GES 102 - PHOTOSYNTHESIS LAB

This week you will investigate the effect of light intensity on the rate of photosynthesis in spinach leaves. Fresh spinach bought in the store is green, and the leaves still have the ability to do photosynthesis. Photosynthesis produces carbohydrates and oxygen and we will use the rate of oxygen production as an indicator of how quickly the spinach leaves actually do photosynthesis.

You will use a hole punch to make a number of small disks of spinach. These disks float when they contain oxygen from photosynthesis. By applying vacuum to the small spinach disks, the oxygen is forced out, and they sink. When you then place the disks in a transparent cup with water, and expose them to light, they will produce oxygen and eventually float. The time it takes the small disks to float is a measure of photosynthesis, and you will test the effect of different light intensities on the floatation time.

Background:

In your first lab this semester you investigated how the UV portion of sunlight was able to cause harm to living cells, which is not so surprising since this shorter wavelength carry enough energy to damage the cell's DNA. In this week's lab we will instead use the portion of radiation from the sun that we call daylight (380-780 nm), plenty of which makes it down to the earth's surface. Our eyes are sensitive to this portion of the spectrum, and it's also the portion plants use for photosynthesis.

The process of photosynthesis can be simplified as:

$$\mathbf{CO_2 + H_2O \rightarrow sugar + O_2.}$$

It is really divided up into two sets of reactions, the **Light Reaction** and the **Calvin Cycle**. In the Light Reaction the plant cells use chlorophyll to capture some of the energy in sunlight. When sunlight hits a chlorophyll molecule, electrons in the chlorophyll

molecule are excited, they 'spin' faster because of the absorbed energy. These excited electrons are passed on down the line and chlorophyll will take a continuous supply of new electrons from H_2O which is broken down, producing O_2. The energy in the excited electrons is eventually used to produce two high energy molecules, called **ATP** and **NADPH**. In the next step, the Calvin Cycle, the energy contained in ATP and NADPH is used to help take CO_2 (carbon dioxide) from the air and manufacture sugar.

Spinach plants normally get their CO_2 from the air, through small openings on the underside of their leaves, called stomata. In our experiment some sodium bicarbonate ($NaHCO_3$) will be added to the water, and provide the carbon needed for the small spinach disks to do photosynthesis. The O_2 produced will be trapped inside the spinach disks and increase their buoyancy until they float.

Alongside photosynthesis, the plants also do cellular respiration. They certainly are **autotrophs** and can produce their own "food", the sugar. But they also need to break down the sugar in order to get the energy required to pay their energy bills, i.e. they need also to be **heterotrophs**, just like animals. It should be no surprise to find that plants cells have both chloroplasts and mitochondria. In our experiment with the spinach disks, some cellular respiration will happen along with the photosynthesis. This will make it take a bit longer for the disks to float, and our measurement of photosynthesis is really a measure of **net photosynthesis**.

You will also place some of your floating spinach disks in the dark. Photosynthesis is obviously impossible in the dark, and now the spinach disks can only do **cellular respiration**, which can be simplified as:

$$\textbf{sugar} + O_2 \rightarrow CO_2 + H_2O.$$

In the dark the spinach disk will consume O_2, and once they have consumed enough O_2, their buoyancy will have decreased enough for the spinach disks to sink. You will determine how long time this takes. **Gross photosynthesis** is the total photosynthesis undertaken by the spinach disks, where you add the net photosynthesis + cellular respiration (as measured in the dark).

Materials:

- 0.2% sodium bicarbonate solution ($NaHCO_3$), with a few drops of dishwashing fluid to lower the surface tension of the water (and allow it to enter the spinach disks)
- 4 clear plastic cups
- Quantity of fresh spinach leaves
- 10 cc plastic syringe
- Timer
- 4 clear plastic cups
- Hole punch
- Light banks with three different light intensities

Experiment:

Preparing the spinach disks for each experiment:

1. Take a spinach leaf and then use a hole punch to punch out at least 10 small spinach disks from the leaf. Make sure to avoid any major veins in the leaf.

2. Now remove the plunger from the syringe and carefully place the 10 spinach disks inside the barrel.

3. Slowly push the plunger back into the syringe, which will make the plunger slowly push the spinach disks into the syringe. Take great care not to crush the disks, and continue until there is only a small amount of air left with the disks at the end of the barrel.

4. Now put the tip of the syringe into the sodium bicarbonate solution and pull a small amount of fluid into the syringe. Tap the syringe and check that the spinach disks free from the walls of the syringe and intact. They should be floating on top of the fluid.

5. You are now ready to apply a vacuum to the disks. Hold a finger over the syringe opening and at the same time very slowly draw back the plunger. This creates a vacuum in the space with the spinach disks. Hold this vacuum for about 10 seconds.

6. The vacuum should draw some sodium carbonate solution into the leaf disks, replace the O_2, and cause the leaf disks to sink. You may have to repeat the vacuum steps 2-3 times to make all the disks sink.

Starting the experiments:

1. There are three light banks in the lab, each with a different light intensity, and it doesn't matter in what order you use them. You need to perform one floatation experiment with 10 spinach leaf disks using each light bank, and measure how long it takes for all the leaf disks to float in each light intensity. What is your hypothesis regarding the results?

2. You also need to do one experiment where you measure cellular respiration in the leaf disks, by putting them in the dark and measuring how long it takes for the disks to sink. This takes longer than making the disks float, and you should start this particular experiment early in the lab period.

3. A good strategy is doing the cellular respiration experiment with the disks you made to float using your first light bank. It doesn't matter what particular light

intensity you happened to do first. After making sure all leaf disks are floating, put them in the dark, and briefly look at them after each minute has passed, noting how many have sunk, and continue until all leaf disks have sunk to the bottom of the cup.

4. For each of the three light intensities, record the number of disks that are floating at the end of each minute, and make sure to swirl the disks to dislodge any that may be stuck against the sides of the cups. Continue up to a total of 15 minutes.

5. An example of a data table is found below, but make sure to enter your results into your lab notebook. For the spinach disks you put in the dark, you need to continue recording until a total of 30 minutes, unless all the leaf disks have sunk.

6. You also need to make a graph of your results from each of the three trials, and make sure to analyze the results.

Minutes	# of disks floating
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	
13	
14	
15	

You need to answer the following questions in the lab notebook. As before, don't copy the question as such, instead write a complete sentence that includes the information asked for.

1) What was the function of the sodium bicarbonate in this experiment?

2) What process causes the leaf disks to rise?

3) Which trial worked the best?

4) What was the purpose of putting some leaf disks into the dark?

5) How does light intensity affect the rate of photosynthesis?

References

Adapted from Biology: Exploring Life. Teaching Resources, by Brad Williamson (http://www.elbiology.com/labtools/Leafdisk.html).