Photosynthesis Lab

Before coming to lab:

1) Use your textbook to review chloroplast structure and photosynthesis;
2) Read this handout - there may be a quiz.

Introduction

Photosynthesis is the process of trapping light energy and converting it into forms of chemical energy that are then used to drive the building of carbohydrates. The vast majority of living organisms depend directly or indirectly on photosynthesis for their food (energy-rich organic molecules), which they then either convert into their own molecules or break down to release energy for metabolic activities.

In eukaryotes, the entire photosynthetic process occurs within each chloroplast. The two distinct parts of photosynthesis - the Light-dependent reactions and the Calvin Cycle - occur in the thylakoids and stroma, respectively. The overall equation is:

$$CO_2 + 2\ H_2O \rightarrow 6\ CH_2O + O_2 + H_2O$$

Table 1: Comparison of the two parts of photosynthesis.

	Location	Energy source	Major reactants	Products	Energy carriers produced
Light-dependent	thylakoids	light	H_20	O_2	ATP, NADPH
Calvin Cycle	stroma	ATP, NADPH	CO_2, RUBP	carbohydrate	n/a

Review the parts of photosynthesis using your text book and Table 1 and note that:

1) O_2 is produced from the breakdown of water;

2) O_2 is a byproduct - it is not used anywhere in the process of photosynthesis;

3) the energy carriers from the Light Dependent reactions drive the Calvin Cycle;

4) NADPH is in short supply in the chloroplast - this is the rate-limiting link that keeps these otherwise separate parts running at the same pace;

5) CO_2 is only used in the Calvin Cycle;

6) the chloroplast will only contain high levels of RUBP or other soluble carbohydrates when it is (or recently has been) rapidly photosynthesizing.

In this lab, we will examine photosynthesis from several angles. These include:

1) the rates of use of CO_2 under different light conditions, and;
2) the presence of various pigments versus the ability of a leaf to perform the entire process

Exercise 1 – Observations of photosynthetic rate

CO_2 use during photosynthesis

- Follow along on the diagram as your instructor points out the components, describes their function, and traces the gas flow. Locate these components in the system your group is using.
- Practice attaching and detaching segments of tubing with the Luer locks – the opposite sections twist together – neither pulling nor pushing is necessary.
- The system currently has a CO_2 scrubber in place – note the CO_2 detector's reading – it should be close to zero.
    - ppm CO_2 _____

- Open the leaf chamber, position a leaf (**leave the leaf attached to the plant**) across the entire chamber, close the chamber and gently tighten the nuts (only until they are finger tight). Try not to breathe into the chamber. Why?
- After 1-2 minutes, what is the ppm CO_2 reading? Has the CO_2 ppm changed from the previous? Why might this happen?
    - ppm CO_2 _____

- Quickly, with instructor supervision, remove and isolate the CO_2 scrubber, attach the previously filled gas bag to the air pump, open the clamp on the gas bag tubing, and reattach the tubing ends where the scrubber was. DON'T allow the pump to push any ambient air into the system!

- Allow about 2 minutes for the ppm CO_2 readout to stabilize – record this reading. Why are you using a previously filled gas bag as an air source?
    - ppm CO_2 _____

- Turn on the light, to full. Note time. Record the ppm CO_2 reading every 2 minutes until it has dropped at least 20 ppm OR for the next 16 minutes. Why does this change?

2 min.	4 min.	6 min.	8 min.
10 min.	12 min.	14 min.	16 min.

- Turn the light off. How fast does the photosynthetic CO_2 use stop? What causes this?
    - time _____ to max. ppm CO_2 _____

- When the CO_2 ppm is back to max, turn the light back on full. How rapidly does photosynthesis reach its previous maximum rate? Is this faster than initially? What could be going on to cause this effect?
    - time _____ to min. ppm CO_2 _____

- Turn the light intensity to about half. How does the CO_2 use change? What is the effect of light intensity on the rate of photosynthesis?
    - ppm CO_2 _____

- Your instructor may have additional variations for you to try. If your group would like to assess the effect of other factors on CO_2 use, ask permission first.

- Stop before the gas bag is completely empty, replacing the CO_2 scrubber before allowing the pump to run ambient air through the system. The CO_2 detector must be left on.

Exercise 2 – Pigments involved in Photosynthesis

CAUTION - you will be heating liquids during this exercise, including highly flammable ethanol.

- These fluids should be simmered, not vigorously boiled.
- Do not boil a beaker dry.
- Use DRY paper towels as hot pads for handling hot beakers AND to protect them from contact with the cold table top.
- Use forceps to remove your leaf from hot fluids.

PROCEDURE:

- Locate the necessary equipment and materials.
- Measure about 75ml of tap water into each of two 250 ml beakers.
- Heat one until the water just begins to simmer, then turn off the hot plate.
- Acquire a leaf of *Coleus* which shows four clearly differently colored areas. Sketch the leaf, noting which areas are each color. You will be removing these pigments, but must still know where they were (see data table below)!
- Compare colors on the top of the leaf and on the lower surface. What does the difference in these patterns suggest about the number of pigments present?

- While the water is heating, soak the leaf in the cool water of the other beaker. After 4-5 minutes, is there any change in the pigments? What does this indicate?

- Place the leaf in the hot water and watch carefully for several minutes. How do the colors change? Which pigment is extracted from the leaf? What precise effect will simmering water have on the parts of a cell? Thus, where is the extracted pigment located?

- Carefully remove the beaker of hot water from the cooling hot plate, and the leaf from the beaker. Allow the beaker to cool until it can be handled, then discard the water in the sink.
- Measure about 75ml of ethanol into a beaker (use the one which contained the cold water).
- Place the beaker with ethanol on the (cooled) hot plate and heat gently to a simmer.
- Add the leaf and observe for 3-5 minutes. How do the colors change? What pigment is extracted? Since these were not extracted in hot water but are extracted in ethanol, what can you conclude about their cellular location?

- Remove the leaf (turn off the hot plate...), place it in a petri dish and soak in 5-10ml of iodine solution. Iodine is a specific test for the presence of starch - iodine causes a unique blue-black staining reaction with starch, not with any other material.
- Match the starch-containing areas to their original colors.

- Discard the cooled ethanol in the waste jug NOT down the sink!

Initial Color	Color after soak in cold water?	Color after soak in hot water?	Color after soak in hot ethanol?	Is starch present?	Pigments present?
white/yellow					
pink					
burgundy					
green					

Additional Questions

- Are the burgundy areas of the leaf the result of a novel pigment or a combination of those present in other areas? What evidence do you have to support your answer?

- Is the pink pigment adequate to drive photosynthesis?

- Are the green pigments required for photosynthesis? Are they adequate for photosynthesis?

- Is the pink pigment involved in photosynthesis? What evidence to do you have to support your answer?

- Where, giving as precise a locality as you can, are the green pigments located? What evidence supports your answer?

Exercise 3 – Pigments involved in Photosynthesis

CAUTION - you are using an acetone:petroleum ether solvent. It is highly flammable, potentially explosive and will cause brain damage if routinely inhaled.

Chromatography is used to separate the components of a mixture based on differences in their physical properties. These properties can include charge, mass, size and solubility. This exercise will use paper chromatography to demonstrate that the green color of leaves actually results from a combination of pigments.

Locate the required materials (test tube, cork with hook, solvent, chromatography paper). Remove the leaf that you previously used to demonstrate photosynthesis (or use another leaf provided by your instructor). Cut out and discard the areas which are covered with stopcock grease. Using a clean area of the leaf, crush the tissues such that the pigments are deposited in a thin line across the chromatography paper, about 2.5 cm above the pointed tip. You should make 10-15 such applications ALWAYS applying the pigments to the same narrow area of the paper.

This should result in a single dark green line that is no more than 3 mm wide.

Add a small amount of solvent to the test tube. Its level MUST be below the pigment line when the paper is attached to the hook, inserted in the tube and the stopper firmly emplaced.

Set the test tube in the rack and watch the solvent line move up the paper. When it reaches the level of the hook, carefully remove the paper, pour the solvent **in the correct waste jug** and replace the paper and stopper in the tube.

How many different bands of color are present?

The **carotene** and **xanthophyll** accessory pigments are yellow. These are both smaller molecules than the chlorophylls and have a radically different structure. They differ from each other in that the xanthophylls include an oxygen atom at each of their ends. How does this difference produce such different mobilities for these compounds?

The chlorophylls are green, with **chlorophyll *a*** being a dull, bluish green and **chlorophyll *b*** a brighter, yellowish green. Use your text to determine how these two molecules differ. Does that provide an explanation for their different mobilities?