LABORATORY EXERCISES
Laboratory 1 - Photosynthesis

NOTE: **YOU WILL BE REQUIRED TO BRING YOUR PROTECTIVE SAFETY GLASSES TO THIS LAB.**

Objective

To introduce aspects of photosynthesis and explore the light-dependence and carbon dioxide requirement of oxygen evolution. For this exercise you should work in pairs.

Introduction

Photosynthesis was the cause of our present atmospheric composition. Without billions of years of photosynthesis, oxygen levels in the atmosphere would be very low, less than 5%. Carbon dioxide levels would be very high, high enough to poison mammalian life. Photosynthesis is one of the defining features of the plant kingdom. It occurs not only in terrestrial environments, but is also the dominant biochemical process in the oceans. Virtually all life depends upon the energy supplied by photosynthesis in the form of carbohydrate ($C_6H_{12}O_6$). In the biosphere, this energy is distributed via food chains to most organisms. This diversity of photosynthetic functions is often encapsulated in the equation:

$$\text{light} + 6(H_2O) + 6(CO_2) \longrightarrow (CH_2O)_6 + 6O_2$$

In terrestrial plants, there are many factors that directly or indirectly influence the rate of photosynthesis. Often, there are competing requirements that affect photosynthetic rates, such that the final morphological and physiological strategy evolved in any particular species is a compromise adaptation to a specific environment. E.g. A large flat horizontal leaf with a monolayer of cells might be optimal for light trapping but structurally vulnerable to mechanical damage by wind, rain, animals etc. Similarly photosynthesis requires maximal access of gaseous CO_2 and O_2 to the photosynthetic cells, which also require an abundant source of H_2O. Large air spaces in the leaf and wide open stomata to enhance gas flow and transpiration are conducive to photosynthesis but also lead to dramatic water loss which may not be sustainable in a water limited environment. In a marine environment, macroscopic plants must evolve a balance between their need for light versus the risk of mechanical damage and competition from other species in the upper layers of the water where light intensity is maximal. Deeper waters are more stable but receive less light. Consequently, any organism adapting to deep water must evolve a way of dealing with low intensity light of incomplete spectral composition.

When light encounters an object, such as a leaf containing chlorophylls and other pigments, it has been observed that wavelengths of light may be absorbed, reflected or transmitted. Sometimes this is written as: A + R + T = 1.

The process of evolution occurs along a very long time line. Yet, with the manipulation of natural environments by humans, especially within the past few thousands of years, there have been dramatic changes in the environment, which may create additional selective pressures on photosynthetic organisms. Most notable is the steady increase in atmospheric carbon dioxide that has been well documented over the past 40 years. Carbon dioxide is a substrate for the photosynthetic process, and with global warming, plants may need to adapt to a changing temperature regime.

In this laboratory exercise we shall investigate the light dependence of the photosynthetic process, and the impact of increased carbon dioxide on oxygen evolution. We will also compare photosynthesis between different types of plants.

Methods

One way of measuring the rate of photosynthesis is to monitor the rate of oxygen production by the plant. In strong light the rate of oxygen evolution from photosynthesis is in the order of 100 times its consumption by respiration so the latter process will not significantly interfere with your results. We will not measure oxygen evolution directly but rather will observe the amount by which the volume of gas is increased above the aqueous solution as O_2 is produced by the photosynthetic organism.

Each pair will have a test tube rack in which the tubes will sit. Use the tube labeled 'C' for your control (artificial pond water only – serves as a blank), 'N' for normal (artificial pond water + plant) and 'T' for the "test" treatment (artificial pond water + $NaHCO_3$+ plant). Take approximately 4-6 g samples of *Cabomba*, *Elodea* or other species as instructed by your TA and place each sample into the N and T tubes. Fill the C and N tubes with artificial pond water. To the T tube, add carbon dioxide in the form of $NaHCO_3$ from the 100 mM solution. Cork each tube with the stopper assembly provided. For each stopper assembly, carefully add a drop of water to the inside of the 1 mL pipette (using a long Pasteur pipette). This water droplet should be a column in the pipette and you will measure the movement of the column during the experiment.

Each lab group will take measurements for two of the three LED (light-emitting diode[1]) lamps (as assigned by your TA). The LED lamp spectra are shown in Figure 1.1. Place the tubes in the rack, and adjust the distance of the lamp: 20 cm for the green LED lamp (λ_{max} 520 nm), 20.75 cm for the blue (λ_{max} 455 nm), and 16.5 cm for the red LED lamp (λ_{max} 630 nm). Turn on the lamp, making sure that all tubes have the same exposure to the light. Determine the rate of the gas (O_2) evolution by measuring how far the water column in the pipette has moved in 15 minutes (in mL per 15 minutes, convert to mL per hour). At the end of each treatment, adjust the water column in the pipette to approximately the same starting point (judge by eye). Repeat for each LED lamp, taking a dark reading (by covering the tubes with a black cloth) before each new LED lamp reading.

[1] In light-emitting diodes electrical current excites electrons to a higher orbital state. When the electron 'relaxes' to the ground state, it emits light. The wavelength of the emitted light depends upon the energy level of the excited orbital state. The spectra of the LED lamps are shown at the end of the lab exercise (Figure 1.1). You can verify their emission spectra with the spectroscopes provided in the lab. If you observe the fluorescent lamps overhead with the spectroscope, you will discover that what we see as 'white light' is not!

Record your results in a table of the following form:

O_2 evolution rate, ml/g/h (compared to dark)

Light Treatment	15 min dark	15 min red	5 min dark	15 min blue	5 min dark	15 min green
Artificial Pond Water	1	3(2)				
			2	4(2)		
					–1	4(5)
Artificial Pond Water	1	5(4)				
Plus $NaHCO_3$			0	2(2)		
					1	5(4)

N.B. The above values are purely fictitious, do not use them as a guide to the sort of values you should be getting.

From this matrix you can calculate an oxygen evolution (= photosynthetic) action spectrum for the photosynthetic species (values of red, green, blue compared to the dark value). You can also calculate the relative efficiencies of photosynthesis at each light colour.

Ideally you should repeat your experiment and get replicate data, to give you more confidence in your results. However, time will be a factor in this lab so the class results will be pooled and the means calculated for the different treatments. Class results will be required to compare photosynthesis between the different plants. You will be expected to compare your results to those of the whole class and to discuss any discrepancies. Remember to discuss any observed variability.

Write Up

Your TA will provide you with the details of your write-up. Attach your (initialed) raw data to your write-up. You should consult at least one relevant, refereed journal (i.e. non-textbook) reference.

In tables, show oxygen evolution as **mL per hour** and standardized for the quantity of the photosynthetic species (**mL per g per hour**) as well as the **difference from the dark control**. Tables should have appropriate headings that describe the data you are presenting.

Include a summary explaining your results, comparing the results seen in the different plants used. Some of the questions you may be asked to address:

1. What is/are the control(s) used in this experiment? Explain.
2. Why is a dark reading taken before each LED reading? Explain the use of the various distances at which the coloured LED lamps were placed.
3. What are your null and alternate hypotheses? Why is the t-test either one-way or two-way?

Figure 1.1. Emission Spectra of the LED lamps.