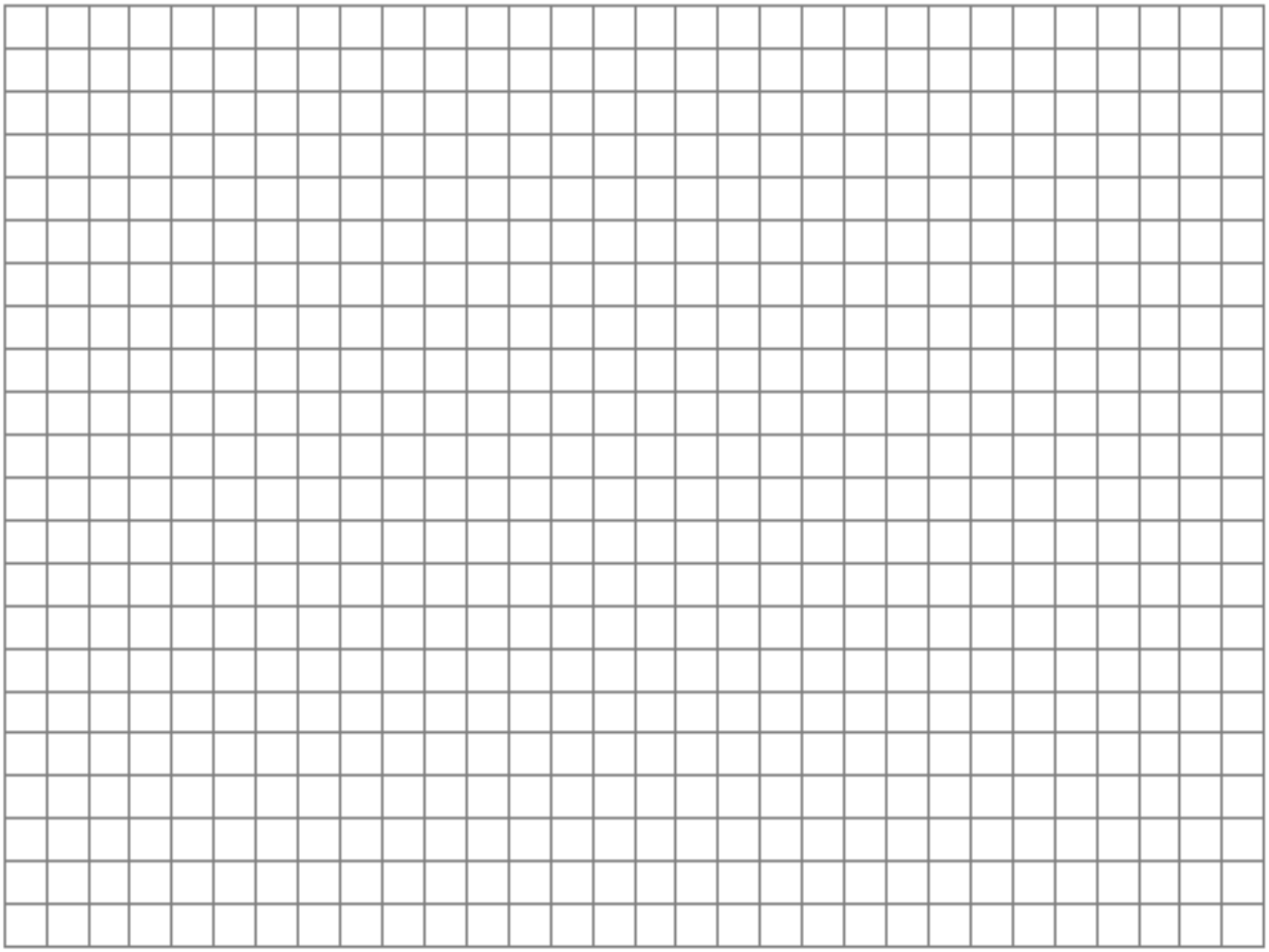
Figure 7b. Disks Floating in Cup with Bicarbonate Solution

Step 5 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.

Step 6 Repeat the set up one more time so that you have four cups total, two cups placed directly under the light source and two cups in the dark (cups are wrapped in foil while not measuring, make a ‘loose’ lid so you can peak at each time point) and start the timer. At the end of each minute, record the number of floating disks. (Swirl the disks to dislodge any that stuck against the side of the cups, if necessary) Continue until all of the disks are floating in the cup with the bicarbonate solution, or for 30 minutes.
Record your data in the data table below. Calculate the ET 50 which is the amount of time it takes for 50% of the disks to float. If 50% of them do not float simply write n/a.

	Direct Light	Dark No Light	Direct Light	Dark No Light
Time (min)	Disk Floating in Bicarbonate	Disk Floating in Bicarbonat	Disk Floating in Water (#)	Disk Floating in Water (#)
0				
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				
29				
30				
ET50				

Explain Appropriately graph <u>your data</u> from all four groups using the graph below.



1) a) List two environmental variables might affect the net rate of photosynthesis? b) Why do you think they would affect it? c) How do you predict they would affect it?

First variable:

a) _____

_____

b) _____

_____

_____

_____

c) _____

_____

_____

_____

Second variable:

a) _____

_____

b) _____

_____

_____

_____

c) _____

_____

_____

_____

2) You are challenged to change the experimental conditions to make all ten disks float as fast as possible. What would you do to change your procedure to make this happen?

_____

_____

_____

_____

_____

Evaluate

Write a brief conclusion explaining a) any issues or difficulties you had during the lab b) any errors you think occurred and explain them (do not simply say that you had human errors) c) the availability of a source of carbon relating the rate of photosynthesis to light

a) _____

_____

_____

_____

b) _____

_____

_____

_____

c) _____

_____

_____

_____

_____

_____

_____

_____