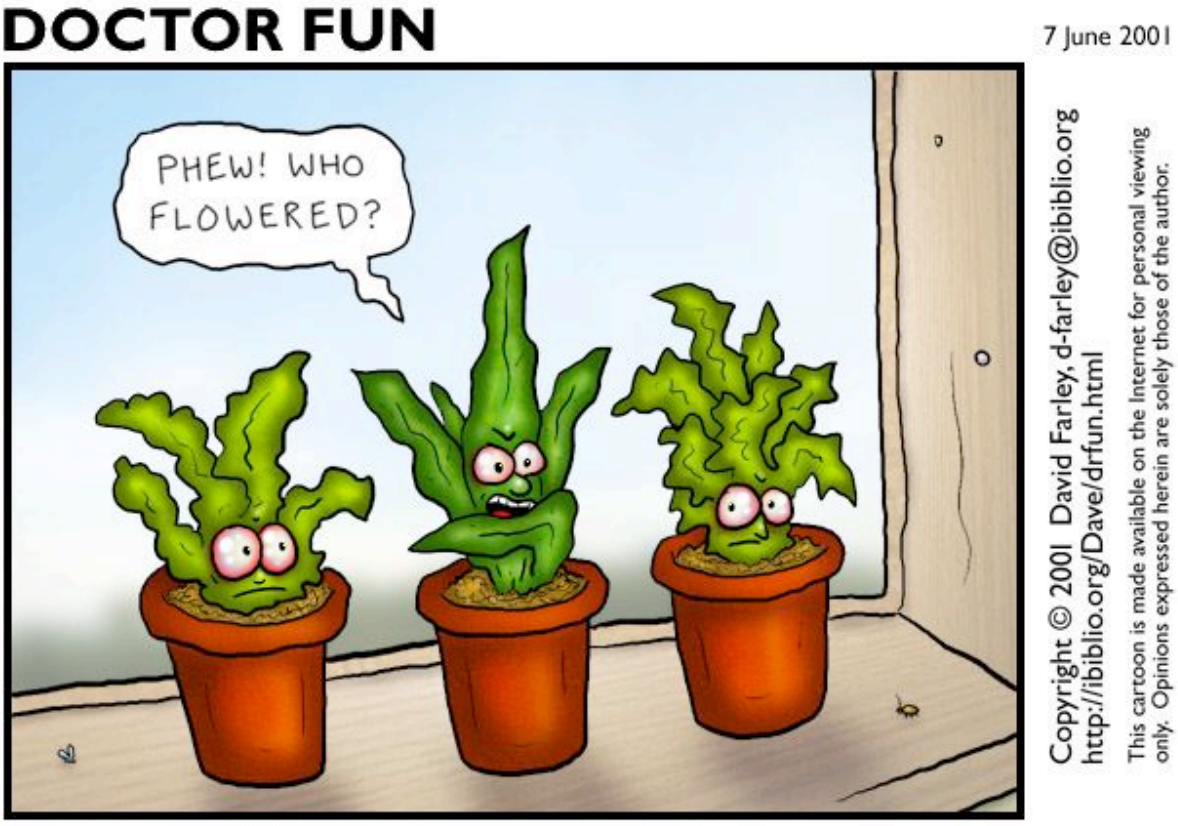
BIO 121 LAB INSTRUCTIONS

Lab 7- Photosynthesis



The secret life of houseplants

All organisms need energy to function. Heterotrophic organisms obtain their energy by oxidizing reduced organic compounds and recovering the energy stored in the C-H bonds. However, those C-H bonds were originally created using energy from sunlight, by a process called photosynthesis. Photosynthesis is therefore the most important biochemical process on earth: virtually all living organisms depend directly or indirectly on photosynthesis for energy, and all aerobic organisms also depend on photosynthesis for oxygen.

The overall reaction for photosynthesis is quite simple:

$$6\ CO_2 + 12\ H_2O + \text{light energy} \iff C_6H_{12}O_6 + 6\ O_2 + 6\ H_2O$$

however, the reactions by which this is accomplished are diabolically complex. Photosynthesis can be separated into two sets of reactions: the **light reactions**, which capture light and use its energy to make ATP and NADPH, and the **light-independent reactions**, which use the ATP and NADPH formed by the light reactions to convert CO_2 into carbohydrates. Both sets of reactions occur in the chloroplasts of higher plants; however, the light reactions occur in the thylakoids, while the light-independent reactions occur in the stroma. Photosynthesis is described in greater detail in chapter 8 of your text.

II. The light reactions

These can be subdivided into four stages: capturing light, electron transport, water-splitting, and chemiosmotic ATP synthesis. Light is captured by **pigments** which **absorb** photons of specific wavelengths. When this occurs, the energy of the photon is added to the energy of an electron within the pigment, which moves to an orbital more distant from the nucleus. Photosynthetic pigments are usually found in a precise array called a photosystem, which is constructed such that energy absorbed by any pigment within the photosystem is transferred by inductive resonance to a special pigment at the center of the array called the **reaction center chlorophyll**. When light energy is transferred to the reaction center chlorophyll an electron within the reaction center becomes "excited" and is transferred to a **primary electron acceptor**. The primary electron acceptor then passes the excited electron down an **electron transport chain** located on the thylakoid membrane. As the electrons move from one carrier to another, energy is released and some is used to pump H+ into the thylakoid lumen. Plants have two photosystems, **Photosystem I** and **Photosystem II**. In Photosystem I the reaction center chlorophyll is called **P700**, while the reaction center chlorophyll in Photosystem II is called **P680**. Photosystem I participates in two reactions: **cyclic photophosphorylation** and **noncyclic photophosphorylation**. In cyclic photophosphorylation electrons transferred from P700 to the electron transport system are returned to P700. In noncyclic photophosphorylation electrons transferred from P700 are passed via a series of intermediates to NADP+ to form NADPH. The electrons lost from P700 are replaced by electrons transferred from P680. When Photosystem II absorbs a photon P680 donates the excited electron to an electron transport system which ultimately donates the electron to P700, after using some of the energy released to pump H+ into the thylakoid lumen. The electrons lost from P680 are replaced by electrons removed from water by the **water-splitting enzyme** located in the thylakoid lumen**.** In the process water is oxidized to oxygen and H+, further increasing the [H+] in the thylakoid lumen. In the final stage of the light reactions, the difference in [H+] between the lumen and the stroma is used to synthesize ATP by chemiosmosis as H+ in the thylakoid lumen diffuses through ATP synthetase into the stroma.

III. The light-independent reactions.

The light-independent reactions use the NADPH and ATP generated by the light reactions to incorporate CO_2 into organic molecules and reduce these to form carbohydrates. This is done by means of a series of reactions called the **Calvin or PCR (photosynthetic carbon reduction) cycle**. The Calvin cycle can be split into 3 phases: **CO_2 fixation**, **reversing glycolysis**, and **regenerating ribulose 1,5-bisphosphate (RuBP)**. CO_2 is fixed by an enzyme called **ribulose 1,5-bisphosphate carboxylase/oxygenase,** or **Rubisco,** for short. Rubisco adds CO_2 to RuBP to form a 6 carbon molecule which is rapidly converted to two molecules of 3-phosphoglycerate (which are also intermediates in glycolysis). 3-phosphoglycerate is next converted to 3-phosphoglyceraldehyde, which is either used for carbohydrate synthesis, or to regenerate RuBP via a fiendishly complex set of reactions.

IV. This week's activities

You will examine five aspects of photosynthesis: photosynthetic pigments, the absorption spectrum of chlorophyll, electron transport, oxygen evolution and CO_2 uptake.

A. Fractionation of photosynthetic pigments

The first event in photosynthesis - the **primary photoevent**- is always the absorption of a photon by a pigment. The most abundant (and effective) pigments are **chlorophylls a and b.** However, as you will see in part B, chlorophylls can only absorb a portion of the visible spectrum. To use light which is not absorbed by chlorophylls plants employ **accessory pigments,** primarily carotenoids and xanthophylls. In this exercise you will examine the pigments extracted from several different plant tissues.

Working with your partner, obtain a TLC plate from the side table. HANDLE THE PLATE ONLY BY THE CORNERS. Lay the plate on a clean piece of paper towel. Lay a ruler across the plate about 1.5 cm from the bottom, and mark four dots 1, 2, 3 and 4 cm from the left edge. Write your initials at the top of the plate, and gently draw a line across the silica gel 1 cm below the top.

4 extracts of plant pigments are located in plastic tubes on the side tables, near the windows. The tubes are marked "green leaf," "yellow leaf," "carrot," and "beets." Each tube has its own capillary tube.

Using the capillary tube, carefully dispense one drop of green leaf extract onto the spot 1 cm from the edge. Then, USING THE PROPER CAPILLARY TUBES, dispense one drop of yellow leaf extract onto the 2 cm spot, one drop of carrot extract on the 3 cm spot and one drop of beet extract on the 4 cm spot. **DO NOT CROSS-CONTAMINATE** THE SOLUTIONS OR YOU WILL BE INFESTED WITH THE FLEAS FROM A THOUSAND CAMELS OR **SOMETHING EVEN WORSE!**

Once your plate is dry, place it in one of the tanks containing solvent so that the spots are towards the bottom. The plate should not touch the sides of the jar. The top of the solvent's surface should still be below the dried spots. If that is not the case, see your instructor. Replace the glass cover onto the tank, so that fumes from the solvent do not escape into the lab.

Let the plate develop until the solvent front reaches the line you drew 1 cm from the top. When your plate is ready, remove it from the jar, replace the cover over the jar, and trace the solvent front using a pencil if it was not exactly at the line you drew. Then allow the solvent to evaporate by placing it in the fume hood. Be sure to handle your plate only by its edges.

When the solvent is completely evaporated note the color and position of each visible pigment. Using a ruler, measure the distance between the front of each spot and the origin (the original spot on which you placed the pigment). Then measure the distance from the origin to the solvent front. Do this for each of your four extracts. Then, for each, calculate the Rf which is defined as the distance that the pigment moved, divided by the distance that the solvent moved (Rf will be a number between 0 and 1). Number your pigments from the bottom, i.e, #1 traveled the shortest distance.

Chlorophyll a will appear blue-green, chlorophyll b will look yellow-green, carotenoids will look yellow-orange, and xanthophylls will look yellow. The order of Rf's should be carotenoids > xanthophylls > chlorophyll a > chlorophyll b. You may wish to examine your TLC plates using a long wave-length UV lamp: chlorophylls will fluoresce a reddish color; you may also detect some fluorescence from other pigments in your extracts.

Report your findings on your datasheet. Your introduction should explain what photosynthetic pigments are, why plants have more than one kind of photosynthetic pigment, and what the purpose of the experiment is. Your results should include sentences saying what you set out to do, where the

results are presented, and what your key results were. It should also include a table of Rf values and colors for each of the pigments detected in the 4 extracts. Your discussion should include a tentative identification of the main pigments in each extract, your reasoning, and some discussion of the differences between the green and yellow leaves.

B. Absorption spectrum of photosynthetic pigments.

Recall that the first event in photosynthesis is always the absorption of a photon by a pigment. Therefore, only light which is absorbed by photosynthetic pigments can be used for photosynthesis. The second experiment determines which wavelengths of light are absorbed by leaves.

1. Turn on the Spec 20 and allow it to warm up.
2. Set the Spec 20 to 400 nm, then adjust it to **0% transmittance** using the **left knob** with **nothing** in the chamber.
3. Insert a blank tube containing 80% acetone and adjust the Spec 20 to **0 absorbance** using the **right knob.**
4. Put 5 mls of **leaf extract** in 80% acetone into a Spec 20 tube, and measure its absorbance. Record the data in Table II of your data sheet.
5. Change the wavelength to 405 nm, and repeat steps 2-4.
6. Repeat, increasing by 5 nm each time until you reach 500 nm.
7. From 500 to 620 nm record the absorbance at 10 nm intervals (as indicated on your datasheet).
8. From 620 to 700 nm record the absorbance at 5 nm intervals, then stop recording once you reach 700 nm.
9. Plot absorbance vs wavelength on figure 1, page 3.

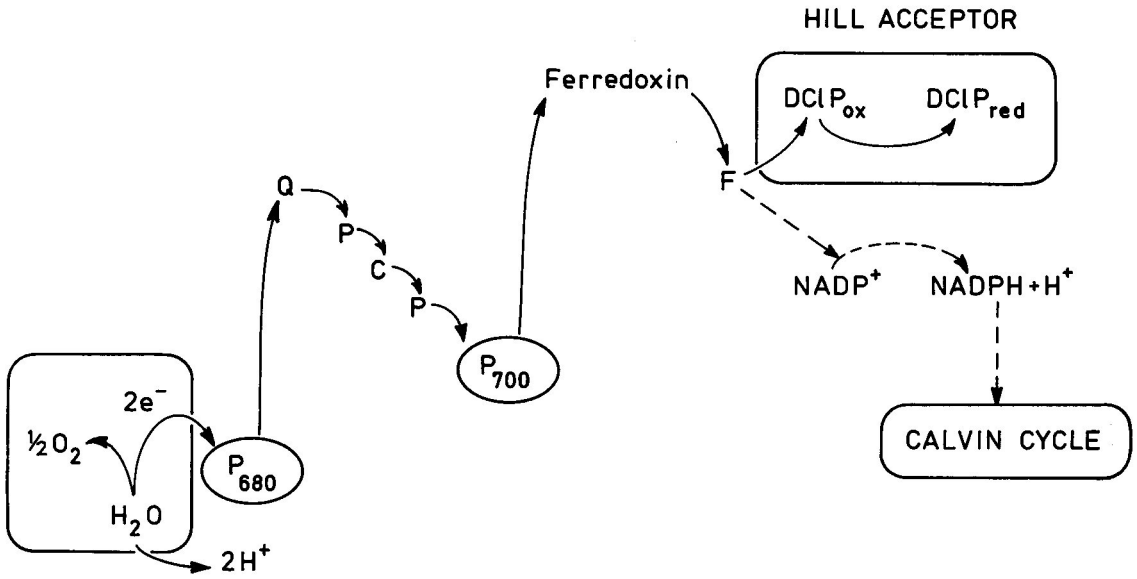
Report your findings on your datasheet in the usual format. In your introduction you should explain (one sentence) what photosynthetic pigments are, why you are interested in determining the absorption spectrum of a leaf, and what you specifically set out to do in this experiment. Your results should explain briefly why you performed the experiment, where the results are, and what the key results were. They should also include a table of raw data, and a figure in which you have plotted absorbance *vs*. wavelength. In your discussion you should explain why some regions of the spectrum are absorbed more effectively than others, and how this might affect photosynthesis.

C. Photosynthetic electron transport (the Hill reaction)

In 1937 Robin Hill demonstrated that isolated chloroplasts could transfer electrons to an artificial electron acceptor and evolve oxygen in the process when they were illuminated. This showed that electron transport and CO_2 fixation (*i.e*. the light and light-independent reactions) are separate reactions. We now know that what happened was that Photosystems I and II used light energy to remove electrons from water and donate them to the artificial electron acceptor.

The Hill reaction can be demonstrated by measuring oxygen evolution, however, it is more conveniently measured by following the reduction of an artificial electron acceptor. We will use the artificial electron acceptor *2,6-dichlorophenolindophenol*, DCIP. The oxidized form of DCIP is blue and absorbs light at 600 nm whereas the reduced form is colorless In the presence of light the Hill reaction can be measured by the change in A_{600} of the reaction mixture. The specific reaction is

$DCIP_{ox} + 2\ e^- <=> DCIP{\cdot}H_{2red}$, as shown in the following figure.



MEASURING THE HILL REACTION

1. Make sure that the Spec 20 is warmed up, adjusted for 0% transmittance, and set to 600 nm.

2. Label five tubes 1-5

3. Fill the tubes as indicated in the table below**.** First, add the correct volume of buffer to all tubes. Then, in the same manner, add the correct volumes of water and chloroplasts. **Do not add the DCIP.** Cover each tube and gently invert twice to mix the contents.

Tube	Buffer	water	chloroplasts	DCIP
1	2.5 ml	1.5 ml	1.0 ml	-
2	2.5 ml	0.5 ml	1.0 ml	1.0 ml
3	2.5 ml	0.5 ml	1.0 ml	1.0 ml
4	2.5 ml	0.5 ml	1.0 ml	1.0 ml
5	2.5 ml	1.0 ml	-	1.0 ml

4. Start the reactions by adding 1.0 ml DCIP to the indicated tubes, cover and mix by inverting twice.

5. Place tubes 1, 2 and 5 in a rack immediately behind the heat-sink (water tank) placed between the light and your samples.

6. Place tube 3 in a rack twice as far from the light as the first rack.

7. Wrap tube 4 with aluminum foil and place it in a dark location.

8. Measure the absorbance at 600 nm at two minute intervals for 12 minutes using tube 1 as blank for adjusting the instrument.

9. Report your findings on your datasheet. Your introduction should explain what the Hill reaction is, why it can be measured using an artificial electron acceptor, and what you specifically set out to do. Results should include a table showing the raw data and the reaction rate (expressed as ΔA_{600}/min), the graphs used to calculate the rates (A_{600} vs time), and a few sentences explaining why you performed the experiment, where the data are presented, and describing the major trends observed. Your discussion should explain the results obtained with each tube.

D. O_2 evolution during photosynthesis

Recall that the overall reaction for photosynthesis is:

$6\ CO_2 + 12\ H_2O + \text{light energy} \iff C_6H_{12}O_6 + 6\ O_2 + 6\ H_2O$

We can therefore measure the rate of electron transport in living plants by measuring the rate of O_2 evolution (note that we can't measure the rate of carbon assimilation this way because some of the carbohydrate that is formed may be consumed by photorespiration).

In this experiment we will measure O_2 evolution by algae using oxygen electrodes. Each pod will be provided with two oxygen electrodes attached to a single sensing unit. One group will use probe I and the other will use probe II.

Experimental:

1. Remove the plunger and cuvette from the stirplate and discard the contents.

2. Add 5 mls of algal culture, then replace the plunger taking care that no bubbles are trapped underneath.

3. Start the stirrer and the water, then gently cover the cuvette with aluminum foil. Wait 2 minutes, then record the % saturation at 1 minute intervals for 5 minutes on your data sheet.

4. Remove the foil and turn on the light, wait 2 minutes, then record the % saturation at 1 minute intervals for 5 minutes on your data sheet.

Your introduction should explain why plants evolve oxygen in the light and consume it in the dark, and what you specifically set out to do in this experiment. Results should include a table showing the raw data and rates of O_2 evolution (Δ% O_2 /min), the graph used to calculate the rates (% O_2 saturation vs time), and a few sentences explaining why you performed the experiment, where the data are presented, and describing the major trends observed. Your discussion should explain the results obtained in light and dark.

E. CO_2 uptake during photosynthesis

Plants take up CO_2 from their environment and incorporate it into carbohydrate during the light-independent reactions of photosynthesis. This can be demonstrated with aquatic plants by measuring

the rise in pH as a plant photosynthesizes. Recall from the previous lab that CO_2 dissolves in water to form carbonic acid:

$$H_2O + CO_2 <=> H_2CO_3 <=> H^+ + HCO_3^-$$

To demonstrate CO_2 uptake we will use our old friend *Elodea*. This time we will use an indicator, phenol red, to demonstrate a change in pH resulting from photosynthetic CO_2 uptake. Phenol red (phenol-sulfonphthalein) turns yellow at pH < 7, and red at pH >7. We will place *Elodea* in a dilute solution of phenol red acidified with carbonic acid, and watch it raise the pH of the solution.

Experimental:

1. Obtain three tubes containing *Elodea* in dilute phenol red.

2. Blow slowly into each solution with a straw until the color changes to yellow. Because excess carbonic acid will prolong the experiment, stop blowing as soon as it changes color.

3. Place one tube just behind the heat-sink 20 cm from the light, the second 40 cm from the light and put the third in the dark.

4. Observe the tubes every 15 min, and note the color in each.

5. Report the findings on your datasheet. Your introduction should explain why CO_2 is taken up during the dark reactions of photosynthesis, why this changes the pH of an aqueous solution, and the hypothesis that you specifically set out to test. Your results should include a sentence recapping the purpose of the experiment, where the data is, and the key results. Your discussion should explain why (hopefully) you observed a color change in some tubes, and account for the differences observed between tubes.

F. Computer simulation of photosynthetic electron transport

1) Start up your computer and **launch Chrome.**
2) Go to https://sites.google.com/site/biologydarkow/photosynthesis-model-with-variable-sample-sizes
3) Select "Background" and answer the questions on your datasheet.
4) Now click "Simulation" and start the interactive exploration.
5) Now construct an action spectrum for photosynthesis.
   - Select "Specifc Spectrum." Leave all the other settings at their default values, and set the light wavelength to 400 nm.
   - Click "Run" and record the µmoles of ATP produced after 15 minutes in the table on your datasheet.
   - Repeat, except this time set the light wavelength to 420 nm.
   - Continue, increasing the wavelength 20 nm each time, until you have reached 700 nm.

Report your findings on your datasheet in the usual format. In your introduction you should explain what an action spectrum is, and what you specifically set out to do in this experiment. Your results should explain briefly why you performed the experiment, where the results are, and what the key results were. They should also include a table of raw data, and a figure in which you have plotted μmoles ATP after 15 minutes vs. wavelength. In your discussion you should explain why some regions

of the spectrum gave more ATP than others (you may wish to compare your action spectrum with the absorption spectrum created in part B).

G. Designing an experiment to study some aspect of photosynthesis.

Your mission will be to design an experiment to study some aspect of photosynthesis, using the techniques which you learned in the first part of the lab. Remember, we are more concerned with your experimental design than with your results.

Some suggestions:
1) test the effects of temperature on the rate of the Hill reaction, on photosynthetic oxygen evolution, or on the rate of photosynthesis by *Elodea*.
2) test the effects of organic solvents, detergents or salts on the rate of the Hill reaction or on photosynthetic oxygen evolution.
3) Determine the effects of different **wavelengths** (colors) of light on the rate of the Hill reaction, on photosynthetic oxygen evolution, or on the rate of photosynthesis by *Elodea*.
4) Determine the absorption spectrum of the beet, carrot, or yellow leaf extract pigments.
5) Use the computer simulation to explore effects of one of the other variables that you can manipulate, such as CO_2 concentration, temperature, or [atrazine].

Start by formulating a hypothesis and write it down in the indicated place on your datasheet.
Next, write down the general predictions that you will make if this hypothesis is correct.

The third step is to think of a way to actually test these predictions, that is, the experiment. Once you have some ideas as to how you might do so, the next step is to think about the expected outcomes of your experiment and how to interpret them. Once you are satisfied with your protocol, write the predicted results and interpretation in the indicated place on your datasheet.

Next you must decide what data you need to collect to test your hypothesis, and enter it in your datasheet. Once you've decided what data you need to collect, you need to decide how to collect them. The next step will be to decide how to analyze your data. The final very important step is to decide what controls you will use.

Now that you have designed the experiment, the hard work is over and the fun begins. Go out and get your hands dirty, record your findings, then run the appropriate tests and tell us in your discussion what you think that you discovered!

H.CLEAN-UP

When you are finished, clean your glassware thoroughly and wipe off your table.