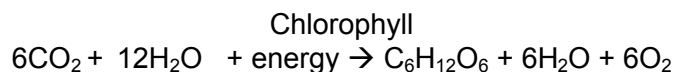


Photosynthesis

During photosynthesis, green plants transform light energy to chemical energy, and use that energy to synthesize organic fuel molecules that can be "stored" in the form of carbohydrates. Carbon dioxide from the atmosphere provides the carbon source for photosynthesis and water provides the hydrogen needed to reduce the carbon dioxide in the formation of carbohydrate molecules. Recall that chlorophyll pigments are necessary to trap the light energy.

The overall chemical reaction for photosynthesis is:



Chlorophyll
(Carbon dioxide + water + light energy \rightarrow glucose + water + oxygen)

Photosynthesis provides virtually all of the energy utilized by living organisms to survive, with the exception of the few food chains based on chemosynthetic bacteria, such as those found in the ocean's abysmal deep.

The process of photosynthesis is responsible for much of the energy we use to sustain our lifestyles as well. Wood and fossil fuels are the result, directly or indirectly, of photosynthetic output, as are the less well-known fuels as gasohol, and the methane generated by biomass conversion.

In this laboratory you will study a few of the factors involved in the process of photosynthesis by observing the utilization of light, oxygen production, and the role of chlorophyll in the process of photosynthesis.

Exercise I Demonstration of Oxygen Production During Photosynthesis

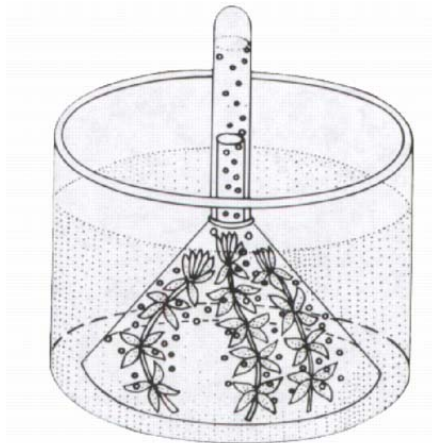
Materials Needed

- Battery jar 3/4 full of 0.2% NaHCO₃ (Sodium bicarbonate)
- Battery jar full of water (heat screen)
- 150-watt Flood lamp
- Several healthy shoots of *Valesneria* or other suitable aquatic plant
- Sharp razor blade
- Large funnel with narrow tip
- Large test tube

The splitting of water (photolysis) during the light reactions of photosynthesis, which provides the hydrogen for the reduction of carbon dioxide during the Calvin cycle, also produces oxygen gas (O₂). You can observe this production of oxygen using the aquatic plant, *Valesneria*.

This demonstration has been set up so that molecules of oxygen produced during photosynthesis are trapped in a test tube attached to the narrow end of a funnel in which several shoots of *Valesneria* have been placed. It is important that the apparatus, except for the top of the test tube, be submerged in water because you want to be sure that the oxygen is trapped in the test tube rather than escaping into the air.

Check the demonstration periodically over the laboratory period to observe the formation of oxygen bubbles as they displace the liquid in the test tube. You may also see oxygen bubbles forming at the cut tips of the *Valesneria*.



O₂ Production during Photosynthesis

Discussion Questions

1. What was the average rate of oxygen production?
2. Why was the *Valesneria* placed in a battery jar of sodium bicarbonate rather than in pure water?

Exercise II The Role of Chlorophyll in Photosynthesis

In most plants, the glucose produced during photosynthesis is converted into starch for storage. So that wherever photosynthesis is occurring in the plant, starch is being formed (for most plants). Production of starch is one way of detecting that photosynthesis has occurred.

Some plants have variegated leaves. Some parts of the plant leaf have chlorophyll and some do not. Portions of leaves that are cream-colored have no chlorophyll. There may be other pigments in the leaf as well, typically anthocyanins. When there are anthocyanins, the leaf may be dark purple or pink. Pink regions lack chlorophyll; darker purple leaves have both anthocyanins and chlorophyll.

You will use variegated *Coleus* or geranium leaves in this exercise to establish the requirement of chlorophyll for the process of photosynthesis. Although each student group will do this exercise, you will share the hot plates and other materials with other student groups.

Materials Needed

- Several large variegated *Coleus* or geranium plants
- Hot plates
- 800-ml Beakers for water
- 800-ml Beakers for alcohol
- 1 Liter alcohol
- Several petri dishes containing concentrated iodine
- Several large forceps
- Hot pads

1. Set up a boiling water bath on a hot plate using an 800-mL beaker.
2. Set up an alcohol bath on a second hot plate using an 800-ml beaker with alcohol. **Note:** Alcohol is flammable. It burns with a colorless flame. Do not allow your alcohol to boil. Watch the alcohol carefully and keep it no hotter than a gentle simmer.
3. Remove a leaf from a *Coleus* or geranium plant.
4. Diagram the variegated pattern of the leaf showing the regions containing chlorophyll in the space below.
5. Put the leaf in boiling water for 1 - 2 minutes.
6. Transfer the leaf to the hot alcohol. Leave the leaf in the alcohol for 3 - 4 minutes, or until all pigment is removed from the leaf.
7. Remove the leaf from the alcohol and blot it on paper toweling. It should be cream-colored (colorless).
8. Transfer the leaf to a petri dish containing iodine. Leave the leaf in the iodine for 1 - 2 minutes.
9. Remove the leaf from the iodine and blot it on paper toweling.
10. Diagram the starch pattern in the leaf as revealed by the iodine stain in the space below. Recall that iodine reacts with starch to form a purple or blue-black color.

Diagrams of Variegated Leaf

Before Treatment

After Treatment

Discussion Questions

1. How does the pattern of starch compare to the original pattern of chlorophyll in the variegated leaf?

2. What can you conclude from this exercise about the role of chlorophyll in photosynthesis?

Exercise III Storage Products in Plants

Whereas most plants store their photosynthetic output as starch, some plants store a variety of sugars, and some seeds store significant quantities of triglycerides. Moreover, many fruits contain sugars to attract potential dispersal agents. This exercise will help you determine the storage molecules in a variety of plants.

Many, but not all, sugars are called reducing sugars because they can cause reductions by donating electrons to a substance that can be reduced. The Benedict's test is used to identify reducing sugars. The Benedict's solution contains a blue soluble form of copper ions (Cu^{++}) that can undergo a reduction (that is the copper ions gain electrons) when heated in the presence of a reducing sugar. When the blue copper ions are reduced, they change from the soluble blue color to reddish color copper ions (Cu^+) that are insoluble.

The color of the test solution changes from blue ---> green ---> orange ---> red-brown or rust color as more reduced copper ions are formed. You will use the Benedict's solution to test for the presence of reducing sugar in various substances.

Iodine, as you know, reacts uniquely with starch to form a blue-black or purple color. Starch in any substance can be identified by this iodine reaction.

Materials Needed

- Concentrated Iodine solution
- Benedict's Solution
- Distilled water
- Test tube rack
- Test tubes
- Hot plate
- 800 ml Beaker
- China marker
- Test tube clamp
- Fresh Plant samples (Students are encouraged to bring additional samples to test)
 - Potato
 - Onion
 - Green banana
 - Very ripe banana
 - Carrot
 - Corn
 - Lima bean
 - Sugar cane or beet

Benedict's Procedure

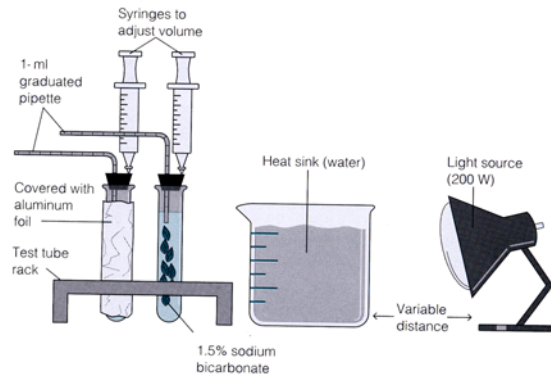
1. Set up a boiling water bath using the hot plate. When the water boils reduce the heat setting to keep the water at a simmer. Leave the stirrer knob turned off.
2. Obtain a number of test tubes equal to the number of plants you are sampling. Label the tubes at the top so the numbers won't wash off in the boiling water bath.
3. Place a small piece of your plant samples in each of the test tubes. Try to make the sample portions equal to each other.
4. Add 3 ml of Benedict's solution to each of the test tubes.
5. Agitate the test tubes gently. Do not shake the test tubes by placing your thumb over the top of the tube.
6. Put the test tubes in the boiling water bath for 5 minutes.
7. Remove the tubes and let them cool.
8. Record your results and conclusions in the Storage Products in Plants Table below.

Exercise IV Light Intensity and Photosynthetic Rate

The rate of Oxygen production, as you have seen, can be used as a measurement of photosynthetic activity. Excess oxygen gas produced during photosynthesis will diffuse from the leaf tissue into the external environment. The rate at which oxygen diffuses can be used to measure the rate of photosynthesis. In this exercise you will investigate the effect of light intensity on the rate of photosynthesis in the aquatic fern, *Azolla*, or the aquatic flowering plant, *Vallisneria*.

Materials Needed

- Healthy living *Azolla* and/or *Salvinia*
- Healthy living *Vallisneria*
- 1.5% NaHCO₃ (sodium bicarbonate)
- 2 Manometers (requires **large diameter** test tubes for the plant material used)
- Flood lamp with 200-watt bulb (Note these get very hot and can cause burns.)
- Aluminum foil
- Meter stick
- Tape
- 1000ml water-filled beaker for heat sink



Manometer Set-up

Procedure

1. Fill the 2 test tubes of your manometer apparatus with 1.5% NaHCO₃.
2. Add the available plants to both test tubes.
3. Place a rubber stopper-syringe-graduated pipette assembly into each test tube. Be sure that the rubber stopper displaces any residual air in the test tube. These are your manometers.
4. Cover one tube with aluminum foil. This is your control.
5. Set the assembled manometers in the test tube rack.
6. Place the heat sink beaker of water in front of the manometer apparatus.
7. Place a piece of tape on the lab table next to the heat sink. This will be your "0" mark.
8. Measure and place pieces of tape at 15cm, 30cm and 45cm from the "0" mark.
9. Put your lamp at the 45cm mark.
10. Use the syringes to set a start point for water in the graduated 1ml pipettes in both test tubes. Record this initial pipette reading in the table below.
11. Turn on the light and wait for 15 minutes. After 15 minutes, record the pipette reading in the table.
12. If needed, readjust the water start point on the pipettes using the syringes, and record the start point.
13. Move the lamp to the 30cm mark and wait 15 minutes. After 15 minutes, record the pipette reading in the table.
14. Repeat steps 12 and 13 for the 15cm and 0cm distance marks.

Volume of Oxygen Produced When Exposed to Different Light Intensities

Distance from Light	Manometer Reading (ml)						Corrected Volume of Oxygen
	Control Tube			Experimental Tube			
	Initial	15 min.	Difference	Initial	15 min.	Difference	
45cm							
30cm							
15cm							
0cm							

Collect class data and record the class data in the table below

Summary of Class Data for Oxygen Production at Different Light Intensities

Group#	Volume of Oxygen Produced			
	45cm	30cm	15cm	0cm
1				
2				
3				
4				
5				
6				
7				
8				
Average				

Discussion Questions

1. Make a graph of the effect of light intensity on oxygen production in *Azolla* or *Valesneria*.
2. Why do you subtract any change in the control tube from the change in the experimental tube?
3. What differences, if any, did you find in rates of photosynthesis in your plants in different light intensities?
4. If you did this experiment using plants that normally live in the shaded understory of the rain forest, would you expect similar results? What about desert plants? How might you design an experiment to test the effect of different light intensities on plants from different habitats?