Catalog No. FB2034

**Introduction**

**Photosynthesis in Leaf Disks**

**AP\* Biology Big Idea 2, Investigation 5**

**An Advanced Inquiry Lab**

Publication No. 11131

A variety of procedures and techniques may be used to study the rate of photosynthesis. However, the most practical method in a high school laboratory setting is the floating leaf disk technique. Examine the factors that affect photosynthesis in land plants.

**Concepts**

• Photosynthesis • Cellular processes

**Background**

The sun plays an important role to life on Earth. Plants contain chloroplasts that capture light energy from the sun and convert

it to chemical energy stored in sugar and other organic molecules. The name of this overall conversion process is *photosynthesis*.

To maintain order, grow, and reproduce, living systems require free energy. Sufficient energy levels are required for individ- ual organisms to grow and also to ensure the survival of populations and ecosystems. Different organisms use various strategies to capture, use, and store free energy. Autotrophic organisms capture free energy from the environment through photosynthesis and chemosynthesis. Heterotrophic organisms harvest free energy and carbon compounds produced by other organisms.

Photosynthesis occurs in the chloroplasts within cells. The photosynthesis process occurs in a series of enzyme-mediated

steps that capture light energy to form energy-rich carbohydrates. The overall process is summarized by Equation 1 below.

2H2O + CO2 + light → carbohydrate (CH2O) + O2 *Equation 1*

The net rate of photosynthesis can be determined in two ways—by measuring the production of oxygen, O2 or the consump- tion of carbon dioxide, CO2. Traditionally the rate of photosynthesis is calculated by measuring the consumption of carbon diox- ide. However, this is difficult to perform with the equipment present in a traditional educational setting. Accurately measuring the production of oxygen is difficult because aerobic respiration occurs at the same time as photosynthesis; thus consuming oxygen as it is produced. Therefore, measuring oxygen production is equivalent to measuring net photosynthesis. A measurement of respira- tion in the same system would allow the estimation of gross production.

The ratio of the rate of photosynthesis (Equation 1) to the rate of cellular respiration (Equation 2) can be indirectly deter- mined using the floating disk assay. The floating disk assay uses the overall rate at which oxygen is produced as a measure of the balance between the two reactions. Disks of leaf tissue are vacuum-infiltrated to replace intercellular air with liquid. As photosyn- thesis takes place, if the rate of photosynthesis exceeds the rate of cellular respiration, the accumulating oxygen imparts buoyancy to the leaf disk, and it floats. Conversely, if the rate of the respiration exceeds the rate of photosynthesis, the decreased oxygen will eventually cause the leaf disk to sink.

 C6H12O6 + 6O2 → 6CO2 + 6H2O + 112 kcal/mol *Equation 2*

**Experimental Overview**

In the *Baseline Activity,* the skills needed to perform a leaf disk assay will be used to compare one variable to a control. The analysis of the results of the baseline activity will provide guidance for open-inquiry, student-designed experiments—see the *Opportunities for Inquiry* section on page 3 for further information. Explore environmental, plant-type and even methodology in the inquiry portion of this lab. The results of the baseline activity will be analyzed and graphed, then a procedure will be devel- oped to study an environmental, method, or plant variable that affects the rate of photosynthesis.

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**Pre-Lab Questions**

1. Draw and label a figure showing a cross-section of a typical dicot leaf.

2. Why, in the *Baseline Activity,* are the floating leaf disks placed in a solution that contains sodium bicarbonate?

3. Why is this method performed using leaves and not roots?

 4. Why is the rate at which the disks float an indirect measurement of the net rate of photosynthesis?

**Materials**

**Baseline Activity**

Soap solution Paper towels Sodium bicarbonate, NaHCO3 Permanent marker Ivy leaves Ruler

Water, distilled or deionized Syringe, 12-mL Balance, 0.01-g precision Syringe tip cap Cups, 10-oz, 2 Support stand Hole-punch, single Timer

Light source

***Safety Precautions***

*Hydrochloric acid is toxic by ingestion or inhalation and is severely corrosive to skin and eyes. Sodium bicarbonate is slightly toxic by ingestion. Wear chemical splash goggles whenever chemicals, heat or glassware is used. Keep water or other solutions away from electrical cords and outlets. Follow all normal laboratory safety guidelines.*

**Baseline Activity**

 1. Using a permanent marker, label one syringe “w/ CO2” and the other “control.” Repeat labels on the plastic cups as well.

 2. Prepare 200 mL of 0.2% sodium bicarbonate solution by dissolving 0.4 g of sodium bicarbonate in 200 mL of distilled or

deionized water.

3. Add one drop of liquid dish soap to the solution. *Note:* Take care to add the smallest possible drop. Too much soap will cause unnecessary suds in the solution.

 4. Prepare 200 mL of the control solution (no carbon dioxide) by adding one drop of soap to 200 mL of distilled or deionized

water.

 5. Pour enough bicarbonate solution into the cup labeled “w/ CO2” so that it is approximately 3 cm full.

 6. Fill the cup labeled “control” 3 cm with the solution made in step 4.

7. Using a single-hole punch, cut out 20 leaf disks. Avoid cutting leaf disks over major veins in the leaf.

8. Remove the plunger from the syringe.

 9. Place 10 leaf disks into the barrel of each syringe.

10. Carefully replace the plunger to avoid crushing the leaf disks. Push the plunger until only a small volume of air and leaf disks remains in the barrel, less than 10% of the volume. *Note:* Steps 10–18 will also need to be performed with the con- trol solution. This may be done simultaneously by another group member or one after the other.

11. Using the syringe labeled “w/ CO2,” pull a small volume of the sodium bicarbonate solution into the syringe, about 3 mL.

Tap and swirl the syringe to suspend the leaf disks in the solution.

12. Place the syringe tip cap on the syringe.

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13. Make sure the disks are suspended in solution by lightly shaking the syringe and then draw back the plunger to create a vacuum. Hold the vacuum for approximately 10 seconds. While holding, swirl the leaf disks to keep them suspended in solution.

14. Release the vacuum. The bicarbonate solution will infiltrate the air pockets in the leaf causing the leaf disks to sink. Repeat step 13 up to three times until all the disks sink. If the disks do not sink after three trials add a second very small drop of soap to the bicarbonate solution and repeat steps 10–14.

15. Add fresh sodium bicarbonate solution (step 3) into a clean 200-mL beaker to a depth of approximately 3 cm.

16. Transfer the leaf disks and the solution in the syringe to the beaker.

17. Place the leaf-disk solution under a light source. The top of the beaker should be approximately 8ʺfrom the light.

18. Start the timer. At the end of each minute, record the number of floating disks. Then swirl the disks to release any disks

that are stuck against the side of the cups. Continue for 10 minutes or until all disks float.

**Analysis**

Calculate the ET50, the time required for 50% of the leaf disks to float by making a graph measuring Number of Disks vs. Time (min) with the disks from the “w/ CO2” cup.

**Opportunities for Inquiry**

 1. Consider the following questions while reflecting upon your knowledge of photosynthesis.

*a.* How might biotic and abiotic factors in the environment, such as light, pH, temperature, etc. affect the rate of photosynthesis?

*b.* Do all leaf types photosynthesize at the same rate? Does the type, color or age of the leaf affect the rate of photosynthe- sis? Do all plant tissues have the same rate of photosynthesis?

*c.* How do method variables such as depth of solution, data collection method, etc vary the rate of photosynthesis?

 2. Plan, discuss, evaluate, execute and justify an experiment to determine how an environmental variable, plant/leaf variable,

or method variable affects the rate of photosynthesis.

*a.* Develop a testable hypothesis.

*b.* Discuss and design a controlled experiment to test the hypothesis.

*c.* List any safety concerns or precautions that will be taken to protect yourself, your classmates and your instructor during

the experiment. Do monocots, dicots, C3, C4 or water plants all have the same ratio of photosynthesis?

*d.* Determine how you will collect and record raw data.

*e.* How will you analyze raw data to test your hypothesis?

*f.* Review your hypothesis, safety precautions, procedure, data tables, and proposed analysis with your instructor prior to beginning your experiment.

*g.* Once the experiment and analysis are complete, evaluate your hypothesis and justify why or why not the hypothesis was supported by your data.

*h.* Present and defend your findings to the class.

*i.* Make suggestions for a new or revised experiment to modify or retest your hypothesis.

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**Teacher’s Notes**

**AP Biology—Photosynthesis in Leaf Disks**

**Materials Included in Kit** (for 8 groups of students)

Hydrochloric acid, HCl, 1 M, 100 mL Hole-punch, single, 4

Soap solution, 30 mL Syringe, 12-mL, 16

Sodium bicarbonate, NaHCO3, 20 g Syringe tip cap, 16

Cups, 10-oz, 16

**Additional Materials Needed**

Water, distilled or deionized Light sources, 8

Ivy or spinach leaves† Support stands, 8

Balance, 0.01-g precision Timers, 8

†*Other leaf types may be required as well, depending on student experimental design.*

**Lab Hints**

• Use a bulb that is at least 40 W. The sample data provided was obtained using a 40 W bulb that was 18 cm from the top of

the lab bench.

• Ivy leaves should ideally be obtained from a plant so they are as fresh as possible.

• Use the freshest possible spinach leaves for optimal results. Pre-cut spinach in the bags will work but it takes 5–10 minutes

longer on average for the disks to float.

• Do not use wilted spinach. The best pieces are found on the stiff leaves and are not cut through veins.

• If students perform the experiment as done in the *Baseline Activity* until the disks float and then place the cup with the floating disks in a dark location the disks will re-sink. This is due to plant respiration consuming oxygen bubbles.

• Rinse plastic cups from the *Baseline Activity* for use in the *Opportunities for Inquiry* portion.

**Teaching Tips**

• Students often have the misconception that plants only undergo photosynthesis and animals undergo cellular respiration. Plants also have mitochondria and respire.

• Have students view prepared microscope slides of leaf cross-sections to review leaf anatomy prior to beginning this lab.

• Complete a stomata peel to study leaves as part of the *Baseline Activity*. Contact Flinn Scientific and request *BioFax*

10226, Lasting Impressions—Counting Stomata.

• Extend the activity using *Mitochondrea in Action,* Flinn Catalog No. FB1823.

**Answers to Pre-Lab Questions**

1. What is the purpose of creating a vacuum with the floating leaf disks?

*When immersed in the experimental solution oxygen bubbles are trapped in the air pockets of the mesophyll layer of the plant leaf. By creating a vacuum the air is drawn out of the leaf and replaced with the bicarbonate solution, allowing the disk to sink.*

2. Why, in this experiment, are the floating leaf disks placed in a solution that contains sodium bicarbonate?

*The bicarbonate ions serve as the carbon source for photosynthesis.*

3. What causes the disks in the bicarbonate solution to rise after they are placed under a light source?

*During the photosynthesis process oxygen is produced changing the buoyancy of the disk causing it to rise.*

 4. Why is the rate at which the disks float an indirect measurement of the net rate of photosynthesis?

*Cellular respiration is occurring simultaneously which consumes the oxygen as it is produced by photosynthesis.*