PHOTOSYNTHESIS & HUMAN RESPIRATION LAB

Introduction:

Photosynthesis in eukaryotes takes place in the chloroplasts, where the pigments absorb light energy from the sun. During the first part of photosynthesis, the light reactions, light energy is absorbed and water is split producing oxygen gas. As a result of the light reactions, the energy rich molecules ATP and NADPH are formed. During the second part of photosynthesis, the dark reactions, carbon dioxide (an energy poor gas) is combined with hydrogen atoms from NADPH to form the energy rich molecule, glucose. The dark reactions are driven using the energy in ATP. The overall chemical reaction of photosynthesis is:

$$\underset{\text{carbon dioxide}}{6\,CO_2} + \underset{\text{water}}{6\,H_2O} \xrightarrow[\text{pigments}]{\text{sunlight}} \underset{\text{glucose}}{C_6H_{12}O_6} + \underset{\text{oxygen}}{6\,O_2}$$

So why, you ask, can you not make sugar water by leaving bottles of carbonated water (H_2O and CO_2) out in the sun for a while? If you do the experiment, all you get is hot club soda, or maybe burst bottles. The reason is that the equation only describes the net result of photosynthesis. It is like saying that the way a television set works is you plug it in and a picture appears on the screen.

The real workings of photosynthesis involve light trapping pigments, particularly chlorophyll a, and a sort of bucket brigade of enzymes which transport the high energy electrons removed from chlorophyll a (and replaced by the splitting of water, which yields electrons, oxygen gas and hydrogen ions) to produce ATP or cell fuel. Hydrogen ions from the splitting of water combine with the electrons at the end of the enzyme bucket brigade to form a complete hydrogen atom. The hydrogen atom then combines with the special hydrogen atom-accepting molecule NADP, which becomes NADPH. These are the light reactions, so called because they require light. The dark reactions use the carbon and oxygen atoms in CO_2 molecules and the hydrogen atoms in NADPH molecules to make glucose. The energy to drive the dark reactions comes from the ATP produced in the light reactions.

Exercise #1 — Plant Pigments

An object may appear to be one substance, but it often is a mixture of many substances. Leaves are an example of an object that is a mixture of many substances, some of which are light absorbing pigments.

You will use an ingenious technique to separate the pigments of plant chloroplasts. The technique is called paper chromatography. Paper chromatography is a chemical process used to separate pigments from each other. During this process, a solvent passes through a sample that has been impregnated on a piece of paper. It works on the principle that different pigments, when dissolved in a solvent (the liquid you place in the test tube) will move or travel through the piece of paper at different rates.

To simplify, as the solvent travels up the paper, the heavier pigments will be left near the bottom, the lighter pigments will be carried up to the top of the paper. The secret to understanding the results you will see is the solvent will pick up each different pigment in the sample and moved up the paper at different rates. This rate of movement is determined by; how soluble the pigment is in the solvent, the degree of adhesion of the pigment to the surface of the paper and the molecular weights of the individual pigments.

After you have performed the experiment, you will see the pigments listed below:

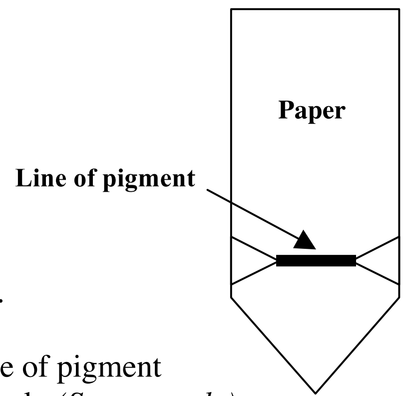
Chlorophyll a	(bright green)
Chlorophyll b	(dull green)
Carotene	(yellow-orange or bright yellow)
Xanthophyll	(yellow)

Materials Needed:

Large test tube
Beaker to hold the test tube
Rubber stopper
Piece of chromatography paper (handle the paper only by the edges)
Spinach leaf
Coin (a quarter works well)
Centimeter ruler

Procedure & Discussion:

1. Trim one end of the chromatography paper strip into a “V”. Cut a nick in each side as shown in the example.



2. Mark the test tube 2 cm from the bottom.

3. Cut the length of the paper so that the line of pigment at the top of the "V" is above the 2 cm mark. *(See example)*

4. Remove the paper from the test tube. Fill the test tube to the 2 cm mark with solvent. The solvent is 90 parts petroleum ether and 10 parts acetone. Be careful as the solvent is flammable.

5. Fold the spinach leaf several times and place on the paper over the top of the “V” *(see diagram)*. Roll the edge of the quarter across the spinach leaf several times. A thin, dark line of spinach leaf pigments should appear on the paper at the top of the “V” making a line of pigment.

6. Carefully insert the paper into the test tube. Make sure the line of spinach pigments is **above** the solvent level. Only the tip of the “V” should be in the solvent.

7. In a few minutes you will see the pigments begin to separate. Keep your eye on the test tube and watch your chromatogram. When the solvent has reached the top of the paper, take the paper out of the test tube and allow it to air dry. Return the solvent to its container.

8. Note the colors of the pigments. One of the bottom pigments is the major pigment in green leaves. Only when it breaks down or is transported out of the leaf, as in fall leaves, do the other pigments become visible.

Name___________________________

Photosynthesis Lab Report Sheet

1. The bottom pigment on the paper is the least soluble. Which pigment is this?

2. The top pigment on the paper is the most soluble. Which pigment is this?

3. White (sun) light contains different colors of light, this can be observed by sending white light through a prism or by looking at a rainbow. What color of light do the chlorophylls absorb least?

   What color of light does the carotenes and xanthophylls absorb least?

4. Why is it beneficial to a plant to have pigments of different colors?

Exercise #2 — Human Respiration & Elodea

This exercise is based on the fact that when carbon dioxide, CO_2, is dissolved in water, hydrogen ions (H^+) are produced, which causes the water to become acidic and the pH to drop. This decreased in pH can be detected with a compound called phenol red. Phenol red is red in non-acidic (basic) solutions and yellow in acidic solutions.

Materials Needed:

Tap water
Phenol red
2 small beakers
Straw
Elodea plant

Procedure:

1. Pour approximately 10 ml of tap water into both beakers.

2. Add 2-3 drops of phenol red solution. Swirl to mix the phenol red with the water. Answer question #1 on the Report Sheet.

3. Take a straw and blow gently into one solution.
   Answer question #2 on the Report Sheet.

4. Next, a piece of elodea plant is placed into the changed color beaker.

5. Place both beakers in front of a light source such as a window.

6. Observe the beakers at the end of the lab period.

Name____________________________

Human Respiration & Elodea Report Sheet

1. What is the color of this solution? What can you conclude regarding the nature of water and its pH?

2. What happens to the color of the water? What does this tell you about the pH?

3. What does a color change back to red indicate?

4. Is CO_2 used up by the plant during photosynthesis? Explain.

5. Why was a beaker with only phenol red solution also placed in the light source?