Photosynthesis Study Technique

Introduction

Numerous procedures and techniques have been employed to teach the basics of photosynthesis. The disk assay method outlined here can be easily incorporated into open-ended, student-designed investigations.

Concepts

- Photosynthesis
- Respiration
- Floating disk assay

Background

Photosynthesis utilizes light energy to reduce carbon dioxide and produce glucose ($C_6H_{12}O_6$), according to Equation 1.

$$6CO_2 + 6H_2O + 112 \text{ kcal/mole} \longrightarrow C_6H_{12}O_6 + 6O_2 \qquad \textit{Equation 1}$$

The reverse process, respiration, takes place in all cells and involves the oxidation of glucose to form carbon dioxide and release energy (Equation 2).

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + 112 \text{ kcal/mole} \qquad \textit{Equation 2}$$

The energy produced during cellular respiration is used to do biological work.

The ratio of the rate of photosynthesis (Equation 1) to the rate of cellular respiration (Equation 2) can be determined using the floating disk assay. The floating disk assay uses the rate at which oxygen is produced or consumed as a measure of the balance between the two reactions. Disks of leaf tissue are vacuum-infiltrated to replace intercellular air with liquid. As photosynthesis 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 disk to sink.

Safety Precautions

Exercise caution when using a cork borer to avoid cuts. Wear chemical splash goggles whenever working with chemicals, heat or glassware in the lab. Please review current Safety Data Sheets for additional safety, handling, and disposal information.

Preparation

Several hours before class, wash fresh spinach leaves in cold running water. Allow the leaves to soak in fresh water at 4 °C until ready to use. Make a 0.2% sodium bicarbonate solution by mixing 0.2 g of sodium bicarbonate ($NaHCO_3$) in 100 mL of distilled or deionized water.

Procedure

1. Use a hole-punch or cork borer to cut leaf disks from a fresh spinach leaf. Do not punch through large veins. Punch only through the large, homogenous, flat parts of the leaf. The disks should be about 4–6 mm in diameter.
2. Place the disks in a 250-mL side-arm filter flask containing 50 mL of 0.2% sodium bicarbonate solution.
3. Cork the flask. Use a piece of tubing to connect the side arm of the flask to an aspirator or other vacuum source. Turn on the vacuum. Air bubbles will be seen escaping from the edges of the disks.
4. After 30 seconds, stop the vacuum and remove the stopper from the top of the flask. The disks should sink. Swirl the flask until all the disks sink. Discard any disks that do not sink.
5. The disks are now ready for transfer to an experimental setup for an oxygen or photosynthesis assay. The disks will float in a liquid environment if photosynthesis produces enough oxygen to buoy the disk in the liquid. The time required to get the disk to float provides a relative measure of the rate of photosynthesis.
6. Use the following general outline to assist students in designing an experiment using their assay disks.
   a. Form a research group to investigate one variable that might affect the rate of photosynthesis. Possible variables include: light intensity or wavelength; oxygen or carbon dioxide concentration; pH or temperature.

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b. Propose a hypothesis for the effect of changing this variable. Write the hypothesis in the form of an "If, then" statement.
c. Design an experiment and write an experimental plan in detail. Be sure to include controls.
d. Discuss the hypothesis, materials requirements, and all safety considerations with your instructor.
e. Obtain all the necessary materials for your experiment.
f. Conduct the experiment and record the data.
g. Write a complete laboratory report. Include: 1) a statement of hypothesis, 2) a description of the experimental design and procedure, 3) data and observations, 4) interpretation, and 5) possible future experiments.

Disposal

Used spinach disks may be disposed of following Flinn Suggested Disposal Method #26a. Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures governing the disposal of any laboratory waste solutions.

Tips

- This disk assay method is a perfect springboard for involving students in higher cognitive level thinking skills as promoted by the National Science Standards. Once the technique has been demonstrated, students will generate numerous experimental designs, most of which are easy to execute.
- One of the most important factors for successful disk assay is the freshness of the spinach. In most cases, fresh spinach from a supermarket will work. The fresher and crisper the spinach, the more responsive the tissue will be. Soak leaves for several hours (at 4 °C) prior to use. This increases turgor pressure and minimizes "limp" leaves. When punching out disks, use only the firm, dark green areas of the leaf. Avoid major veins or damaged areas. Disks that are about 4 mm in diameter work best.
- Do not over vacuum–infiltrate leaf disks. Too little vacuum treatment causes the disks not to sink; too much vacuum treatment, however, may kill the cells.
- One obvious experiment is to place disks in sodium bicarbonate solution in Petri dishes, expose them to various light intensities, and measure how long it takes for the disks to float with each light intensity. Other variables that might be tested include different colors of light, varying sodium bicarbonate concentrations, varying amounts of chlorophyll (by using leaves other than spinach), leaf maturity, pH, temperature, and photosynthesis inhibitors.

Connecting to the National Standards

This laboratory activity relates to the following National Science Education Standards (1996):

Unifying Concepts and Processes: Grades K–12
Evidence, models, and explanation
Constancy, change, and measurement
Content Standards: Grades 5–8
Content Standard A: Science as Inquiry
Content Standard C: Life Science, regulation and behavior
Content Standards: Grades 9–12
Content Standard A: Science as Inquiry
Content Standard C: Life Science, matter, energy, and organization in living systems; behavior of organisms

Reference

Fox, M.; Gaynor, J. J.; Shillcock, J. *Floating Spinach Disks—An Uplifting Demonstration of Photosynthesis*; JCST, January, 1999.