# Lab 8: Measuring the Effect of Light Manipulation on Photosynthesis

## Purpose

To analyze the effect of varying light conditions on the rate of photosynthesis.

## Learning Objectives

At the conclusion of this exercise, students will be able to:

* Measure the rate of photosynthesis under varying levels of light intensity.
* Measure the rate of photosynthesis under varying wavelengths of light.
* Explain the role of photosynthesis in the global carbon cycle.

## Why It’s Relevant

Most of the energy available to humans and other animals on Earth originates from photosynthesis. Photosynthetic organisms convert atmospheric carbon dioxide into sugars that serve as food and become fuels used to power machines. By understanding photosynthesis, scientists can better track how energy flows through living systems. Photosynthesis is a chemical process through which light, water, and carbon dioxide are transformed into glucose and oxygen. This process not only provides food and energy but also plays a crucial role in regulating Earth’s atmosphere. It helps reduce greenhouse gases by removing carbon dioxide from the air and produces the oxygen that animals need to breathe. Environmental factors such as light intensity and wavelength can significantly influence the rate of photosynthesis, affecting both energy production and gas exchange on a global scale.

## Introduction

The sun provides the most abundant and readily available energy resource on earth. Solar energy delivers a constant supply of more than 10,000 times the world’s energy use each second (U.S. Department of Energy, 2021). This tremendous source of power serves as the foundation for nearly all the energetic transformations occurring on earth. Even fossil fuels are the stored remnants of the impact solar energy has had on our planet. Most of the solar energy made available on earth is captured through a process called photosynthesis. Photosynthesis converts light energy to chemical energy and is carried out by living organisms such as plants, algae, and some bacteria. These organisms subsequently make chemical energy available to other ecosystems through their bodies, which we call biomass energy. The chemical reaction for photosynthesis is 6 carbon dioxide (CO2) + 6 water (H2O) + light energy → glucose (C6H12O6) + 6 oxygen (O2). In this reaction, the movement of light waves serves as kinetic energy that drives production of potential energy stored in the chemical bonds of glucose.

Photosynthetic organisms harness the power of light through the presence of pigments that are energized by varying wavelengths of light. Light that strikes special molecules in pigments drives the motion of electrons, producing electricity via the photoelectric effect.

Electricity generated from the photoelectric effect is used to make a universal form of cellular energy called adenosine triphosphate (ATP). ATP is subsequently used to make sugar destined to be incorporated into an organism’s biomass, for use as energy to facilitate growth and cellular activity, or for long-term storage in specialized structures. Thus, photosynthesis is called a coupled reaction. The coupled reactions include the light-dependent reactions (photo), in which light is used to harvest electricity, and the light-independent reactions (synthesis), where that energy is used to synthesize sugars. During the light-dependent reactions, light and water serve as inputs to yield ATP (energy) and electrons that act like a type of molecular glue in later reactions. This is followed by the light-independent reactions, where sugars like glucose are assembled by spending ATP and binding CO2 absorbed from the air together with electrons from the light reactions.

Light is energetic because it is made of particles that move. For example, low-energy infrared wavelengths of light are used to keep food warm at fast food restaurants. If you have ever seen the orange glow above a batch of French fries, you have viewed light energy in the form of heat. While this is amazing, light can do way more than simply transfer heat.

Humans are currently investigating ways light can be used to generate clean alternative forms of energy. One approach makes use of photovoltaic cells that mimic the actions of plant pigments by converting light to electricity. Newer technologies harness hydrogen and methane gas byproducts of algal photosynthesis for use as fuel. In other cases, compounds are extracted directly from plants to create biofuels such as ethanol, which is often used as a fuel additive that significantly extends the range of combustible fuel. The opportunities for innovation around light as a power source present exciting ways to reduce human impact on climate and protect our environment. In the following lab, you will investigate how varying levels of light intensity and wavelength impact the effectiveness of photosynthesis by performing a leaf disc assay.

## Develop a Hypothesis

In this lab you will measure the effect of different variables on the rate of photosynthesis using the leaf disc assay method. An assay is simply a method of analyzing or detecting something. You will find the median time it takes for 50% of leaf discs to float in a solution of sodium bicarbonate. This will represent the amount of oxygen gas released per unit of time. In this procedure you will measure the median instead of the mean. Refer to the Scientific Method Lab for a refresher on descriptive statistics and when it is appropriate to report the median. Start by making some predictions.

* Prediction #1. Light intensity will [INCREASE / DECREASE] the rate of photosynthesis. Explain the rationale for your prediction.
* Prediction #2. The rate of photosynthesis will be like the control (no light) when exposed to [RED / BLUE / GREEN] light. Explain the rationale for your prediction.
* Prediction #3. Choose your own parameters, e.g., type of light source (LED, CFL, incandescent, halogen, sunlight), water temperature (10 °C, 20 °C, 30 °C, 40 °C), baking soda (CO2) concentration (e.g., 0.5% w/v, 1% w/v, 2% w/v), plant species, leaf color, or pH. Explain why you chose the parameter and the rationale for your prediction.

## Materials

* Straws or hole punch
* Sodium bicarbonate (baking soda)
* Liquid dish soap
* Spinach leaves
* Water
* Plastic syringe
* Permanent marker
* Light source (bulb with shield e.g., desk lamp)
* Timer
* 3 beakers
* Black mesh 50% shade cloth (more precise) or cheesecloth (less precise)
* Luxmeter
* Red plastic sheet
* Blue plastic sheet
* Green plastic sheet

## Procedure

1. Prior to the start of lab, soak spinach leaves in water maintained at 4 °C or 39.2 °F for 2-3 hours to increase turgor pressure and minimize limp leaves. Be sure to select fresh crisp leaves.
2. Prepare 300 mL of a 0.2% (w/v) sodium bicarbonate solution by combining 0.2 g of sodium bicarbonate (NaHCO3) per 100 mL of distilled water. Add one drop of liquid soap into the solution and gently mix to dissolve everything completely. Note: Avoid the creation of bubbles so all the baking soda is available as dissolved CO2.
3. Fill three beakers with 300 mL of sodium bicarbonate and soap solution.
4. Using a hole puncher or the circular edge of a straw, cut out 50 equal-sized leaf discs about 4 mm in diameter from firm, dark green leaf regions away from the major veins. Store the leaf discs in a wet paper towel to keep them from drying out until you are ready to inject them with the sodium bicarbonate solution.
5. Prepare the syringe by removing the plunger. Place 10 leaf discs into the base of the syringe and replace the plunger by gently pushing it down. Remove all the air from the syringe without crushing the leaf discs.
6. Holding the syringe vertically, draw up 1.5 mL of the sodium bicarbonate / detergent solution from the beaker so that the leaf discs float on top.
7. Invert the syringe and carefully remove all the air by slowly pushing the plunger up until the water is level with the tip of the syringe.
8. Place your finger on the tip of the syringe, then slowly pull the plunger down to create a strong vacuum inside the syringe.
9. Hold the vacuum for 10 seconds, agitating the leaf discs to suspend them in the solution.
10. Release the plunger and remove your finger from the tip of the syringe, ending the vacuum.
11. Repeat steps 7-10 until all the leaf discs have sunk to the bottom of the inverted syringe. Sunken leaf discs have been injected with the sodium bicarbonate solution.
12. Pour the leaf discs into beaker #1. Leaf discs that have sunk to the bottom have had all the air removed and replaced with sodium bicarbonate solution. When exposed to light, the leaf discs will use the infused sodium bicarbonate as a source of carbon to perform photosynthesis until it is completely consumed. Photosynthesis rate will be measured by the time it takes for enough oxygen to build up in the leaf discs that the leaf discs no longer sink to the bottom.
13. Repeat steps 4-11 two more times so that you have triplicates. Place 10 leaf discs in beakers #2 and #3.
14. Make two negative controls (no leaf discs expected to float) with the remaining 20 leaf discs. Make the first control by injecting leaf discs using a solution of only soap and water, excluding sodium bicarbonate. Make another negative control by creating the same leaf disc setup with the sodium bicarbonate solution, but instead of exposing leaf discs to the light, put the leaf discs in the dark for the duration of the experiment. Negative controls are known conditions that produce no photosynthesis.
15. Divide the experimental treatments among the class so that each group investigates one treatment. Each class should investigate 50% shade, normal light, blue light, red light, green light, and 80-90% shade.
16. Adjust the light intensity by using a 50% shade cloth or 1-2 cheesecloths. Increase the number of cloths to reduce the intensity of light. Use a luxmeter to record the percent shade for each treatment. Make sure the light source is completely covered by the shade.
17. Adjust the wavelength by using a blue, red, or green colored plastic sheet to test how wavelength impacts the photosynthetic rate. Ensure the light source is completely covered by the colored plastic sheet and that the distance of the beaker from the light is the same for each treatment.
18. Place each beaker under your light source, ensuring that each is positioned to receive an equal amount of light. It is best to use the same distance for each treatment to minimize the effect of heat on your results. For LED bulbs, select a consistent distance of between 10 and 30 cm; for fluorescent bulbs (T5/T8), select a distance between 10 and 20 cm; and for incandescent bulbs, place your treatments 35 cm away from the light source. Be consistent in all your trials.
19. Set a timer for 15 minutes and record the number of floating discs in each beaker at 1-minute intervals until all the leaf discs have floated to the surface of the water. Keep an eye on leaf discs to make sure attachment to the walls of the beaker does not prevent them from moving up the water column.
20. Identify the independent and dependent variables, then create a graph depicting the number of floating leaf discs over time, with time (in minutes) on the x-axis and number of floating leaf discs on the y-axis.
21. Determine the time it takes for 50% (the median number) of the discs to float by drawing a horizontal line parallel to the x-axis starting at leaf disc number 5 until you reach slightly above the closest point on your graph. This will be your Effective Time (ET50), which represents the median, rather than the mean. The median provides a more accurate representation in the event that there are outliers (leaves that floated too quickly or too slowly).
22. Calculate the average of the three ET50 values. The average ET50 value serves in place of a direct oxygen gas measurement. Express your reaction rate as the concentration of product formed per unit of time by taking the reciprocal of ET50. Divide ET50 by 1 as follows: 1/ET50, using minutes-1 as your unit of measurement. Report your reaction rate as the value you calculated per unit min-1.
23. Compare the photosynthetic reaction rate measured for each experimental treatment to the negative control and normal light conditions using the class data.
24. Identify the independent and dependent variables then create a bar graph that shows the photosynthesis rate for each variable tested. Plot the variables tested on the x-axis and the rate of photosynthesis (1/ET50) on the y-axis.
25. Optional extension activity: Perform a non-parametric single sample sign test to compare the median photosynthetic rate of your group’s experimental treatment to the standard rate determined from normal light conditions trial.

## Extension Activity: Performing a sign test (optional)

The sign test is used to compare the median value of a data set to a hypothesized value. Sign tests are statistical tools used when data is not symmetrical around the mean and/or the sample size is small. The purpose of the sign test is to measure the direction of change rather than magnitude of change. For example, in this study you might state your null hypothesis as: The median photosynthetic rate of the experimental treatment is equal (no difference) to the standard rate. The standard rate is the average photosynthetic rate calculated from all trials conducted under normal light conditions. Your null hypothesis states there is no difference (faster or slower) in the photosynthetic rate between your hypothesized standard (average rate under normal light conditions) and each individual experimental photosynthetic rate. Fill in **Table 8.1** to calculate the sign test.

**Table 8.1. Stepwise instructions for calculating a sign test.**

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| --- | --- | --- | --- |
| Normal light photosynthetic rate (xi) | Experimental light photosynthetic rate1(ei) | Difference2(ei – xi) | Sign of difference3(+ or -) |
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|  |  |  | # positive \_\_\_# negative \_\_\_ |

1e.g., 50% intensity, blue, green, red, 80% intensity
2experimental photosynthetic rate (ei) – normal photosynthetic rate (xi)
3Write a positive or negative symbol to indicate when the rate of photosynthesis for experimental light conditions is greater or less than normal light conditions.

Count the number of positive signs and write the value in the last row of the table. Count the number of negative signs and write the value in the last row of the table. Ignore zeros. For statistical analysis, the test statistic is the sign with the smaller number counted. Use a sign table or binomial distribution to find the critical value based on your sample size (n) at alpha = 0.05. The sample size (n) includes all comparisons excluding any zeros.

If the critical value is greater than your test statistic, the experimental treatment impacted the rate of photosynthesis. The conclusion you are drawing is that there is a statistically significant difference between the median photosynthetic rate of the experimental treatment and the hypothesized value (standard rate).

If the critical value is less than your test statistic, the experimental treatment had no effect on the rate of photosynthesis. The conclusion you are drawing is that there is no statistically significant difference between the median photosynthetic rate of the experimental treatment and the hypothesized value (standard rate).

Based on your statistical inference:

* How does light intensity affect photosynthesis?
* How does light wavelength affect photosynthesis?

## Questions

1. What are some potential sources of error in this procedure? Hint: Do plants respire aerobically?
2. How could this procedure be modified to collect a more accurate representation of photosynthetic rate?
3. What do you conclude from your data about how your manipulated variable impacted the rate of photosynthesis?
4. If you decided to grow spinach in your backyard, what light intensity and/or wavelength of light would produce the highest yield based on class data?
5. Why was soap added to the bicarbonate solution?
6. What are the inputs and outputs of photosynthesis? How do the inputs and outputs of photosynthesis relate to the prefix “photo-” and root word “synthesis”?
7. What role does water play in supporting photosynthesis?
8. What is the purpose of having negative/positive controls?
9. List the independent variable, dependent variable, and controlled variable in the study you performed.
10. Does the data support the predictions you made earlier in the lab?

## Alternate Procedure for Online Courses

Follow the same procedure as above, but substitute household containers for your experimental setup. Most household measuring devices represent only standard units, so you will need to convert the quantities from the procedure above from SI units to standard units. Refer to the metric conversion lab for guidance on how to perform scientific conversions. As you think about your experimental design, parameters from prediction #3 under the “develop a hypothesis section” may provide you with interesting options to consider investigating.