Lab 7. PHOTOSYNTHESIS: Floating Leaf Disk Photosynthesis Lab

Overview

During this lab, you will learn the factors that affect the net rate of photosynthesis such as different lighting conditions (light, dark), and CO_2 concentrations. You will also measure the CO_2 concentrations of the water samples by looking at the net rate of photosynthesis in these samples.

Learning objectives

1. Understand that photosynthesis is a measurable process.
2. Understand factors affecting photosynthesis.
3. Understand the correlation between cell respiration and photosynthesis.
4. Understand the correlation between organic materials and the rate of photosynthesis.
5. Learn to measure the concentration of CO2 in the water samples by using the standard curve and titration assay.

Materials and equipment

- Sodium Bicarbonate (baking soda) solutions
- Liquid Soap
- Syringe (20 ml or larger)
- Leaf (baby spinach from grocery store)
- Hole Punch
- Timer
- Light Source
- Stir sticks
- Plastic cups (should be clear)/ 10 cups per group
- Graduate Cylinder (100 ml)
- Tin Foil

Background

Photosynthesis is a process that converts carbon dioxide into sugars such as glucose using energy from the sun. When light is absorbed by pigments in a leaf, the energy absorbed is used to incorporate the carbon dioxide into organic molecules in a process called carbon fixation.

The process of photosynthesis can be expressed by the following word equation and chemical equation.

Carbon dioxide + Water → Glucose + Oxygen

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$$

In this lab, you will be using leaf disks, to assay the net rate of photosynthesis under various lighting conditions. Leaf disks normally float, however if you remove the air from the air spaces, the overall density of the leaf disk increases, and the leaf disk sinks. When sodium bicarbonate is added to the water, the bicarbonate ion acts as a carbon source for photosynthesis. When the disks are exposed to sufficient light, photosynthesis proceeds, and oxygen is released into the interior of the leaf, which changes its buoyancy causing the disk to rise. As a result, the rate at which the disks rise is indirectly proportional to the net rate of photosynthesis.

Terminology

Make sure you have a clear understanding of the following concepts after reviewing the provided videos and reading materials:

- ADP
- ATP
- Autotroph
- Chloroplast
- Chlorophyll
- Calvin Cycle
- Light reaction
- Oxidation
- Reduction

Procedures

LAB ACTIVITIES: Every student must do all the calculations, record all the results and draw the graphs during the lab activity. Your instructor will write her/his initials once you have completed recording your results.

Before starting the experiment, answer the following questions based on your background knowledge that you acquired through the lecture or the posted videos on Blackboard about photosynthesis.

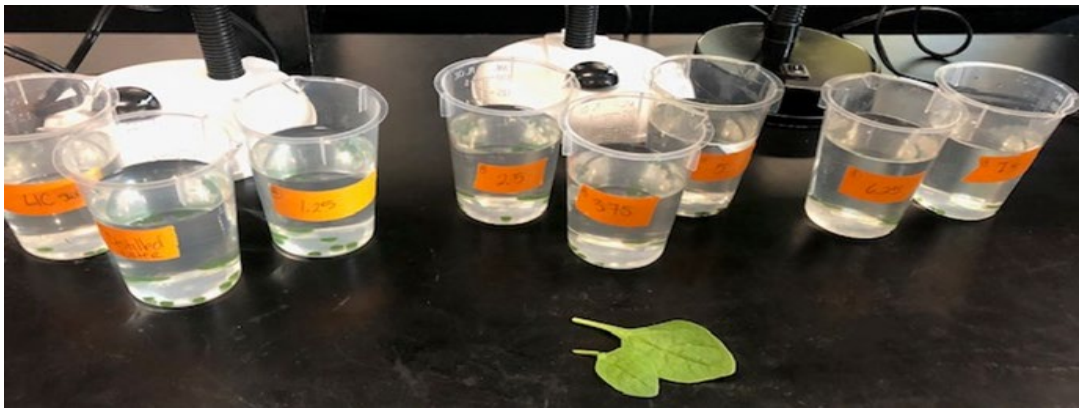
1. **Which condition light/dark will produce the fastest rate of photosynthesis?** State your hypothesis in the following form: If…..then….because…

2. **Which concentrations of CO2 (high/low) will produce the fastest rate of photosynthesis?** State your hypothesis in the form: If…..then….because…

3. **Which temperature 0°C, 37°C or 95°C will produce the fastest rate of photosynthesis?** State your hypothesis in the form: If…..then….because…

A. Effect of Carbon Dioxide Concentration on the Rate of Photosynthesis

Figure 1. Spinach disks immersed in different concentration of sodium bicarbonate



1. Obtain 8 clear cups. Label six of them according to the following amounts of baking soda: 0 g/100 ml, 0.5 g/100 ml, 1g/ml, 1.5g/ml, 2g/ml, and 2.5g/ml. Label the seventh cup as “unknown” water sample.

2. Add approximately 100 ml of each one of the solutions of baking soda into the labeled cups.

3. Add approximately 100 ml of your water sample to the cup labelled with the name of your water sample.

4. Add 1 drop of liquid soap to each beaker of bicarbonate solution and to your water sample. Make sure not to add too much soap to your solution to prevent making bubbles.

5. Hole punch at least 40 leaf disks, from fresh spinach leaves. Choose areas that are uniform in texture and thickness, avoiding major leaf veins. You will need to use a minimum of 5 disks for each trial; if you wish to use more, make sure you use the same number of disks for each solution.

6. Remove the plunger of the syringe and place 5 leaf disks in the syringe barrel.

7. Replace the plunger, being careful not to crush the leaf disks. Push on the plunger until only a small volume of air and leaf disk remain in the barrel.

Figure 2. Removing air from the spinach leaves

8. Draw a small volume (about 5 ml) of the sodium bicarbonate solution into the syringe. Start with the lowest concentrations of bicarbonate solution, which is

2.5g/100 ml. After you have added all the bicarbonate solutions, rinse the syringe, and repeat the procedure with your water sample.

9. Invert the syringe and tap the syringe to suspend the leaf disks in the solution.
10. Push the plunger, removing as much air as possible from the syringe.
11. Hold a finger over the syringe opening and draw back on the plunger to create a vacuum. Hold this for 10 seconds while swirling the syringe to further suspend the leaf disks in solution.
12. Let off the vacuum and repeat steps 8-10, if needed, 2-3 more times until all leaf disks sink. If the leaf disks do not sink, add one more drop of soap to the bicarbonate solution and repeat the vacuum steps.
13. Pour the disks and solution back into the labeled cup for each concentration of bicarbonate solution and water sample.
14. Once all your cups are ready with the disks, put them under the lamp; record the time (**this will be time 0**).
15. Record the number of floating disks at the end of each time period (every five minutes) in the table in your notebook. Keep time for 30 minutes. Gently swirl the cup with a stir stick to dislodge any disks that are stuck to each other or on the side of the cups.

Figure 3. Floating disks after 30 min under light

(Picture credit: Meghan Pierce and Laura Vimos, 2018. CC-BY 4.0)

16. For the dark trial, obtain 1 cup, label it “dark 7.5%” and add 100 mls of the 7.5 % bicarbonate solution. Wrap the beaker with tin foil to cover the plastic cup when not taking observations.

17. **Record your data in Table 1.**

18. Graph a **standard curve** for different concentrations of bicarbonate under light conditions. Make sure to label the X axis (bicarbonate concentration) and Y axis (rate of photosynthesis measured in number of disks floating/unit of time) with the appropriate units.

    You can use Excel, Google Sheet or some other graphing tool. If you draw on the graph paper provided (Figure 4), you will need to upload or attach an image file if you submit your graph electronically.

B. The Effect of Temperature on the Rate of Photosynthesis

This experiment will be done by one group (volunteer) to demonstrate to the rest of the class.

1. Obtain 3 clear cups. Label them according to the different temperatures of the solution that contains 15 g of baking soda.

2. Obtain solutions that contain 15 g of baking soda from the ice bucket, 37°C and 95°C water bath and 100 ml of these solutions to each cup.

3. Prepare 5 leaf disks for each trial as you did for the previous experiment. Once all your cups are ready with the disks, put them under the lamp; record the time (this will be time 0).

4. Record the number of floating disks at the end of each time period (every five minutes) in the table in your notebook. Keep time for 30 minutes. Gently swirl the cup with a stir stick to dislodge any disks that are stuck to each other or on the side of the cups.

5. Record your data in **Table 2**.

Table 1. Net rate of photosynthesis at different concentrations of bicarbonate solutions under light

Time (minute)	0 g/100ml	0.5 g/100ml	1.0 g/100ml	1.5 g/100ml	2.0 g/100ml	2.5 g/100ml	Unknown	Notes
0								
5								
10								
15								
20								
25								
30								

Figure 4. Graph paper

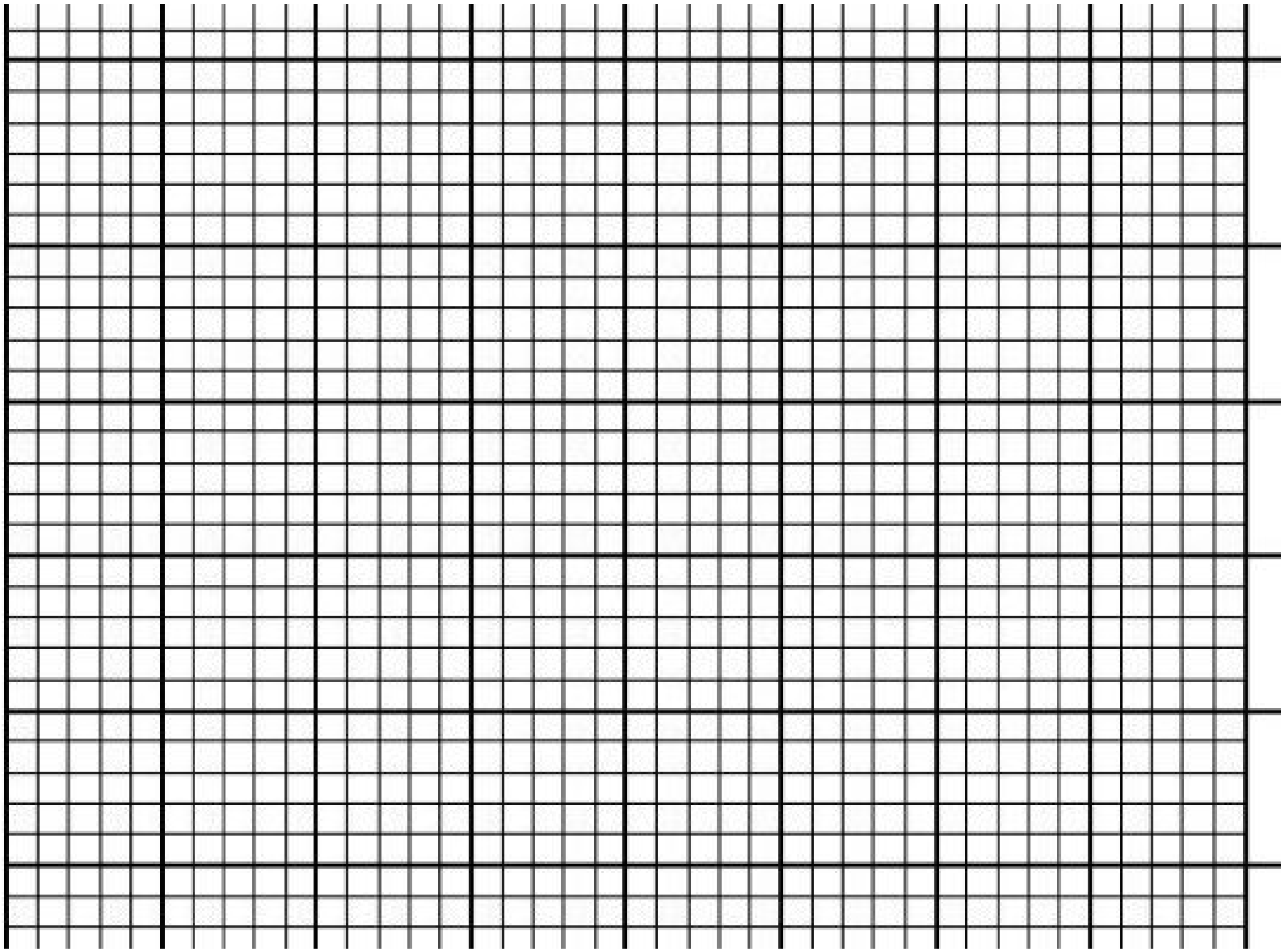


Table 2. Number of floating disks at three different temperatures, in degree Celsius by minutes

Time (minute)	0 °C	37 °C	95 °C
0			
5			
10			
15			
20			
25			
30			

Questions

Answer each one of the questions in the space provided below.

1. What was the purpose of adding the baking soda in this experiment?

2. How do different concentrations of bicarbonate affect the rate of photosynthesis? Why?

3. Which trial resulted in all the leaf disks floating the fastest? Explain why you think this happened.

4. Explain the process that caused the leaf disks to rise.

5. How does light affect the rate of photosynthesis? Why?

6. How does temperature affect the rate of photosynthesis?

7. If the leaf disks were boiled, what kind of result would you expect? Hint: think about the enzymes that assist this reaction.

C. Direct Measurement of Amount of Dissolved Carbon Dioxide in Water Samples

Measure the concentration of CO_2 in the water sample, with the kits provided, by using the titration assay. Follow the instructions provided by the manufacturer.

Figure 3. LaMotte Carbon Dioxide titration kit

Record your data in **Table 3**. You need this data later on for your final presentation.

Table 3. Data for measurement of the concentration of CO_2 in the water sample

Description	Data
Water sample NAME of area of collection	
Date of collection	
Time of collection	
Temperature at time of collection	
pH	
CO_2 concentration (make sure to give the units)	
Temperature at time of measurement	

First and last name: _____ (required)

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