Photosynthesis

Introduction

The process of photosynthesis can be summarized in the equation below.

$$6\,CO_2 + 12\,H_2O \xrightarrow[\text{Light}]{\text{Chloroplasts}} C_6H_{12}O_6 + 6\,H_2O + 6\,O_2$$

This simple equation conceals a multitude of complex processes, which are divided into two phases. In the first phase, known as the light-dependent reactions, the pigment chlorophyll absorbs light energy, which is then invested in the high-energy chemical bonds of ATP and NADPH molecules. The second phase of photosynthesis, known as carbon fixation, does not require light, since the energy has already been captured. In this light-independent phase, ATP and NADPH, along with CO_2, are used to make the carbohydrate glucose, which is a six-carbon monosaccharide (see Lab Topic 3, Macromolecules). So the result of photosynthesis is that a small amount of light energy is absorbed and then stored in the chemical bonds of glucose. The entire process takes place within the chloroplasts of plants. The chloroplast contains the chlorophyll, the enzymes, and the other molecules that are required for photosynthesis. (The prokaryotic Cyanobacteria, which can also photosynthesize, do not have their photosynthetic apparatus enclosed in an organelle.)

As you learned in Lab Topic 6 (Cellular Respiration), virtually all of the energy used by organisms on the earth, with the exception of a few chemosynthetic organisms, is made available by photosynthesis. In the process of cellular respiration, the energy stored in the chemical bonds of carbohydrates is released and repackaged in ATP, the usable "energy currency" of the cell.

In this lab topic you will learn a method of measuring the rate of photosynthesis. You will then use this method to design your own investigation of photosynthesis.

Outline

Exercise 7.1: Measuring Photosynthetic Rate in Spinach Leaf Disks

Exercise 7.2: Designing an Experiment

Exercise 7.3: Performing the Experiment and Interpreting the Results

EXERCISE 7.1

Measuring Photosynthetic Rate in Spinach Leaf Disks

Objectives

After completing this exercise, you should be able to

1. Explain how the experiment measures the occurrence of photosynthesis in spinach.
2. Interpret the results of the experiment.

The light-dependent reactions of photosynthesis are summarized in the equation:

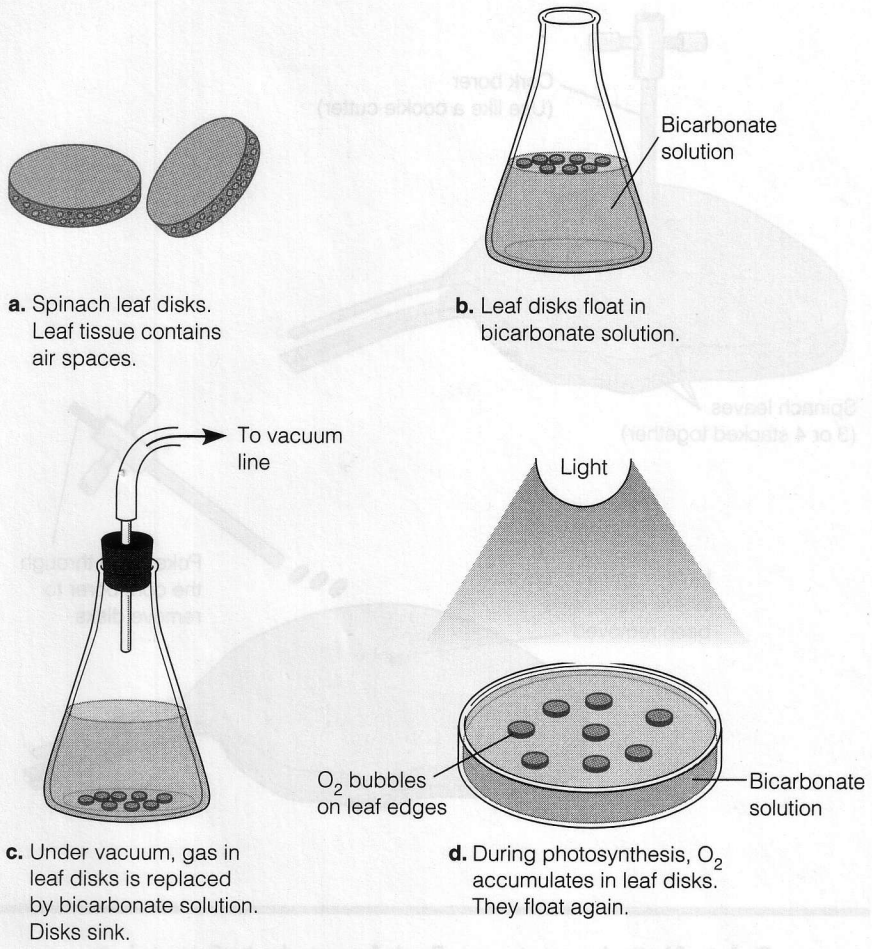
$$H_2O + ADP + \textcircled{P} + NADP^+ \xrightarrow[\text{Light}]{\text{Chloroplasts}} \frac{1}{2}O_2 + ATP + NADPH + H$$

Light energy is used to split water molecules. Some of that energy is captured by adding P (phosphate) to ADP to make ATP and by adding hydrogen to $NADP^+$. O_2 is released as a by-product. The rate of these reactions can be estimated by measuring O_2 production in disks cut from spinach leaves.

Leaf tissue is riddled with gas-filled intercellular spaces (see Figure 7.1a), so they float when they are placed in solution (Figure 7.1b). But when leaf disks in a solution are subjected to a vacuum, the gases in the leaves are pulled out and the spaces are filled by the liquid. Since fluids are heavier than gas, replacing the gas causes the leaf disks to sink to the bottom of the flask (Figure 7.1c). Then as O_2 is produced by the light reactions of photosynthesis, it diffuses into the intercellular spaces and replaces the liquid with gas. When enough O_2 has accumulated, each leaf disk will regain its buoyancy and turn on edge or float to the surface (Figure 7.1d). You will use this technique as a method of measuring photosynthesis. Sodium bicarbonate ($NaHCO_3$), which supplies CO_2 for photosynthesis, will be the solution used to infiltrate the leaf disks.

Procedure

1. Attach the lamp to the support stand so that the lamp is approximately 25 cm from the base of the stand.
2. Fill a large (1- or 2-L) beaker with cold water to act as a heat filter for the dish you will place under the lamp. The lamps produce a lot of heat, which can affect the rate of photosynthesis. Set the beaker aside for the moment.



a. Spinach leaf disks. Leaf tissue contains air spaces.

b. Leaf disks float in bicarbonate solution.

c. Under vacuum, gas in leaf disks is replaced by bicarbonate solution. Disks sink.

d. During photosynthesis, O_2 accumulates in leaf disks. They float again.

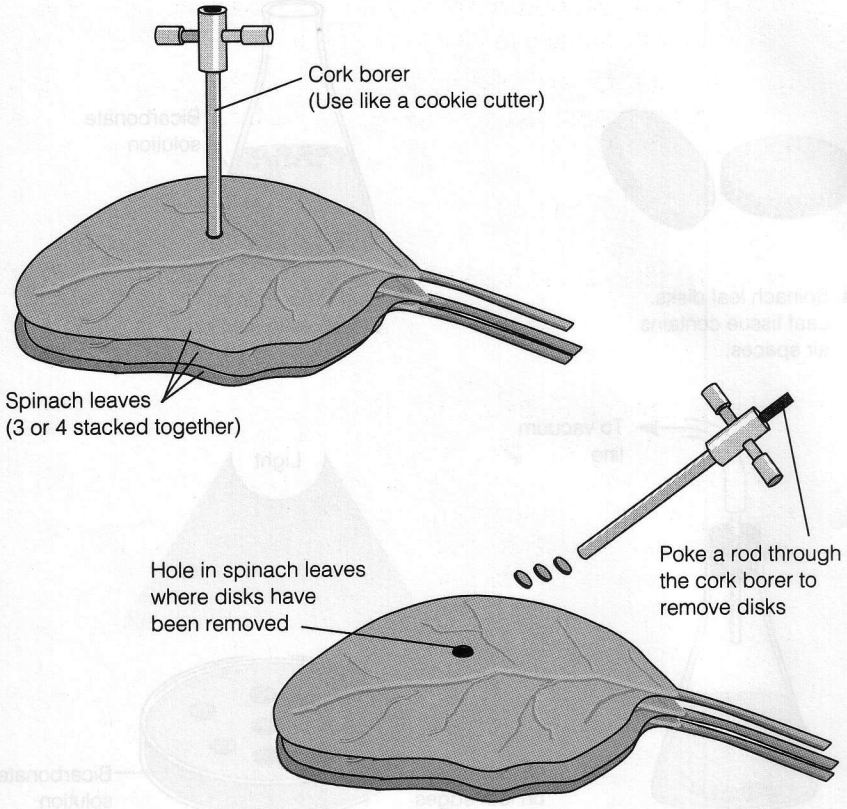
Figure 7.1.
Leaf disk method for measuring photosynthetic rate.

3. Pour 0.2% $NaHCO_3$ solution into three petri dishes so they are about 2/3 full. Pour approximately 100 mL of 0.2% $NaHCO_3$ solution into a 250-mL flask. The exact amount is not important, so you can use the markings on the flask to measure.
4. Get several spinach leaves and cut 40–50 disks with a cork borer or other circular cutting instrument. (See Figure 7.2 on the next page.) Do not include the large veins. Use a paper towel or Styrofoam board to cut on. As the disks are cut, put them in the flask with the bicarbonate solution.

Stack three or four leaves together so you can cut through them all at the same time. But don't cut so many leaves that the edges of the disks are ragged.

5. Use a water aspirator or other vacuum source, as directed by your instructor, to sink the disks.

Figure 7.2.
Cutting disks from spinach leaves.



Safety Note: Inspect your flask for cracks before using. Flawed glassware may implode under vacuum. Wear safety goggles while using the aspirator.

a. Attach the vacuum tubing to the sidearm of the flask or the glass tube in the rubber stopper. Put the rubber stopper firmly in the mouth of the flask and press the tape securely over the hole in the stopper.

Hold the flask steady while you apply the vacuum.

b. Turn on the water faucets all the way. After several seconds, you should see bubbles coming out of the edges of the disks. Leave the disks under vacuum for approximately 15–20 seconds; then release the vacuum by peeling back the tape.

c. Swirl the flask and wait to see whether the disks sink. Remember, the leaves will continue to float as long as they are under vacuum.

d. You will probably have to apply the vacuum two or three times. It is not necessary to sink all the disks. You will damage the tissue if you overaspirate.

Keep the disks away from bright light!

6. Pour the contents of the flask into a large dish. *Discard any disks that are floating.* With forceps, gently transfer 10–15 disks to each petri dish. Put the lids on the petri dishes. Put one dish in a dark place (drawer or cabinet); put another one under the lamp. Set the large beaker of water on top of the petri dish; then turn on the lamp. Place the third dish on a bench where it will receive only room light.
7. Wait 20 minutes for photosynthesis to occur. While the experiment is running, answer the questions below and work on Exercise 7.2.
8. After 20 minutes, count the number of disks that are either floating or turned on edge in each petri dish. Record your data in Table 7.1.

Table 7.1
Results of Subjecting Spinach Leaf Disks to Different Light Conditions

Light	# Disks Floating	% Disks Floating
Dark		
Room light		
Under lamp		

What hypothesis is being tested with this experiment?

Predict what the results will be if your hypothesis is supported.

What is the independent variable in this experiment?

What are some other independent variables that could affect photosynthesis?

What is the dependent variable?

What is the control treatment?

Was your hypothesis supported or proven false by the results? Explain.

EXERCISE 7.2
Designing an Experiment

Objective

After completing this exercise, you should be able to

1. Design an original experiment to investigate some factor that affects the production of oxygen in photosynthesis.

In Exercise 7.1 you learned a method of measuring photosynthetic rate in spinach leaf disks. In Exercises 7.2 and 7.3 your lab team will design an experiment using this method, perform your experiment, and present and interpret your results. Review the factors that could affect photosynthesis that you listed in Exercise 7.1 to get some ideas for possible independent variables.

The basic materials that are needed to perform an investigation will be supplied for you. Your instructor will tell you what additional materials will be available.

fresh spinach
0.2% sodium bicarbonate (1 L)
250-mL flask with 2-hole rubber stopper
vacuum source
#3 cork borer or other cutting instrument
glass stir rod
6 petri dishes
2 reflector lamps
2 support stands for lamps
Two 1-L or 2-L beakers
culture dish
forceps
cutting board

Describe your experiment below.

Question or Hypothesis

Dependent Variable

Independent Variable

Explain why you think this independent variable will affect photosynthesis.

Control Treatment(s)

Replication

Brief Explanation of Experiment

Predictions

What results would support your hypothesis? What results would prove your hypothesis false?

Method

Include the levels of treatment you plan to use.

Design a Table to Collect Your Data

List Any Additional Materials You Will Require

EXERCISE 7.3

Performing the Experiment and Interpreting the Results

Objectives

After completing this exercise, you should be able to

1. Perform the experiment your lab team designed.
2. Present and interpret the results of your experiment.

Before you do the experiment, be sure that everyone on your lab team understands the techniques that will be used. You may want to divide up the tasks before you begin work.

Be thorough in collecting data. Don't just write down numbers; record what they mean as well. Don't rely on your memory for information that you will need when reporting on your experiment later! If you have any questions, doubts, or problems during the experiment, be sure to write them down, too.

Results

Before you begin to prepare your results for presentation, decide on the best format to use. Remember, you want to give the reader a clear, concise picture of what your experiment showed. Refer to the data presentation section of Appendix A (Tools for Scientific Inquiry) for help. If you are drawing graphs, use graph paper. Complete your tables and/or graphs before attempting to interpret your results.

Write a few sentences *describing* the results (don't explain why you got these results or draw conclusions yet).

Discussion

Look back at the hypothesis or question you posed in this experiment. Look at the graphs or tables of your data. Do your results support your hypothesis or prove it to be false? Explain your answer, using your data for support.

Did your results correspond to the prediction you made? If not, explain how your results are different from your expectations and why this might have occurred.

Describe how your data are supported by information from other sources (for example, textbooks or other lab teams working on a similar problem).

If you had any problems with the procedure or questionable results, explain how they might have influenced your conclusion.

If you had an opportunity to repeat and extend this experiment to make your results more convincing, what would you do?

Summarize the conclusion you have drawn from your results.

Questions for Review

1. What is the function of each of the following in photosynthesis?
   Light:

CO_2:

Chlorophyll:

2. In the leaf disk method, spinach tissue is infiltrated with bicarbonate solution so that it sinks. Why do the disks float again after being exposed to light?

3. Reflotation of leaf disks by O_2 is one method of measuring photosynthesis. Review the equations for photosynthesis and suggest at least one other method that could be used (assume that you have the means of measuring any substance you choose).

4. A team of students performs an experiment to determine whether different wavelengths of light are equally effective at producing photosynthesis. Their results are shown in Table 7.2. What conclusion would you draw from these results?

Table 7.2
Effects of Wavelength on Photosynthesis

Light	# Disks Floating	% Disks Floating
Dark	1/30	3
White light	25/30	83
Blue light	24/30	80
Yellow light	3/30	10
Green light	2/30	7
Red light	25/30	83

Acknowledgments

The leaf disk technique was modified from Witham, F., D. Blaydes, and R. Devlin. *Experiments in Plant Physiology.* New York: Van Nostrand Reinhold, 1971. Those authors acknowledge the following paper for the original technique: Wickliff, J. L., and R. M. Chasson. "Measurement of Photosynthesis in Plant Tissues Using Bicarbonate Solutions." *Bioscience* 14:32–33, 1964.