

# PHOTOSYNTHESIS WITH TECHNOLOGY

## GENERAL INTRODUCTION

*You really need to read this introduction before coming to this lab exercise. The purpose of this handout is to provide background information on the source of the O<sub>2</sub> produced in photosynthesis, and describe how measurement of this O<sub>2</sub> can be used to determine photosynthetic rate. It also describes how the O<sub>2</sub> sensor works and provides instructions for setting up the experimental system that will be used. There will not be sufficient time in the laboratory for reading this material!*

### What is Photosynthesis?

Photosynthesis is the primary, and arguably the most important, source of energy input into the majority of terrestrial and aquatic ecosystems. It is carried out by green plants and algae, as well as certain bacteria and cyanobacteria.

As its name suggests, photosynthesis is a process by which photons (packets of light energy) are converted to chemical energy and used to synthesize metabolites within the organism

Photons captured by light-sensitive chlorophyll molecules within the chloroplasts of the leaf excite electrons within these molecules, raising them to a higher energy level. When the P700 chlorophyll molecule within Photosystem I is energized, the electrons may be passed through a series of electron carriers in the chloroplast membrane before being returned to the P700. ATP is generated during this process, which is known as **cyclic photophosphorylation**. Alternatively, the electron carriers may not return the electrons to P700, but transfer them to NADP<sup>+</sup>, reducing this to NADPH. This leaves the P700 molecule with a net positive charge. Electrical neutrality is restored to P700 by accepting electrons from light activated P680 chlorophyll molecules in Photosystem II. These electrons also are passed through an electron transport chain, which generates ATP, before being accepted by P700. This process is called **non-cyclic phosphorylation**.

Non-cyclic phosphorylation results in the production of P680 molecules having a net positive charge. Electrical neutrality is restored to the P680 molecules by accepting electrons derived from the splitting of water (photolysis). Oxygen gas is generated as a product of the water-splitting reaction, and this is evolved by the leaf.

The ATP and NADPH generated in the light reactions of photosynthesis are required for the reduction of CO<sub>2</sub> gas to sugars within the leaf. These sugars are then used as an energy source by the plant, and to provide the building blocks for more complex metabolites.

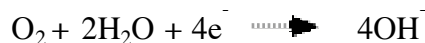
The process of photosynthesis involves the exchange of gases between the leaf and the environment. CO<sub>2</sub> is taken up by the leaf, and O<sub>2</sub> is evolved, usually in a 1:1 ratio.

Therefore, if the leaf were to be enclosed in a sealed chamber, the atmosphere within the chamber would be enriched in O<sub>2</sub> and depleted in CO<sub>2</sub>. By measuring the rate at which these gases are exchanged between the leaf and its environment, we can determine the rate of photosynthesis. The project you will carry out will involve the use of a Plexiglas leaf chamber with an oxygen sensor incorporated into its upper lid. The sensor does not measure the rate of photosynthesis directly, but monitors the changes in O<sub>2</sub> concentration of the atmosphere within the leaf chamber. Using data acquisition software, you will record these changes in O<sub>2</sub> concentration and will calculate the rate of change of O<sub>2</sub> as a measurement of photosynthesis.

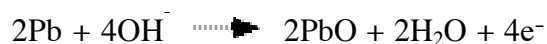
### The Oxygen Sensor: Principle of Operation

The O<sub>2</sub> sensor incorporated into the upper surface of the leaf chamber is a galvanic cell (a lead-oxygen battery) consisting of a lead anode, an O<sub>2</sub> cathode and an acid electrolyte. It also incorporates an O<sub>2</sub>-permeable Teflon FEP membrane with a gold electrode bonded to its surface. O<sub>2</sub> diffusing through this membrane is reduced electrochemically at the gold electrode. Within the sensor, the following reactions occur:

#### Cathode:



#### Anode:



#### Overall:



A resistor and a thermistor (for temperature compensation) are connected between the anode and the cathode, so that the battery is always discharged. Current flowing through the resistor and thermistor is proportional to the partial pressure of O<sub>2</sub> in contact with the Teflon FEP membrane (i.e. the partial pressure of O<sub>2</sub> in the leaf chamber), and by measuring the current flowing through the resistor and thermistor, the pO<sub>2</sub> can be determined.

## STANDARD SET-UP PROCEDURE FOR EXPERIMENTS

*To save time, and to avoid damage to the components of the photosynthesis lab, the equipment described here will be set up prior to your use. However, the following set of instructions for preparing the experimental set-up is provided for your information.*

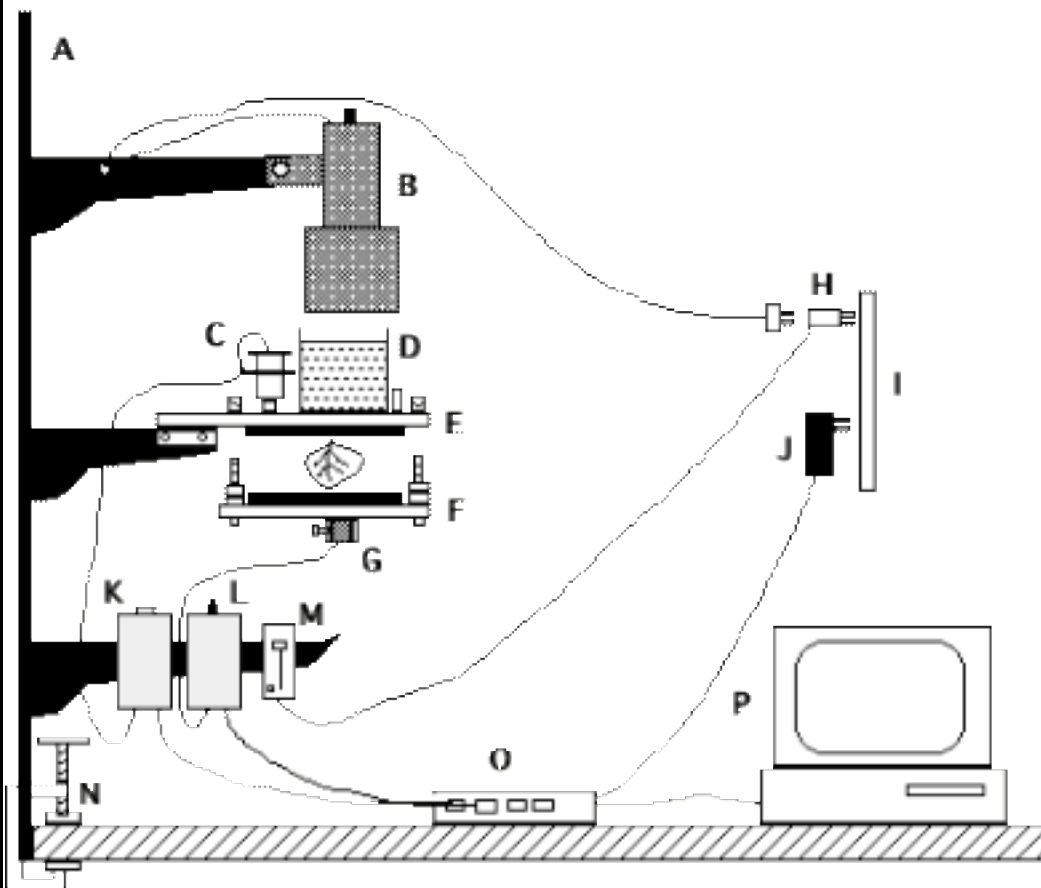
### Components of the Photosynthesis Laboratory Package

- A laboratory stand to which other components are attached.
- A light fitting with a 50W halogen bulb.
- A sliding dimmer control into which the light source is plugged.
- A leaf chamber incorporating an oxygen sensor in its upper lid.
- A light sensor attached to the bottom of the chamber.
- A 200 mL beaker for use as a heat filter (when filled with water).
- An acetate grid for leaf area measurement.
- A nylon-polyethylene gas bag.
- Drinking straws to fill the gas bag.
- A Lab Pro interface (analog to digital converter).
- A power supply for the Lab Pro interface.
- A USB cable to connect the Lab Pro interface to the computer.

Assemble these components as shown in the diagram on the following page, according to the following instructions:

- (a) Fasten the laboratory stand to the edge of the lab bench. If the bench is narrow, with an accessible lip on the far side, it may be convenient to mount the stand on that side. Otherwise, mount the stand with the mounting brackets facing away from you and towards the bench.
  - (b) Attach each of the mounting brackets to the stand before attaching the components of the system that they support (see next paragraph). For example, you must attach the leaf chamber bracket to the stand before mounting the upper lid of the leaf chamber on it using the wing nuts provided. Never apply force to the leaf chamber itself when it is attached to the laboratory stand. Ensure that the leaf chamber and lamp are the correct distance apart: there should be six empty slots on the stand between the brackets supporting the light source and the upper lid of the leaf chamber. Also ensure that the leaf chamber is attached at an appropriate height for the plant you are using in your experiment.
- The mounting brackets are attached to the stand by pushing their projections into the slots on the stand and applying a downward force. You will feel a click as the projections engage. Test that the brackets are firmly mounted by tapping each side to side and up and down: they should stay attached to the stand. If a bracket is difficult to mount, use a small hammer to tap the top of it at a point close to the stand to apply the downward force. (When disassembling the system, detach the components from the brackets before removing the brackets from the stand. Brackets are detached by applying an upward force underneath each bracket close to the point of attachment to the stand. Again, this can be done using a small hammer if a bracket is difficult to remove).
- (c) If the halogen bulb is not screwed into the light fitting, do this now before plugging the light fitting into the dimmer control.
  - (d) Plug the light fitting into the dimmer control socket, and then plug the dimmer control into a 110 VAC outlet. Slide the dimmer control to maximum to ensure the halogen lamp is functioning, and then slide it back to minimum. A red light appears on the dimmer control when it is set at minimum.
  - (e) Attach the plug from the oxygen sensor amplifier to Ch 1 on the Lab Pro interface, and the plug from the light sensor amplifier box to Ch 2 of the interface.
  - (f) Plug the power supply into a 60-hertz, 110 VDC power outlet, and then attach this to the back of the Lab Pro interface by the jack plug. A green LED will light on the top of the Lab Pro interface when power is supplied.
  - (g) Plug the computer cable into the USB socket on the side of the Lab Pro interface. Connect the other end of the cable to the USB port of the computer.
  - (h) Calibrate the oxygen and light sensors as described in the next sections.

# Photosynthesis Laboratory Set Up



- A - Laboratory Stand with Mounting Brackets
- B - Light Fitting with 50 W Halogen Source
- C - Oxygen Sensor
- D - Water Filter (200 mL Beaker)
- E - Upper Surface of Leaf Chamber
- F - Lower Surface of Leaf Chamber
- G - Light Sensor in Mounting Cylinder
- H - Socket for Dimmer Control and Light Source
- I - Power Supply
- J - Power Supply for Lab Pro Interface
- K - Amplifier for Oxygen Sensor
- L - Amplifier for Light Sensor
- M - Dimmer Control
- N - Clamp for Mounting Stand to Desk
- O - Lab Pro Interface
- P - Macintosh or PC Computer (not supplied).

## Calibration of the Oxygen Sensor

- (1) (a) Turn on the computer, and use the mouse to double-click on the "Logger Pro" icon on the desktop. This will activate the Logger Pro software for data collection and analysis.  
  
(b) Select the "Close" option from the "File" menubar item. Click "Don't Save" in the dialog box. This will put away the dummy lab that appeared on screen. Now Select the "Open" option from the "File" menubar item. Select the file: PH1\_setup.mbl and click the "Open" button. Click "OK" in the next dialog box.
- (2) Two graphs will appear on the screen. The upper graph will display data from the O<sub>2</sub> sensor, and the lower graph will display data collected from the light sensor. At the bottom of the screen, the outputs of the two sensors will be displayed numerically. A data table appears at the right edge of the screen for holding collected data.
- (3) Click on the "Collect" button at the top center of the screen to activate data collection from the sensors. The O<sub>2</sub> sensor should read 20.7% in normal room air. The instructor will assist you in making this calibration...with a fine screwdriver and perhaps some settings.
- (4) The oxygen sensor is now calibrated.

## Calibration of the Light Sensor

- (1) The light sensor is calibrated to give readings of irradiance in units of  $\mu\text{mol quanta/m}^2/\text{s}$ , but because the sensor is below the leaf, the calibration is made assuming green light penetrating the leaf. If the leaf is normal, you will want to set the amplifier for the light sensor to Medium and be sure that the software is set for 0-100  $\mu\text{E/m}^2/\text{sec}$  under the "Calibrate" option of the "Experiment" menubar item. If the leaf is very thick (succulent) then you should set the amplifier to Low and the software for 0-60  $\mu\text{E/m}^2/\text{sec}$ .
- (2) The light sensor is now calibrated.