



Using Spectrophotometers to Examine Photosynthetic Rates Under Various Qualities of Light

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1. Overview

This lesson focuses on allowing students to examine how photosynthetic rates can change by using different qualities of light. It is meant to supplement an existing knowledge of the processes of photosynthesis and the factors that can contribute to proper function. In addition, the students learn how to use a spectrophotometer and get a better understanding of the scientific method. Photosynthetic rates can easily be indirectly measured by using an isolated chloroplast suspension and a chemical, 2,6-dichlorophenol-indophenol (DCIP). DCIP is a blue colored compound that is capable of being oxidized and reduced. As the compound becomes reduced it becomes less blue and the color change can be measured using a spectrophotometer. A greater decrease in absorbance values indicates that a greater amount of photosynthesis is taking place in the chloroplast suspension. The photosynthetic rate can then be measured under different amounts (Dark Vs. Light treatments) and qualities (Red Vs. Blue Vs. Green) of light.

2. Objectives

The objective of this lesson is to allow the students to become familiar with the process of photosynthesis. They are able to observe the importance of light in the process and how various colors of light affect the efficiency of photosynthesis. In addition, the students become familiar with using a spectrophotometer, a standard piece of laboratory equipment, and become more familiar with using and understanding the scientific method.

3. Indiana Standards Met

Standard 1 The Nature of Science and Technology

Students further their scientific understanding of the natural world through investigations, experiences, and readings. They design solutions to practical problems by using a variety of scientific methodologies.

The Scientific View of the World

- 7.1.1 Recognize and explain that when similar investigations give different results, the scientific challenge is to judge whether the differences are trivial or significant, which often takes further studies to decide.

Scientific Inquiry

- 7.1.2 Explain that what people expect to observe often affects what they actually do observe and provide an example of a solution to this problem.
- 7.1.3 Explain why it is important in science to keep honest, clear, and accurate records.

- 7.1.4 Describe that different explanations can be given for the same evidence, and it is not always possible to tell which one is correct without further inquiry.

Standard 2 Scientific Thinking

Students use instruments and tools to measure, calculate, and organize data. They frame arguments in quantitative terms when possible. They question claims and understand that findings may be interpreted in more than one acceptable way.

Manipulation and Observation

- 7.2.6 Read analog and digital meters on instruments used to make direct measurements of length, volume, weight, elapsed time, rates, or temperatures, and choose appropriate units.

Communication Skills

- 7.2.7 Incorporate circle charts, bar and line graphs, diagrams, scatterplots, and symbols into writing, such as lab or research reports, to serve as evidence for claims and/or conclusions.

Standard 3 The Physical Setting

Students collect and organize data to identify relationships between physical objects, events, and processes. They use logical reasoning to question their own ideas as new information challenges their conceptions of the natural world.

Matter and Energy

- 7.3.11 Explain that the sun loses energy by emitting light. Note that only a tiny fraction of that light reaches Earth. Understand that the sun's energy arrives as light with a wide range of wavelengths, consisting of visible light and infrared and ultraviolet radiation.

Forces of Nature

- 7.3.19 Explain that human eyes respond to a narrow range of wavelengths of the electromagnetic spectrum.
- 7.3.20 Describe that something can be "seen" when light waves emitted or reflected by it enter the eye just as something can be "heard" when sound waves from it enter the ear.

Standard 4

The Living Environment

Students begin to trace the flow of matter and energy through ecosystems. They recognize the fundamental difference between plants and animals and understand its basis at the cellular level. Students distinguish species, particularly through an examination of internal structures and functions. They use microscopes to observe cells and recognize that cells function in similar ways in all organisms.

Interdependence of Life and Evolution

- 7.4.7 Describe how plants use the energy from light to make sugars from carbon dioxide and water to produce food that can be used immediately or stored for later use.

4. Methods and Procedures

Each lab station will need the following:

- Spectrophotometer
- 4 Cuvettes
- Light Source
- 4 Test Tubes
- 1 Test Tube Rack
- 3 Small Beakers (1 covered in aluminum foil, 1 covered in either red, blue, or green cellophane filter paper)
- Stopwatch

The following protocol is used to obtain the chloroplast suspension:

1. Weigh out 42g of fresh spinach (*Spinacia oleracea*) after removing the major veins and petioles.
2. Tear the leaves into small pieces and place in a blender with 160ml of ice-cold Tris-Sucrose buffer (0.3M sucrose, 0.2M Tris-HCl, 5mM MgSO₄; pH 7.5).

Amounts to add for Tris-Sucrose Buffer, pH 7.5 – per liter

- 102.7g Sucrose
 - 1.23g MgSO₄
 - 25.3g Tris-HCl
 - 4.75g Tris-OH (Basic)
3. Blend at medium speed for 10 seconds and check to make sure all the leaves are in the liquid and blend again for 10 seconds. **Be Careful NOT to over-homogenize the sample.**
 4. Filter the homogenate through approximately 8 layers of cheesecloth into a cold beaker or flask.
 5. Divide the homogenate equally between chilled centrifuge tubes and centrifuge for 5 minutes at 1000g at 2-4°C.
 6. Discard the supernatant and carefully resuspend the chloroplast pellet in 12ml of cold Tris-Sucrose buffer.

- Store this in a dark container and keep on ice until ready for use. This should be made up NO MORE than 2 days before the experiment.
Note: This protocol should be repeated to attain the amount needed for the class. For a 42 (7 lab stations for 6 periods) lab station set-up approximately 160ml of the chloroplast suspension will be needed.
- Prepare the DCIP solution (2,6-dichlorophenol-indophenol; final concentration of 4×10^{-4} M). Dissolve 0.012g of DCIP in 100ml of distilled water. Store in a dark container at 4°C until ready for use.

For each lab station, 3 samples and a blank will need to be prepared:

This should be done the morning of the experiment. The samples can be made in a test tube and should be thoroughly mixed before use. The samples can then be carefully poured into the cuvettes and subsequent experimentation can be done for each treatment (light, dark, and filter color) in the cuvettes. This will save time because the students will not have to pour the sample into the cuvettes at each sampling time

Amounts to Add to Each Test Tube:

<u>Tube</u>	<u>Buffer</u>	<u>DCIP</u>	<u>Distilled Water</u>	<u>Chloroplast Suspension</u>
Blank	3.5ml	-	1.0	0.5ml
Tube #1 – Light	3.5ml	1.5ml	0.5ml	0.5ml
Tube #2 – Dark	3.5ml	1.5ml	0.5ml	0.5ml
Tube #3 – Filter Color	3.5ml	1.5ml	0.5ml	0.5ml

Experimental Protocol:

- Set up the spectrophotometers and light source at each lab station. The lights should be off in the classroom as well as closing window blinds if possible. This will reduce the amount of stray light that could potentially hit each tube.
- Each sample from the blank, tube #1, #2, and #3 should be carefully poured into the cuvette right before each class is about to begin.
- The students will first zero each spectrophotometer (See section on Operating Instructions for a digital spectrophotometer) by changing the wavelength to 600nm and placing the Blank cuvette in the spectrophotometer (this should be done after each time interval to ensure the correct readings are being obtained). Each lab group will be assigned a color filter (either red, blue, or green) that will be covering a small glass beaker. Each group will also have two additional glass beakers that will be used to measure photosynthetic rates of the chloroplasts in a light source (clear glass beaker) and the photosynthetic rate in the dark (aluminum foil wrapped beaker).
- The light and colored beakers should be placed approximately 12in in front of the light source (Note: This distance may vary with the type of light source used. If the tubes are too close to the light source, the chloroplasts may get too hot and the membranes could be destroyed) and the dark beaker should remain in the dark.
- Before the light source is turned on, the students should obtain an initial reading for the light, dark, and colored samples and record the Absorbance values in a data table.
- The light source should then be turned on and time monitored. At every 2 minute interval, the students should take another Absorbance reading for each of the trials and record their data. The students should continue collecting data for all tubes for the full 10 minutes.

Expected Results:

There should be a steady decrease in the cuvette that is exposed to light. This indicates that photosynthesis is actively occurring in the sample. The cuvette that is kept in the dark should show little or no change in the Absorbance values because light was not present for the reactions of photosynthesis to occur. However, slight changes may be observed and this is due to the amount of light that is exposed to the tube as it removed from the dark treatment and placed into the spectrophotometer to obtain the reading. The cuvettes with the red and blue filter paper should show a decrease similar to what is observed in the light treatment tube. This is because the chloroplasts are able to absorb both red and blue light and use it for photosynthesis. There should, however, be a slight difference between the red and blue filter. This is due to that fact that the chlorophyll absorbs red light better than blue light. The tube with the green filter should show little to no change in the Absorbance values. Green light is reflected by the chloroplasts and therefore can not be used for photosynthesis. Any decreases in absorbance values that are observed are again due to the light the tube is exposed to when it is removed from the filter and put into the spectrophotometer for a reading.

Operating Instructions for the Spectrophotometer:

1. Turn on the instrument and let it warm up for at least 5-10 minutes.
2. Select the wavelength with the dial next to the sample compartment.
3. With the sample compartment closed and empty, adjust the % Transmittance (zero percent transmission of light) to read 0% T using the left front dial.
4. Place a clean (no fingerprints), dry cuvette filled approximately $\frac{3}{4}$ full of blank sample (solvent only) into the sample compartment.
5. Close the sample compartment and adjust the % Transmittance to read 100% using the front right dial.
6. Remove the blank cuvette and place the cuvette containing the sample into the sample compartment.
7. Close the sample compartment and read and record the value registered on the meter.

5. Scope

One class period prior (at least 45 minutes) is required to introduce how to use the spectrophotometers and have the students collect their data for each treatment (light, dark, and color filter). The following class period was used to discuss the results that were obtained from the experiment and helping the students construct their graphs.

6. Evaluation

Each student should share their data with the class so that each group will have all the color data (Red, Blue, and Green). The students then construct a graph to show the changes in Absorbance that occurred for each treatment over time. The students also answer several questions regarding what is occurring during each treatment as it relates to the process of photosynthesis (See worksheets and handouts; Data Tables and Follow-Up Questions) to help them understand why the treatments they applied to the chloroplast suspension produced the data that they collected.

7. Comments and Reflection

A test run of the experiment should be done before the students perform the experiment to allow for optimization of the amount of DCIP that is added to each test tube. To ensure that the students will get absorbance values greater than 0 for approximately the full 10 minutes, more or less DCIP may need to be added to the test tube. This variation is due to how successful the isolation process was for the initial chloroplast suspension (The more chloroplasts that are present, the faster the DCIP will be reduced resulting in obtaining low Absorbance readings in less than 10 minutes). This lesson does require an extensive amount of preparation if it needs to be completed in a two day time period. However, if a longer amount of time is available the students could be more involved in the preparation by making the solutions as well as the chloroplast suspension. This lab was very effective for all of the groups and demonstrated very well the importance of light for the process of photosynthesis. In addition, the students were able to learn how to use the spectrophotometers and had a better understand the scientific method after the lesson.

8. References

Bregman, A. 1990. Laboratory Investigations in Cell and Molecular Biology, 3rd ed.
John Wiley and Sons, New York.

9. Worksheets and Handouts

Name: _____

Date: _____

Hour: _____

Photosynthesis Lab Data Sheet – Absorbance Values

<u>Treatment</u>	<u>Initial (Time 0)</u>	<u>2 Minutes</u>	<u>4 Minutes</u>	<u>6 Minutes</u>	<u>8 Minutes</u>	<u>10 Minutes</u>
Tube #1 = Light						
Tube #2 = Dark						
Tube #3 = Red						
Green						
Blue						

Class Absorbance Values for the Red, Blue, and Green Filters

<u>Treatment</u>	<u>Time 0</u>	<u>2 Minutes</u>	<u>4 Minutes</u>	<u>6 Minutes</u>	<u>8 Minutes</u>	<u>10 Minutes</u>
RED						
BLUE						
GREEN						

Name: _____ Date: _____ Hour: _____

Photosynthesis Lab – Follow-Up Questions

1) On a piece of graph paper, graph the data that you and your classmates collected from the Photosynthesis Lab. Make only ONE graph and include all 5 treatments (Light, Dark, Red Light, Green Light, Blue Light) on the graph.

Remember to include the following on your graph:

- A. Title
- B. Label X and Y axis (with proper units)
- C. Key (Legend)

2) What happened to the Absorbance values in the 5 different tests tubes?

3) What evidence do you have that light is needed for Photosynthesis?

4) What happened to the Absorbance values with the different colored filters (Red, Blue, Green)?

5) Why is there a difference between the Absorbance values for the 3 different colors? Explain.