Photosynthesis

The process of photosynthesis involves the use of light energy to convert carbon dioxide and water into sugar, oxygen, and other organic compounds. This process is often summarized by the following reaction:

6 H2O + 6 CO2 + light energy C6H12O6 + 6 O2

This process is an extremely complex one, occurring in two stages. The first stage, called the *light reactions of photosynthesis*, requires light energy. The products of the light reactions are then used to produce glucose from carbon dioxide and water. Because the reactions in the second stage do not require the direct use of light energy, they are called the *light independent reactions of photosynthesis*.

In the light reactions, electrons derived from water are “excited” (raised to higher energy levels) in several steps, called photosystems I and II. In both steps, chlorophyll absorbs light energy that is used to excite the electrons. Normally, these electrons are passed to a cytochrome-containing electron transport chain. In the first photosystem, these electrons are used to generate ATP. In the second photosystem, excited electrons are used to produce the reduced coenzyme nicotinamide adenine dinucleotide phosphate (NADPH). Both ATP and NADPH are then used in the dark reactions to produce glucose.

In this experiment, a blue dye (2,6-dichlorophenol-indophenol, or DPIP) will be used to replace NADPH in the light reactions. When the dye is oxidized, it is blue. When reduced, however, it turns colorless. Since DPIP replaces NADPH in the light reactions, it will turn from blue to colorless when reduced during photosynthesis.

OBJECTIVES

 In this experiment, you will

* Use a Colorimeterto measure color changes due to photosynthesis.
* Study the effect of light on photosynthesis.
* Study the effect that the boiling of plant cells has on photosynthesis.
* Compare the rates of photosynthesis for plants in different light conditions.



Figure 1

MATERIALS

|  |  |
| --- | --- |
| computer | 250 mL beaker  |
| Vernier computer interface\* | two small test tubes |
| Logger *Pro* | 5 mL pipet |
| Spectrometer or Colorimeter | pipet pump or bulb |
| two cuvettes with lids | two eyedroppers or Beral pipets |
| aluminum foil covered cuvette with lid | 10 mL DPIP/phosphate buffer solution |
| 100 watt floodlight | unboiled chloroplast suspension  |
| stopwatch | boiled chloroplast suspension  |
| 600 mL beaker  | ice |

 \* Not necessary if using a Spectrometer.

PROCEDURE

1. Obtain and wear goggles.

2. Make sure at your station there are: plastic pipets, cuvettes with lids, and one aluminum foil covered cuvette with a lid. Mark one pipet with a U (unboiled) and one with a B (boiled). Mark the lid for the cuvette with aluminum foil with a D (dark). For the remaining two cuvettes, mark one lid with a U (unboiled) and one with a B (boiled).

3. There should be cuvette with distilled water with the lid marked H for H20. To correctly use a cuvette, remember:

* Wipe the outside of each cuvette with a lint-free tissue.
* Handle cuvettes only by the top edge of the ribbed sides.
* Dislodge any bubbles by gently tapping the cuvette on a hard surface.
* Always position the cuvette so the light passes through the clear sides. Clear facing the front.

Colorimeter Users Only

4. Connect the Colorimeter to the Vernier labquest.

 5. Calibrate the Colorimeter.

1. Open the Colorimeter lid.
2. Holding the blank cuvette by the upper edges, place it in the cuvette slot of the Colorimeter. Close the lid.
3. Press the < or > button on the Colorimeter to select a wavelength of 635 nm (Red) for this experiment. **Note:** If your Colorimeter has a knob to select the wavelength instead of arrow buttons, ask your instructor for calibration information.
4. Press the CAL button until the red LED begins to flash, then release. When the LED stops flashing, the calibration is complete. Proceed to Step 6.



Figure 2

6. Add 2.5 mL of DPIP/phosphate buffer solution to each of the 3 cuvettes. **Important**: Perform the following steps as quickly as possible.

 Locate the unboiled and boiled chloroplast suspension prepared by your instructor. Before removing any of the chloroplast suspension, gently swirl to resuspend any chloroplast which may have settled out. Using the pipet marked U, draw up ~1 mL of unboiled chloroplast suspension.

 a. Cuvette U: Add 3 drops of *unboiled* chloroplasts. Place the lid on the cuvette and gently mix; try not to introduce bubbles in the solution.

 b. Cuvette D: Add 3 drops of *unboiled* chloroplasts. Place the lid on the cuvette and gently mix; try not to introduce bubbles in the solution. Wrap foil around the cuvette entirely. Make sure that no light can penetrate the cuvette.

c. Cuvette B: Add 3 drops of *boiled* chloroplasts. Place the lid on the cuvette and gently mix; try not to introduce bubbles in the solution.

 8. Take absorbance readings for each cuvette. Invert each cuvette two times to resuspend the chloroplast before taking a reading. If any air bubbles form, gently tap on the cuvette lid to knock them loose.

1. Cuvette U: Place the cuvette in the device (close the lid if using a Colorimeter). Allow 10 seconds for the readings displayed in the meter to stabilize. Click  and record the absorbance value in Table 1 for time 0. Remove the cuvette.
2. Cuvette D: Remove the cuvette from the foil sleeve and place it in the device (close the lid if using a Colorimeter). Wait 10 seconds and record the absorbance value in Table 1 for time 0. Remove the cuvette and place it back into the foil sleeve.
3. Cuvette B: Place the cuvette in the device (close the lid if using a Colorimeter). Wait 10 seconds and record the absorbance value in Table 1 for time 0. Remove the cuvette.

9. Obtain a 600 mL beaker filled with water and a flood lamp. Arrange the lamp and beaker as shown in Figure 2. The beaker will act as a heat shield, protecting the chloroplasts from warming by the flood lamp.

 10. Turn on the lamp.

 11. Repeat Step 8 when 5 minutes have elapsed.

 12. Repeat Step 8 when 10 minutes have elapsed.

 13. Repeat Step 8 when 15 minutes have elapsed.

 14. Repeat Step 8 when 20 minutes have elapsed.

PROCESSING THE DATA

1. There is a laptop in the front of the room with a spreadsheet on it. You are to take the data from Table 1 and type the data into the spreadsheet. The spreadsheet will show you the graph of the data and provide a linear regression for each solution.

 2. In Table 2, record the slope of the line, *m*, as the rate of photosynthesis for each data set.

DATA

|  |  |  |
| --- | --- | --- |
| Table 1 |  | Table 2 |
| Time (min) | Absorbanceunboiled | Absorbancein dark | Absorbanceboiled |  | Chloroplast | Rate of photosynthesis |
| 0 |  |  |  |  | Unboiled |  |
| 5 |  |  |  |  | Dark |  |
| 10 |  |  |  |  | Boiled |  |
| 15 |  |  |  |  |  |  |
| 20 |  |  |  |  |  |  |

Questions

1. Is there evidence that chloroplasts were able to reduce DPIP in this experiment? Explain.

2. Were chloroplasts able to reduce DPIP when kept in the dark? Explain.

3. Were boiled chloroplasts able to reduce DPIP? Explain.

4. What conclusions can you make about the photosynthetic activity of spinach?

extension - Plant Pigment Chromatography

Paper chromatography is a technique used to separate substances in a mixture based on the movement of the different substances up a piece of paper by capillary action. Pigments extracted from plant cells contain a variety of molecules, such as chlorophylls, beta carotene, and xanthophyll, that can be separated using paper chromatography. A small sample of plant pigment placed on chromatography paper travels up the paper due to capillary action. Beta carotene is carried the furthest because it is highly soluble in the solvent and because it forms no hydrogen bonds with the chromatography paper fibers. Xanthophyll contains oxygen and does not travel quite as far with the solvent because it is less soluble than beta carotene and forms some hydrogen bonds with the paper. Chlorophylls are bound more tightly to the paper than the other two, so they travel the shortest distance.

The ratio of the distance moved by a pigment to the distance moved by the solvent is a constant, *Rf*. Each type of molecule has its own *Rf* value.

 *Rf* = distance traveled by pigment

 distance traveled by solvent

OBJECTIVES

In this experiment, you will

* Separate plant pigments.
* Calculate the *Rf*values of the pigments.

MATERIALS

|  |  |
| --- | --- |
| 50 mL graduated cylinder | cork stopper |
| chromatography paper | pencil  |
| spinach leaves | scissors |
| coin | solvent |
| goggles | ruler |

PROCEDURE

*Obtain and wear goggles!* ***Caution:*** *The solvent in this experiment is flammable and poisonous. Be sure there are no open flames in the lab during this experiment. Avoid inhaling fumes. Wear goggles at all times. Notify your teacher immediately if an accident occurs.*

1. Obtain a strip of chromatography paper with one end of the paper into a point.
2. Draw a pencil line 2.0 cm above the pointed end of the paper.
3. Use a coin to extract the pigments from the spinach leaf. Place a small section of the leaf on top of the pencil line. Use the ribbed edge of the coin to push the plant cells into the chromatography paper. Repeat the procedure 10 times making sure to use a different part of the leaf each time.
4. Pour 5 mL of solvent into a 50 mL graduated cylinder.
5. Place the chromatography paper in the cylinder so the pointed end just touches the solvent. Make sure the pigment is not in the solvent.
6. Stopper the cylinder and wait until the solvent is approximately 1 cm from the top of the paper. Remove the chromatography paper and mark the solvent front before it evaporates.
7. Allow the paper to dry. Mark the bottom of each pigment band. Measure the distance each pigment moved from the starting line to the bottom of the pigment band. Record the distance that each of the pigments and the solvent moved, in millimeters.
8. Identify each of the bands and label them on the chromatography paper.
* beta carotene: yellow to yellow orange
* xanthophyll: yellow
* chlorophyll *a*: bright green to blue green
* chlorophyll *b*: yellow green to olive green

9. Discard the solvent as directed by your teacher.

DATA

|  |
| --- |
| Table 1 |
| Band number | Distance traveled(mm) | Band color | Identity |
| 1 |  |  |  |
| 2 |  |  |  |
| 3 |  |  |  |
| 4 |  |  |  |
| 5\* |  |  |  |
| Distance solvent front moved = mm |

\* The fifth band may not appear.

Processing the DATA

Calculate the *Rf* values and record in Table 2.

|  |
| --- |
| Table 2 |
| Molecule | *Rf* |
| beta carotene |  |
| xanthophyll |  |
| chlorophyll *a* |  |
| chlorophyll *b* |  |

questions

1. What factors are involved in the separation of the pigments?
2. Would you expect the *Rf* value to be different with a different solvent?

3. Why do the pigments become separated during the development of the chromatogram?