**Photosynthesis Lab**

**Objectives:**
1. To understand the role of light and photosynthetic pigments in photosynthesis.
2. To demonstrate which wavelengths of light are used for photosynthesis.
3. To learn the function of CO2 in photosynthesis.
4. To demonstrate the production of O2 in photosynthesis.

Photosynthesis is arguably the most important chemical reaction that occurs on Earth. You, and (nearly) all other living organisms, are ultimately dependent upon photosynthesis. Organisms that accomplish this complex process convert light energy from the sun into chemical energy (sugar). Although photosynthesis involves many chemical steps, an overall summary reaction can be simply stated as follows:

**Light energy +** **6 CO2 + 6 H20 ---------------> C6H1206 + 6 O2**

Photosynthesis is the conversion of carbon dioxide and water to sugar and oxygen. In plants and photosynthetic protists, photosynthetic reactions occur within an organelle called the **chloroplast.** Photosynthesis requires light to begin the reactions that produce sugar. Special **pigments**, embedded in the **thylakoid membranes** of chloroplasts, are responsible for absorbing this light. Each pigment can absorb light wavelengths from slightly different ranges of visible light.

  **Exercise 1:**
 **Use of carbon dioxide (CO2) in photosynthesis**

During this experiment, you will be able to visualize CO2being consumed for photosynthesis in an aquatic plant, *Elodea*. To do this, we will take advantage of the fact that CO2 dissolves in water to form a weak acid:
 CO2 + H2O ---------> H2CO3  --------------> H+ + HCO3-

 Carbon dioxide water carbonic acid hydrogen bicarbonate

**We will also use a color indicator, phenol red, to show when a pH change has taken place.**
 **Phenol red is yellow in an acidic solution and red in a neutral or basic solution**

**Materials:**
two 50 ml beakers
phenol red solution
straw
*Elodea* sprigs

**Procedure:**
1. Fill each beaker with approximately 30 mL of tap water and add several drops of phenol red to each, until the solutions turns red.
Add the same volume of phenol red to each beaker.
2. Using a straw, gently blow carbon dioxide into each beaker until the solution turns yellow (indicating the solution has become slightly acidic).
3. Place several *Elodea* sprigs in one of the beakers and place both beakers in front of the light source or on the light table for at least 60 minutes.
4. Observe the beakers for color changes and explain what happened when the solutions were exposed to the light.
 Color after 60 minutes Why did the color change or not change?

|  |  |  |
| --- | --- | --- |
| Beaker without *Elodea* |  |  |
| Beaker with *Elodea* |  |  |

 **Exercise 2:**
 **O2 production in photosynthesis**

During this experiment, you will be able to visualize O2produced by photosynthesis in spinach.
**Materials:**
Sodium bicarbonate dissolved in tap water
500 mL plain tap water
Liquid soap
Two syringe tubes
Light box from an overhead projector
20 spinach leaf discs (cut with a hole punch)

1. **Procedure:**
Place 10 spinach leaf disks into one of the syringe tubes and put in the plunger. Push the plunger all the way down, but do not squash the spinach disks.
**Label this syringe “control.”**
2. Put a scant drop of liquid soap on the tip of your finger and stir it into the tap water with your finger. You do not want bubbles to appear – if the water begins to suds, dilute it with more water.
3. Slowly pull the soap solution into the control syringe containing the leaf disks until the syringe contains 30 mL of water. Turn the syringe upside down and expel any air contained in the tube.
4. Vacuum all of the air out of the spinach leaf disks. This is done by putting your thumb or finger over the tip of the syringe tube and pulling back on the plunger. You will notice bubbles coming out of the leaf disks while you apply the vacuum. After applying the vacuum for a few seconds, stop pulling on the plunger and slowly release your thumb from the tip. When you do this, water will flow into the leaf disks, replacing the air that has been vacuumed out. This should cause the leaf disks to begin to sink. Gently shake the tube to help them along. You may need to repeat this step 2 or 3 times to fully sink all of the disks.
5. Place 10 spinach leaf disks into the other syringe tube and put in the plunger. Push the plunger all the way down, but do not squash the spinach disks.
**Label this syringe “CO2 added.”**
6. Put a scant drop of liquid soap on the tip of your finger and stir it into the sodium bicarbonate/tap water solution with your finger. You do not want bubbles to appear – if the water begins to suds, dilute it with more water. (This step may already be done for you – check with your instructor).
7. Slowly pull the soap/sodium bicarbonate solution into the CO2 added syringe containing the leaf disks until the syringe contains 30 mL of water. Turn the syringe upside down and expel any air contained in the tube.
8. Follow step 4, above, to cause the leaf disks in the CO2 added syringe to sink.
9. Place both syringes on the light table and turn the light on.

As the spinach leaf discs undergo photosynthesis, they produce O2. When the O2 is produced and trapped within the leaf discs, they will float to the surface of the water. Observe the discs in the “control” and “CO2 added” syringes for 5 minutes.
**What differences do you see between the two syringes?**
**Can you explain what you see?**

 **Exercise 3:**
 **Paper chromatography**

Chloroplasts contain several photosynthetic pigments. The principal one is **chlorophyll a** (bluish green). Other accessory pigments are **chlorophyll b** (yellow green), **carotenes** (yellow orange), and **xanthophylls** (yellow). In the following exercise, a mixture of these pigments will be obtained from fresh leaves. Individual pigments contained in the mixture will be separated and visualized using a method known as paper chromatography.

In chromatography. a smear of photosynthetic pigments is applied to a strip of paper, and the molecules cling to the fibers of the paper. The tip of the paper is then immersed in an organic solvent and the solvent is absorbed by the paper. As the solvent moves up through the smear of pigment, the photosynthetic pigments may dissolve in the moving solvent. A sort of tug of war is continuously waged between solvent and paper for the pigment molecules. Molecules of a given pigment will spend a portion of the time attached to the paper and the remainder of the time they will be dissolved in the solvent. Pigments move differently because each one has a characteristic tendency to stick more or less to the fiber of the paper or to dissolve more or less in the solvent, depending on its molecular size, polarity, and solubility. When the pigment molecules are in the solvent they are being swept upward by the flow of the solvent.

Not all plant pigments are soluble in these organic chromatography solvents. Pigments that are water soluble and located in the cytoplasm or the central water vacuole such as the betacyanin in beet cells will not dissolve in the chromatography solvent and will therefore remain at the spot where they were applied.

**Materials:**
spinach leaf
other leaf
test tube
test tube rack
chromatography solvent
chromatography paper

**Procedure:**
1. Cut a piece of chromatography paper to fit into the test tube so that at least 2 cm of paper extends past the top of the tube. Be careful to handle the paper only by its edges so the oil from your fingers does not contaminate the paper. Cut a tip on one end of the paper and mark a pencil line 1.5 cm from the tip. Make sure your paper fits into the test tube without folding by trimming the width, if necessary.

2. Apply spinach pigment to the paper by placing a spinach leaf on the chromatography paper.
Roll the coin over the leaf to leave a green pigment line over the line you have drawn.

3. Add 2 ml of chromatography solvent (9 parts petroleum ether: 1 part acetone) to the test tube. Place the paper strip into the test tube. Only the tip of the paper should contact the solvent and the line of spinach leaf pigment should be ABOVE the surface of the solvent (see Figure 1, above).

4. Place the tube in a rack and do not disturb. Observe what happens at 5 min. intervals. Remove the strip when the solvent front reaches the top (15 to 30 min.) Allow the strip to dry. You should see four bands of color on the strip (see Figure 2, above).

5. Repeat steps 1 – 4 with the other leaf. Depending upon the leaf chosen, you will see
 different results. List and compare the results, below.



1. Which pigments were observed in the spinach leaf?
2. Which pigments were observed in the other leaf?
3. Can you explain the differences?

 **Exercise 4:**
 **Creating an absorption spectrum for photosynthetic pigments**

In exercise 3, you saw four types of photosynthetic pigments that can be extracted from fresh spinach leaves. Each of these pigments is important for absorbing light during photosynthesis. Each absorbs a slightly different range of the spectrum of visible light, and combined, they can absorb most wavelengths of visible light.

In exercise 3, you used an organic solvent to dissolve photosynthetic pigments for chromatography. In this exercise, you will use a bulk extraction of these pigments that I prepped for you by mashing spinach leaves and mixing them with acetone. The liquid was poured off (approximately 25 mL acetone for 5-6 spinach leaves). This liquid contains a concentrated solution of photosynthetic pigments. You will use a spectrophotometer and this pigment solution to construct an **absorption spectrum** for photosynthetic pigments. An absorption spectrum is a graph that shows which wavelengths of light are being absorbed, and at what strength.

**Procedure:**

1. Go to the spectrophotometer and get two glass cuvettes. Fill one cuvette with acetone only. This will serve as the blank. Fill the other cuvette with the pigment extract.
2. Set the spec to read absorbance at 400 nm (see table below). Blank the machine, and then measure the absorbance of the pigment sample. Record the value in the table on the next page.
3. Measure the absorbance of the pigment sample at each of the wavelengths in the table below. Record the data in the table on the next page.
4. Use the data you have recorded in the table to graph an absorption spectrum for photosynthetic pigments. Use the graph paper on the last page of this laboratory exercise. Set up the graph as follows:
	1. The x-axis is the light wavelength. Number the darkest graph lines with the wavelength values in the table.
	2. The y-axis is absorbance. Number the darkest graph lines as increments of 0.1, beginning with 0.
5. Plot points 1 – 14 on the graph, where wavelength is the x-coordinate and absorbance is the y-coordinate for each point.
6. Use a curving line to connect points 1 – 14.

|  |  |  |
| --- | --- | --- |
| Point number for graph | Wavelength of light | Absorbance of photosynthetic pigments |
| 1 | 400 |  |
| 2 | 425 |  |
| 3 | 450 |  |
| 4 | 475 |  |
| 5 | 500 |  |
| 6 | 525 |  |
| 7 | 550 |  |
| 8 | 575 |  |
| 9 | 600 |  |
| 10 | 625 |  |
| 11 | 650 |  |
| 12 | 675 |  |
| 13 | 700 |  |
| 14 | 725 |  |

After completing the graph, locate the light spectrum poster on the laboratory wall to answer the following questions about your data and the graph:

1. Which wavelengths have peak absorbance values? To what color(s) of light does this correspond?
2. Which wavelengths have the lowest absorbance values? To what color(s) of light does this correspond?

 **Questions:**

1. What happens to CO2 that is absorbed during photosynthesis?
2. In exercise 1, why did the solution become acidic when you blew into it? Why did the solution become less acidic after the *Elodea* was exposed to the light?
3. In exercise 2, why did the leaf discs float? What explains the difference between the results in the two syringe tubes?
4. Name the pigments found in spinach leaves. What is the function of these pigments?
5. Why do most plants appear green to our eyes?
6. What wavelengths of light do plants absorb the best? What are these wavelengths of light being used for in the plant?
7. Form a hypothesis for what would happen if you repeated exercise 2, with the following variation: There are two “CO2 added” syringe tubes. One is placed on a light table that shines only blue light, and the other is placed on a light table that shines only green light. What results do you expect for each syringe tube after waiting for a few minutes?

