Principles of Biology I Laboratory

Week 12

Photosynthesis and Respiration in Plants

Assignments

1. [10 points] Pre-lab assignment, found at the end of this handout
2. [5 points] In-lab quiz, covering the lab handout
3. [5 points] In-lab assignment (graph from the first set of measurements; turn in one lab per group before you leave the lab)
4. [18 points] Post-lab assignment, found at the end of this handout

Suggested Reading

1. **Before Lab:** Openstax Chapter 8 figures
2. **After Lab:** Page 40 of the SM on Results sections

Objectives:

1. Set up an experimental system to measure photosynthesis and respiration in leaf disks
2. Investigate the effect of an independent variable of your choice on photosynthesis and respiration
3. Articulate an independent research question and hypothesis and design an experiment

Concepts in Action:

1. Plants possess mitochondria capable of respiration just like human cells
2. There is a delicate balance between photosynthesis and respiration in the plant cells

Introduction:

Autotrophic organisms, such as green plants, algae and cyanobacteria, capture light energy and convert it into chemical energy, stored in the bonds of organic molecules such as monosaccharides. In plants, this process (**photosynthesis**) takes place in the chloroplasts of green leaves. Review Openstax Ch. 8 for an overview of photosynthesis.

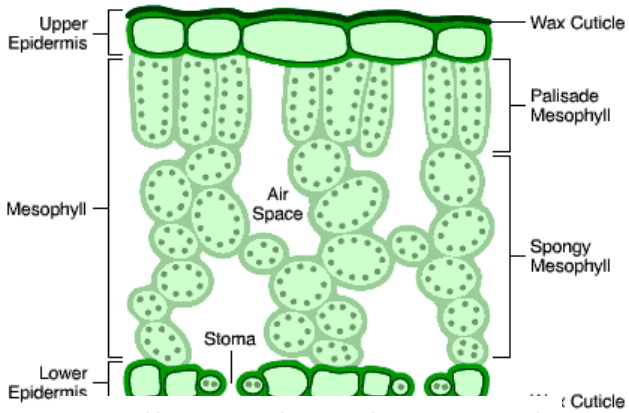
All living organisms, including plants, extract energy from organic molecules through redox reactions. **Cellular respiration** (Openstax, Ch. 7.5) is a series of reactions that utilize the energy in the bonds of organic molecules such as glucose ($C_6H_{12}O_6$) to produce ATP, which is then used to power cellular activities.

Oxygen gas (O_2) is produced in the process of photosynthesis and consumed in aerobic respiration. While there are many sophisticated techniques to monitor photosynthesis and cellular respiration, measuring the production and utilization of oxygen by leaf tissue is a convenient and effective "low-tech" approach.

In this laboratory activity, you will use leaf disks obtained with a paper punch; you will monitor photosynthesis by (indirectly) measuring net oxygen production in the disks, and respiration by estimating oxygen use during cellular respiration.

Experimental Setup

To monitor changes in the amount of oxygen in the intercellular spaces of leaf disks (the spaces between mesophyll cells, see **Fig. 1**), first you will need to vacuum-infiltrate the leaf disks with a buffer.



http://wdict.net/gallery/palisade+cell/

Figure 1. A Leaf Section. The mesophyll cells, packed with chloroplasts, are loosely arranged leaving abundant air spaces in between them. This allows direct exchange of gases (O_2 and CO_2) between cells and atmospheric air.

To accomplish this, you will submerge the leaf disks in a buffer containing bicarbonate (HCO_3^-) and apply a vacuum: the air in the intercellular spaces will be forced out and replaced with the buffer. Leaf disks infiltrated with buffer will sink. As photosynthesis proceeds, the oxygen gas produced by the mesophyll cells will form bubbles in the spaces between the cells. Oxygen will then displace the liquid from the intercellular space, and the disks will float. Dissociation of bicarbonate in the solution will provide the necessary CO_2 for photosynthesis:

$$H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \rightleftharpoons H_2O + CO_2$$

Aerobic respiration occurs in all cells when oxygen is available, including plant cells that are actively photosynthesizing. As you know, the process of respiration consumes O_2 gas. To measure **net** respiration (oxygen consumption) with this experimental setup, you will need to effectively stop photosynthesis in your leaf disks. Think about what will happen to your leaf disks if they stop producing O_2 by photosynthesis and keep consuming O_2 by respiration.

Do you expect them to sink or float? Why?

References:

Juliao, F. and H.C. Butcher IV. 1989. Further improvements to the Steucek and Hill assay of photosynthesis. Am. Bio. Teacher 51(3): 174-176.

Morris JR, Hartl DL, Knoll AH, Lue RA, Berry A, Biewener AA, Farell BD, Hobrook NM, Pierce NE, Viel A. 2013. Biology: How Life Works. 1st ed. New York (NY): Freeman. 1200 p.

Pitkin, R. B. 2004. Photosynthesis/respiration in leaf disks. Pages 347-351, in Tested studies for laboratory teaching, Volume 25 (M. A. O'Donnell, Editor). Proceedings of the 25th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 414 pages.

Steucek, G.L. and R.J. Hill. 1985. Photosynthesis I: an assay utilizing leaf disks. Am. Bio. Teacher 47(2): 96-99.

Laboratory Activity

Work in groups of four. Complete in-lab as a group with all names on the assignment.

Objective 1

Set up an experimental system to measure photosynthesis and respiration in leaf disks and test the system.

You will be provided with 20cc syringes (no needles!) and a rack to support them. To perform your test in triplicates, you will need 3 syringes to begin with. Cut at least 10 leaf disks per syringe (at least 30 total) with a paper punch and keep them on a moist paper towel until you are ready to begin. When punching leaves, avoid the large veins!

To prepare the syringes as your "assay chambers":

1. Remove the plungers and drop 10 leaf disks into the barrel of each syringe.
2. Tap gently until the disks fall to the bottom.
3. Carefully replace the plunger, making sure that you do not crush the disks.
4. Pull 10 mL of sodium bicarbonate solution (infiltration buffer) into the syringe. This solution contains a small amount of a "surfactant" agent (similar to soap), to facilitate vacuum-infiltration.
5. Invert the syringe, tap a few times to let air bubbles float, then push the plunger as close as you can to the 10 mL mark, to eliminate as much air as possible from the barrel.
6. Vacuum-infiltration: hold the syringe barrel firmly against your gloved finger, pull the plunger and hold in this position. The buffer will begin infiltrating the leaf disks.
7. Swirl the syringe and repeat step 6 until all disks are infiltrated and sinking.
8. Cover the syringes with foil (to keep the contents of the syringe in the dark) until you are ready to start the experiment.

Data Collection and Representation

Work in groups of four. In the interest of time, make sure that **while** three group members collect the data, one group member plots the data on a graph.

Part I: monitoring photosynthesis. Position the goose-necked lamp so that the light shines onto your assay chambers. Measure and record the distance of the light source from your samples. At 1 – minute intervals, record the number of disks floating in each of the syringes (note that *floating* doesn't necessarily mean a disk will go all the way up to the surface; use your judgment to establish consistent criteria for floating). Remember to swirl the syringes often, because disks may get on top of each other and prevent the disks below from floating. Continue recording data until all of your leaf disks are floating (or for 30 minutes, maximum).

Part II: monitoring respiration. Turn off the light and cover your syringes with foil. Continue to monitor the leaf disks at 2-minute intervals, and record at each time point the number of disks that are floating. Continue recording data until all of your leaf disks sink (or for 20 minutes, maximum).

Represent the data as totals of the three replicates. Plot the **total number** of floating disks versus time in minutes. Clearly mark on the graph the time point when the leaf disks were switched from light to dark. Turn in one graph per group. This graph will be graded as an in-lab assignment and will be worth **5 points**.

Objectives 2 & 3

Independent Investigation

What variables may affect photosynthesis and/or respiration? What do you think would happen if you changed the light intensity, or wavelength, or the availability of CO_2, or the pH of the buffer?

Do all types of leaves photosynthesize and respire at the same rate?

You will be provided with a number of possible independent variables to choose from:

- Light intensity (you can place the assay chambers at various distances)
- pH of the bicarbonate buffer (you can choose between pH 7.4, pH 8.0 and pH 8.4)
- Type of leaves (Swiss chard, Spinach, Ivy, Turning Tree Leaves…)
- Light wavelength (colored light bulbs will be available)

In your groups, choose a variable that you want to test.

Formulate a question, a hypothesis and a prediction; design an appropriate experiment to test your hypothesis.

Make sure that you set up and monitor your experimental conditions. They will need to be performed in triplicates; you will need at least 6 syringes for this experiment.

Before proceeding: Verify with your TA that your experimental setup is feasible; Measurements may need to be at 1 or 2-minute intervals for your variable. Or if your experiment requires two lamps (e.g., you are measuring photosynthesis and respiration under a red light vs. white light), let your TA know and make sure you can split into two groups working with the two different lights.

Data Collection and Analysis

Start fresh! Punch out new leaf disks, clean the syringes if you need to re-use them, and obtain all the materials you need.

Repeat the procedures you applied in your test run. However, this time, you will need to create a graph separately for reference for Objective 4.

Objective 4

Post-Lab activity: write a Results Section and Explanation of Results.

1. Work individually!

2. Use the rubric provided at the end of this handout to guide your writing.

3. Represent your data as **mean of floating disks at each time point**(at 1 or 2-minute intervals). This should be a table and a line graph (or scatterplot) reporting your treatments on the same graph.

On your graph, mark clearly the time point at which you switched from light to dark.

An Explanation of results analyzes and compares the data but also steps back and addresses a broader context, namely the variable studied and how it relates to Photosynthesis.

Pre-Lab Assignment

Worth 10 Points, Complete on your own in your carbonless notebook before going to lab. Turn in your pre-lab worksheet to the TA as you walk in.

The Quiz starts 5 minutes into the lab.

Q1. [5 points] Outline the procedure that you will use to observe photosynthesis and respiration. Be sure to include all of the materials you will need and a description of your methods. You may answer this question outline form, in a flow chart, in a diagram, or in any format you choose, as long as all necessary information is included.

Q2. [2 points] In this experiment, when you place leaf disks in the light, the disks will float. Briefly explain in your own words **why** the disks will float when they are placed in the light.

Q3 [3 points] Both photosynthesis and respiration are occurring in the leaf disks when they are in the light. If the disks float, is more O_2 being produced by photosynthesis or consumed by respiration? Explain your reasoning.

Post-Lab Assignment

Worth 16 Points

For your post-lab assignment this week, you will be writing a Results section. The rubric that will be used to grade results section can be found below. your

	Category/Expectation	Good	Fair	Inadequate
Results [14points]	- Experimental results are summarized in the text in a logical and coherent manner	2	1	0
	- Sample sizes (n) and statistical analysis of the data are reported in the text	2	1	0
	-Table is visually appropriate with Title (no raw data, sig. fig.)	2	1	0
	- Results include Figure(s) of the experimental results. Each graph included will need to have:			
	1. Title and caption below graph	2	1	0
	2. Dependent and independent variables identified with titles on the appropriate axes. Label and units on both axes	2	1	0
	3. A legend, if necessary (or otherwise the graph can stand on its own).	2	1	0
	- the graphs (Figures) are appropriately cited in the text and the information in text and figures is coherent.	2	1	0
Explanation of Results [4pts]	-Based on Results, formulate scientific explanation of what you observed	4	2	0