

LAB 3: RESPIRATION AND PHOTOSYNTHESIS IN PLANTS

OBJECTIVES

In this laboratory exploration, you will

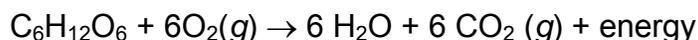
- Use a pH probe to measure the pH of water.
- Use pH measurements to make inferences on the amount of carbon dioxide dissolved in water.
- Use the inferences about the amount of carbon dioxide in the water to make conclusions about whether plants consume or produce carbon dioxide in the light.
- Use the inferences about the amount of carbon dioxide in the water to make conclusions about whether the plant is respiring or photosynthesizing more in the light.
- Reinforce concepts about respiration and photosynthesis.

PREPARATION

Before coming to class, it is very important that you read this handout. After reading the handout, fill out the “**Worksheet**” below. ALSO fill out hypotheses for Tables 3 and 4 at the end of the lab.

INTRODUCTION

♦ **We can measure rates of respiration** in several ways, all of which come from the basic equation of cellular respiration:



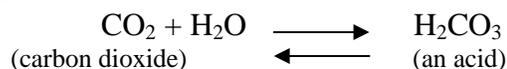
Thus, we can measure

- Rates of the disappearance (consumption) of glucose
- Rates of the disappearance (consumption) of oxygen
- Rates of the production of water
- Rates of the production of carbon dioxide
- Rates of the production of energy

Because we are measuring respiration in living organisms, it is not easy to measure the consumption of glucose or the production of water molecules or energy. Also, remember that some of the energy produced is captured as ATP (and some is lost as heat). If we want to measure respiration, the easiest things to measure, is either the consumption of oxygen or the production of carbon dioxide. In this laboratory exploration, we will concentrate on the production of carbon dioxide.

To test for cellular respiration, you will need to determine the presence of carbon dioxide, and changes in carbon dioxide levels. We will measure carbon dioxide indirectly. We will make use of the fact that aquatic organisms that respire directly in the water (like *Elodea*) give off carbon dioxide directly into their watery surroundings. When carbon dioxide is produced by an aquatic plant, the carbon dioxide dissolves in water.

As it dissolves, some of the carbon dioxide forms carbonic acid, H_2CO_3 . This **reversible** reaction is shown by the equation:



When carbon dioxide (CO_2) in the water increases, the pH decreases, due to the formation of carbonic acid (H_2CO_3). If carbon dioxide is removed from the water, the amount of carbonic acid decreases and the pH increases. Therefore, we have an indirect measure of the amount of carbon dioxide in the water: the pH of the

water. A pH probe can be used to monitor pH and thus determine whether carbon dioxide is released into the water or is removed from the water.

Since plants are composed of cells, and all cells must undergo cellular respiration, it stands to reason that we should be able to measure respiration in a plant.

◆ **We can measure rates of photosynthesis** in several ways, all of which come from the basic equation of photosynthesis:



Thus, we can measure

- Rates of the appearance (production) of glucose
- Rates of the appearance (production) of oxygen
- Rates of the disappearance (consumption) of water
- Rates of the disappearance (consumption) of carbon dioxide
- Rates of the consumption of light energy

Of these possibilities, again the easiest to measure is the appearance of oxygen or the disappearance of carbon dioxide. For this lab exploration, we will measure the disappearance of carbon dioxide.

To perform the necessary tests, you will need to determine the presence of carbon dioxide. We will make use of the fact that aquatic organisms that photosynthesize directly in the water consume carbon dioxide directly from the water. As in the respiration experiment, we have an indirect measure of the amount of carbon dioxide in the water: the pH of the water. A pH probe can be used to monitor pH and thus measure how much carbon dioxide is released into the water.

Since plants also photosynthesize, we should also be able to measure rates of photosynthesis. However, remember that plants are made of cells and so they must also undergo cellular respiration. Therefore, we will be measuring both respiration and photosynthesis by measuring pH changes under light conditions. Based on what you know about photosynthesis and respiration, which would you expect to be more prevalent in a plant under light conditions compared to dark conditions? What would you expect to happen to pH levels in light compared to dark conditions? These are questions you will address as part of this exploration.

◆ Discuss these questions with your lab partners and **fill-in the worksheet** that follows

Worksheet

- Do plants respire in light? _____
- Do you expect that plant photosynthesize in the light? _____
- Why do plant cells respire? To produce _____.
- Why do plant cells photosynthesize? To produce _____, which will be used in the set of reactions called _____.
- Given your answer to the last question, what would happen to a plant cell that did not photosynthesize and why?

f. Given your answer to the previous question, which should be faster, the rate of photosynthesis or the rate of respiration and why?

g. Given your answer to the previous question, what do you predict will happen to the pH in the light, and why?

◆ NOW: Propose and enter into Tables 3 and 4 the appropriate hypotheses and predictions for our experiments, testing whether photosynthesis and/or respiration occur in a plant in light and/or dark conditions. Also indicate which beaker you would use to test the prediction, and indicate which beaker is the control beaker. Be prepared to present what you came up with and why to the class. Remember that a prediction is an "If...then..." statement.

MATERIALS (per group)

4 large test tubes	2 sprigs of <i>Elodea</i>	scale
400-ml beaker to rinse probe into	wax pencil	1 weigh boat
distilled wash water in squirt bottle	well water	1 pH probe

PROCEDURE

1. Work in groups of 4. To work efficiently, split up the work!
2. Obtain and label 4-250 ml test tubes. Using a wax pencil, label them "Light-Control", "Light-Experimental", "Dark-Control", and "Dark-Experimental". Also label all tubes with your group name.
3. Obtain a pH meter, and turn it on to warm up.
4. Fill each test tube with well water (about $\frac{3}{4}$ full).
5. Obtain 2-4 large sprigs of *Elodea* (or other aquatic plant). You will eventually divide your sprigs between the "Light-Experimental" and "Dark-Experimental" tubes. Obtain enough plant to fill the water in the test tube. Pat the plants dry with a paper towel, **weigh them** and record the data in Table 1.

6. Place 1-2 sprigs in test tube “Light-Experimental”, and the other sprigs in test tube “Dark-Experimental”. The sprigs should be submerged (under water).
7. Remove the cap on the pH probe. Rinse the probe thoroughly with distilled water (you may rinse into a beaker).
8. Place the probe into beaker “Light-Control” and gently swirl briefly to allow water to move past the probe’s tip. When the reading stabilizes, or after 1 minute, record the pH value in Table 1; do not wait longer than 2 minutes. Assume that the pH of beaker “Light-Experimental” is the same as for beaker “Light-Control”, since you used the same well water, and fill in the pH in the spaces in Table 1.
9. Place the probe into beaker “Dark-Control” and gently swirl briefly to allow water to move past the probe’s tip. When the reading stabilizes, or after 1 minute, record the pH value in Table 1; do not wait longer than 2 minutes. Assume that the pH of beaker “Dark-Experimental” is the same as for beaker “Dark-Control”, since you used the same well water, and fill in the pH in the spaces in Table 1.
10. When all readings have been taken, rinse the pH probe with distilled water, replace the cap, and turn off the meter.
11. Place beakers “Light-Control” and “Light-Experimental” under light conditions in the light rack. Leave the test tubes for 40 minutes.
12. Place test tubes “Dark-Control” and “Dark-Experimental” under dark conditions in the cabinet at the front of the lab. Leave the test tubes for 40 minutes.
13. After 35 minutes, turn on your pH meter, so that it has time to warm up.
14. After 40 minutes, measure pH (using the pH probe) for **each** of the 4 test tubes. Remember to rinse the probe after each reading! Record the data in Table 1 below.
15. Repeat step 10, this time putting a drop of distilled water in the cap to prevent the probe from drying out.
16. Clean up by returning the well water and *Elodea* to the *Elodea* container, and putting your beakers, wax pencil, squirt bottle and the pH meter back at the front of the room. Wipe up any spilled water, and throw away paper towels and the weigh boat.
17. Before leaving the lab, be sure to record both your own data and class data in the table below.

PROCESSING THE DATA

- ◆ Calculate the change in pH, **ΔpH** , for your tubes. Do this by subtracting the starting pH from the ending pH. (**ending pH – starting pH**) Record your results in Table 1.
- ◆ Calculate the **Corrected ΔpH** . You must correct for any changes that occurred to the pH of the water that were NOT the direct result of photosynthesis or respiration by *Elodea*. Do this by subtracting the ΔpH of the control tube from that of the *Elodea* tube. (**$\Delta\text{pH Elodea} - \Delta\text{pH control}$**) Record this “**Corrected ΔpH** ” in Table 1.
- ◆ Calculate the **Corrected $\Delta\text{pH/g}$** . Do this by dividing the “Corrected ΔpH ” for the *Elodea* tube by its weight, in grams. Record in Table 1.
- ◆ Report your findings to the class. Report only your **Corrected $\Delta\text{pH /g}$** on the table on the wipe board at the front of the classroom.

Neatly copy all class data from the wipeboard to Table 2.

- ◆ Calculate the “Average Corrected $\Delta\text{pH/g}$ ” from the class data for both Light and Dark *Elodea* tubes. For each condition (Light or Dark), find the average by adding all class data (across the table) and then dividing by the number of groups.

DATA

1. Table 1: Data from respiration/photosynthesis experiment with *Elodea*

beaker	treatment	weight (g)	starting pH	ending pH	ΔpH	Corrected ΔpH	$\frac{\text{Corrected } \Delta\text{pH}}{\text{g}}$
“Light-Control”	control						
“Light-Experimental”	<u>Elodea</u>						
“Dark-Control”	control						
“Dark-Experimental”	<u>Elodea</u>						

2. Table 2. Corrected $\Delta\text{pH/g}$ from the different class groups, and average corrected $\Delta\text{pH/g}$.

	Corrected $\Delta\text{pH/g}$								
Condition	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Average
<i>Elodea</i> -Light									
<i>Elodea</i> -Dark									

- 3. Graph the averaged class data, the “Average Corrected $\Delta\text{pH/g}$ ” for BOTH the light and dark data. USE GRAPH PAPER (or a computer). Be sure to correctly label the x- and y-axes. Attach your labeled graph to this handout. Remember that the X-axis will probably contain the group categories, while the Y-axis will contain the experimental data. If you need help generating your graph, please ask me! You cannot receive full credit for the lab without a labeled graph.**

INTERPRETING THE DATA AND DRAWING CONCLUSIONS

In the following table (Table 3), you **should have already** filled in the predictions and indicated which is the experimental test tube and which is the control test tube. Now:

- Interpret the class data to support or reject the hypothesis.
- Explain your reasoning. How did you interpret your data to make the conclusion?
- If rejected, write a corrected hypothesis.

Table 3. Hypothesis table about whether respiration rate or photosynthetic rate is greater in the “**Light**” conditions.

Hypothesis 1:		
Prediction 1:		
Experimental Test Beaker:	Control Test Beaker:	Interpretation (circle one): support or reject ?
Reasoning (for your choice of "support" or "reject")		
If rejected, a corrected hypothesis:		

In the following table (Table 4), you **should have already** filled in the hypothesis, your reasoning for the hypothesis, and the prediction addressing the question of which would be greater, the rate of respiration or the rate of photosynthesis. Now:

- Interpret the class data to support or reject the hypothesis.
- Explain your reasoning. How did you interpret your data to make the conclusion?
- If rejected, write a corrected hypothesis.

Table 4. Hypothesis table about whether respiration rate or photosynthetic rate is greater in the “**Dark**” conditions.

Hypothesis 2:
Reasoning for your hypothesis: Why did you propose the particular hypothesis you did?
Prediction 2:
Interpretation (circle one): support or reject ?
Reasoning (for your choice of "support" or "reject")
If rejected, a corrected hypothesis:

Additional Questions:

1. Calculate what the corrected $\Delta\text{pH/g}$ would have been if we could have prevented the plant from respiring in the “light” conditions (i.e., in the absence of respiration, so that the plant was only photosynthesizing).

2. Why do we divide ΔpH by the weight of the organism?

3. What was the purpose of the empty tubes in your experiment? If the pH changed in these tubes, what might have caused these changes? Write down two possibilities!

4. So... do plants both respire and photosynthesize in the light (yes or no)?

5. How did your group data (Corrected $\Delta\text{pH/g}$) compare to the class data (Average Corrected $\Delta\text{pH/g}$)? Was it identical? In general, why might it be beneficial to report the “Averaged Data” from many experimental trials versus data from just one single experiment?

6. Suppose that our pH meters were unavailable the day of the lab. Instead of canceling lab, we drove to the UW and borrowed their highly sensitive oxygen-sensors. We do the *Elodea* experiment, exactly as planned, but instead of measuring the pH, we measure levels of oxygen in the test tubes. **Write two new hypotheses for this experiment.** Your new hypotheses should pertain to the changes in oxygen levels you would expect to see following *Elodea* exposure to either Light or Dark conditions.

Hypothesis 1 (light condition) _____

Hypothesis 2 (dark condition) _____
