EDVOTEK®

AP BIOLOGY

BIG IDEA 2
INVESTIGATION
5

EDVO-Kit: AP05

Photosynthesis

See Page 3 for storage instructions.

EXPERIMENT OBJECTIVE:

In this experiment, students will learn how to measure the rate of photosynthesis indirectly by using the floating leaf disk method. They will also investigate several factors that might affect the photosynthesis process.

Experiment Components

- Sodium Bicarbonate (baking soda)
- Liquid soap
- Plastic syringes
- Transfer pipets
- Plastic cups

Store the entire experiment at room temperature.

This experiment is designed for 10 lab groups.

Requirements

- Leaves (i.e. spinach, ivy, pokeweed)
- Timer
- Light source (60 watt light bulb recommended)
- Hand-held hole punch
- Beakers

Background Information

Photosynthesis is the process by which plant cells use light energy for the biosynthesis of cellular components. Photosynthetic organisms form the basis of the food chain. These life forms include higher plants, algae, dinoflagellates, euglenoids, diatoms and certain bacteria. Photosynthesis consists of two biochemical phases. The general equation for the first phase is:

H_2O + NADP+ + Pi + ADP + Light → O_2 + NADPH + H+ + ATP

The first phase is light dependent. NADP+ and NADPH are oxidized and reduced forms, respectively, of nicotinamide adenine dinucleotide phosphate. The reduced form is an essential cofactor in the biosynthesis of many types of molecules such as carbohydrates. Chemical energy of ATP is required for many biochemical reactions and for maintenance of cellular integrity and function. ATP is generated from ADP and inorganic phosphate (Pi). The reaction also generates protons (H+) and molecular oxygen from water. The second phase of photosynthesis can be generally written as:

CO_2 + NADPH + H+ + ATP → glucose + NADP+ + ADP + Pi

The second phase is not light dependent. The reaction fixes atmospheric carbon dioxide into organic linkage (glucose). Each phase consists of many separate chemical steps. First phase steps are called light reactions and second phase steps are called dark reactions.

Light reactions in eukaryotic cells occur in organelles called chloroplasts. Chloroplasts contain DNA and are self-replicating. These organelles consist of an outer membrane and a folded inner membrane. Stacked, disk- like structures called thylakoids form part of the inner membrane and it is here that light dependent photosynthetic systems are found. The primary photosynthetic pigments are green chlorophylls. Chloroplasts contain chlorophyll 'a' and 'b', magnesium-porphyrin complexes, and are specifically bound to proteins that reside on and within the inner membrane. Pure chlorophyll 'a' maximally absorbs light at wavelengths of around 420 and 660 nm. Chlorophyll 'b' absorbs primarily at approximately 480 and 640 nm.

The absorption spectrum of chlorophylls can be shifted depending on with which type of protein they are associated. Other pigments found in chloroplasts include ß-carotene and xanthophylls. These pigments have an accessory light harvesting function and absorb at wavelengths in between the maxima of the chlorophylls. They all capture light energy and transfer it to the chlorophyll a at the reaction center. All these pigments are sensitive to light and oxygen in the purified state and eventually breakdown. Extremely pure preparations required for chemical and biological studies are stored under vacuum, in the dark, at -20° C.

Photosynthetic System

There are two photosynthetic systems in chloroplasts, termed Photosystem i and ii. These physically distinct systems contain different proteins and ratios of chlorophylls and accessory pigments. Photosystem i is not responsible for oxygen evolution and is activated by longer wavelengths of light. Photosystem ii is activated by shorter wavelengths of light and is required for oxygen and ATP production. Both systems contribute high energy electrons for the reduction of NADP+. Both photosystems are required for maximal photosynthetic activity. When light is absorbed by the chlorophyll-protein complex of photosystem I, chlorophyll becomes excited and enters a higher energy state. During return

Background Information

from high energy to ground state, an electron is boosted to a higher energy level and is sequentially transferred, via several membrane associated protein transport molecules, to the final electron acceptor NADP+. The NADP+ is reduced to NADPH through the action of a reductase enzyme. Since this is not normally a spontaneous (energetically favorable) reaction, input of light energy is required to convert NADP+ to NADPH.

Electron transport proteins containing iron and sulfur are called ferredoxins. Other transporters are called cytochromes. A high energy electron generated by light absorption in photosystem II is donated, via a specific sequence of transporters, to the electron deficient photosystem I. Photosystem II then receives an electron through a series of transport proteins from H_20. Water is oxidized to molecular oxygen during this process. Water is the electron donor in photosynthesis. Conversion of water, a very stable molecule, to oxygen is energetically unfavorable and would not occur to any significant extent without input of light energy at photosystem II. Production of ATP occurs along the sequence of electron transfer steps. ATP is a high energy compound and requires energy for its synthesis. This energy is siphoned from the high energy electrons through a complex series of events involving membrane proteins and formation of pH gradients across chloroplast membranes to drive ATP synthesis.

The dark reactions of photosynthesis occur simultaneously with the light reactions in plant cells. The dark reactions are a set of seven enzyme catalyzed metabolic steps that synthesize glucose. The key metabolic step in plant glucose synthesis is catalyzed by the abundant enzyme ribulose diphosphate carboxylase. The majority of these reactions take place outside the chloroplast in the cytoplasm. Most of the glucose is polymerized into starch and cellulose. The reaction involves the fixation of carbon dioxide.

This experiment uses the floating disk leaf assay to explore the process of photosynthesis in plants. Leaf disks generally float due to the many intercellular spaces used for exchange of gases. When the air spaces are infiltrated with solution, the overall density of the leaf disk increases, causing the disks to sink. The infiltration solution includes a small amount of Sodium bicarbonate. Bicarbonate ion serves as the carbon source for photosynthesis. By providing the components needed for photosynthesis (light, CO_2, and H_20), oxygen will be produced in the leaf. As photosynthesis takes place, oxygen is released into the interior of the leaf which changes the buoyancy and causes the disks to rise. Since cellular respiration takes place and also consumes oxygen, the rate that the disks rise is an indirect measurement of the net rate of photosynthesis.

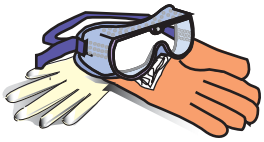
Respiration, which uses the oxygen produced by photosynthesis, is also observed in the leaf disk assay. Some of the oxygen will be used in the leaf's respiration process. Leaf disks float, because the net result is that more oxygen is produced by photosynthesis than is used in respiration. In this investigation, the rate at which leaf "disks" rise will be used as an indirect measure of the net production of oxygen produced by photosynthesis.

Experiment Overview and General Instructions

EXPERIMENT OBJECTIVE:

In this experiment, students will learn how to measure the rate of photosynthesis indirectly using the floating leaf disk method. They will also investigate several factors that might affect the photosynthesis process.

LABORATORY SAFETY GUIDELINES



1. Wear gloves and goggles while working in the laboratory.
2. Exercise caution when working in the laboratory – you will be using equipment that can be dangerous if used incorrectly.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Always wash hands thoroughly with soap and water after working in the laboratory.
5. If you are unsure of something, ASK YOUR INSTRUCTOR!

LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:
- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:
- Record your observations.

After the Experiment:
- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

Investigation I: Observation of Cellular Photosynthesis

Notes:

- For Module I, you will receive 2 plastic cups – (1) cup containing water / liquid soap solution, and (1) cup containing the bicarbonate / liquid soap solution.
- Throughout this module, you will be preparing material for the "Light - Control soln" and "15 cm Light - CO_2 soln" simultaneously. Follow the steps below for preparation of materials.

Procedure

1. Label the plastic cup containing water / liquid soap solution provided by your lab instructor as "Light - Control soln." This is your control cup.

2. Label the plastic cup containing the bicarbonate / liquid soap solution provided by your lab instructor as "15 cm Light - CO_2 soln." This is your experimental cup.

3. Prepare 10 uniform leaf disks for each trial using the hole punch. Try not to include the major veins in the leaf disks (Fig. 1).

4. Remove the plunger of the syringe and carefully transfer leaf disks in the barrel. Shake or tap the barrel on the lab bench to collect the disks to the bottom (near the opening) of the barrel.

5. Replace the plunger back into the barrel. Push the plunger until only a small volume of air and leaf disks remain in the barrel (Fig. 2). Be careful not to damage the leaf disks.

6. Using the transfer pipet provided, put a small volume of sodium bicarbonate solution (4-5 cc) into the syringe. Gently shake or tap the syringe to suspend the leaf disks in the solution.

7. While placing a finger over the syringe opening tightly, draw back slowly on the plunger to create a vacuum and hold it for 10 seconds (Fig. 3).

8. While holding the vacuum, swirl the leaf disks to suspend them in solution. Turn the syringe upright and slowly let the plunger spring back to release the vacuum.

9. If the disks don't sink, repeat steps 7-8. You may have to repeat this procedure two to three times in order to get all the disks to sink to the bottom (near the opening) of the syringe barrel (Fig. 4).

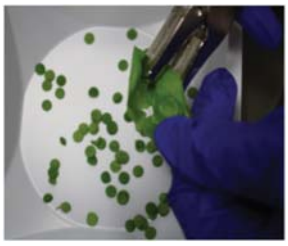


Fig. 1 - Making leaf disks.

Fig. 2 - Placing leaf disks into the syringe.

Fig. 3 - Creating vacuum in the plunger.

Fig. 4 - Sinking leaf disks.

Investigation I: Observation of Cellular Photosynthesis, continued

Fig. 5 - Sinking leaf disks in bicarbonate solution.

Fig. 6 - Placing cup under light source.

Fig. 7 - Disks floating in bicarbonate solution.

10. Once all the disks have sunk, remove plunger from the barrel. Swirl and quickly pour the disks and the solution into the cup labeled "15 cm Light - CO_2 soln" (Fig. 5).

    If the disks stick to the side of the syringe, add a small amount of Sodium Bicarbonate Solution into the syringe. Slowly swirl the syringe to dislodge the disks and pour it into the plastic cup (or beaker).

11. Repeat steps 3 – 9 for the cup labeled "Light – Control soln." Remember to replace the bicarbonate solution with the diluted soap solution in the plunger.

12. Place both cups under light located about 15 cm away and begin timing (Fig. 6).

13. Use Table 1 to record the number of disks that are floating in the "Light – Control soln" cup at the end of each minute (Fig. 7).

14. Use Table 2 to record the number of disks that are floating in the "15 cm Light - CO_2 soln" at the end of each minute.

15. Continue to record the number of floating disks at the end of each minute until all of the disks are floating or you have reached 30 minutes.

Investigation I: Observation of Cellular Photosynthesis, continued

Table 1: Light – Control Solution	
Minutes	# of leaf disks floating
1	
2	
3	
4	
5	
6	
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21	
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24	
25	
26	
27	
28	
29	
30	

Table 2: 15 cm Light – Co_2 Solution	
Minutes	# of leaf disks floating
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	
13	
14	
15	
16	
17	
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19	
20	
21	
22	
23	
24	
25	
26	
27	
28	
29	
30	

Investigation II - Observation of Plant Respiration

Question:
What happens if photosynthesis is not occurring, but cellular respiration continues?

Notes:

- For Investigation II, you will receive 1 plastic cup containing the bicarbonate / liquid soap solution

Procedure

1. Label the plastic cup containing bicarbonate / liquid soap solution provided by your lab instructor as "30 cm Light - CO_2 soln."
2. Prepare 10 uniform leaf disks using the hole punch. Remember to avoid the major veins in the leaf when making leaf disks.
3. Remove the plunger of the syringe and carefully transfer your leaf disks in the barrel. Shake or tap the barrel on the lab bench to collect the disks to the bottom (near the opening) of the barrel.
4. Replace the plunger back into the barrel. Push the plunger until only a small volume of air and leaf disk remains in the barrel. Be careful not to damage the leaf disks.
5. Using the transfer pipet provided, put a small volume of sodium bicarbonate solution (4-5 cc) into the syringe. Gently shake or tap the syringe to suspend the leaf disks in the solution.
6. While placing a finger over the syringe opening tightly, draw back slowly on the plunger to create a vacuum and hold it for 10 seconds.
7. While holding the vacuum, swirl the leaf disks to suspend them in solution. Turn the syringe upright and slowly let the plunger spring back to release the vacuum.
8. If the disks don't sink, repeat steps 7-8. You may have to repeat this procedure two to three times in order to get all the disks to sink to the bottom.
9. Once all the disks have sunk, remove plunger from the barrel. Swirl and quickly pour the disks and the solution into the cup containing bicarbonate / liquid soap solution.

   If the disks stick to the side of the syringe, add a small amount of Sodium Bicarbonate Solution into the syringe. Slowly swirl the syringe to dislodge the disks and pour it into the plastic cup (or beaker).
10. Place the cup under light located about 30 cm away and begin timing (Fig. 6).
11. Use Table 3 to record the number of disks that are floating at the end of each minute until all of the disks are floating or you have reached 30 minutes.
12. Once all the leaf disks have floated, remove the cup from the light source and place it in the dark. Suggestions include covering the beaker with an empty box or a piece of aluminum foil. Use Table 4 to record how many disks are still floating at the end of each minute over the next 15 minutes.

Investigation II - Observation of Plant Respiration

Table 3: 30 cm Light – Co_2 Solution

Minutes	# of leaf disks floating
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	
13	
14	
15	
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25	
26	
27	
28	
29	
30	

Table 4: Dark – Co_2 Solution

Minutes	# of leaf disks floating
1	
2	
3	
4	
5	
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12	
13	
14	
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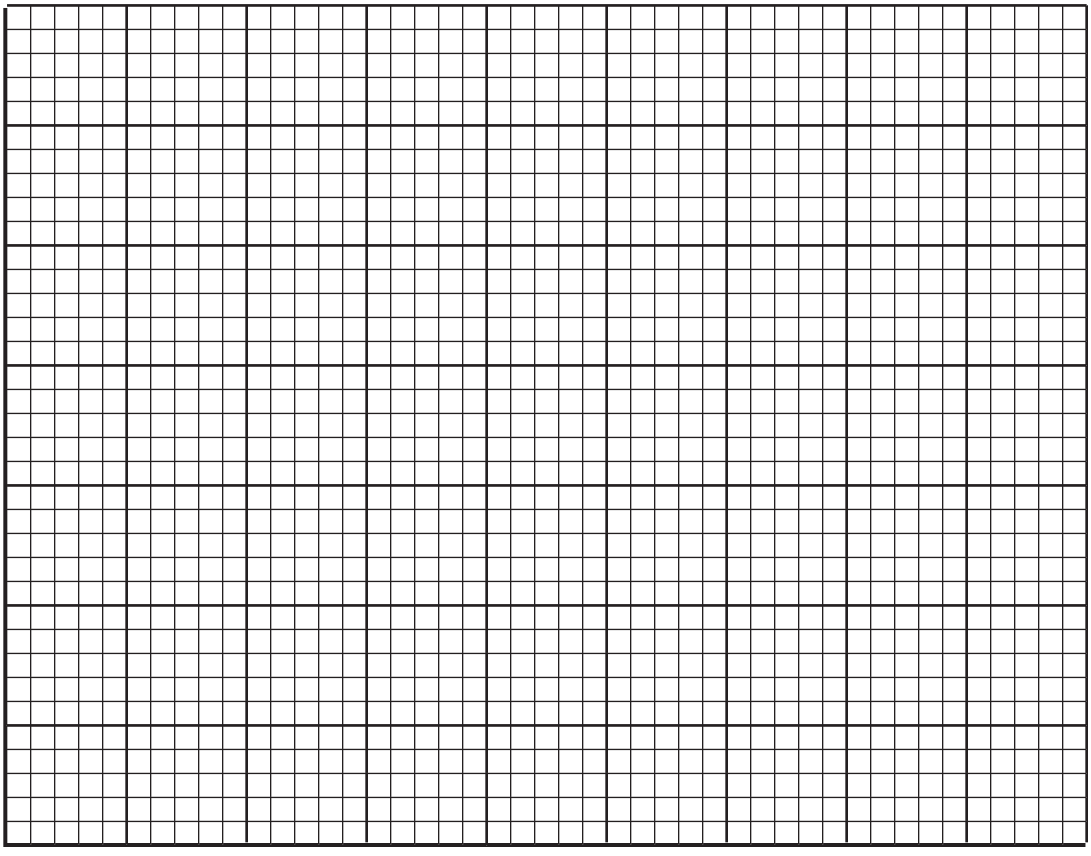
Data Collection and Analysis

Graph your results for **each** of the trials on the graph paper provided. Graph the results from your class average.

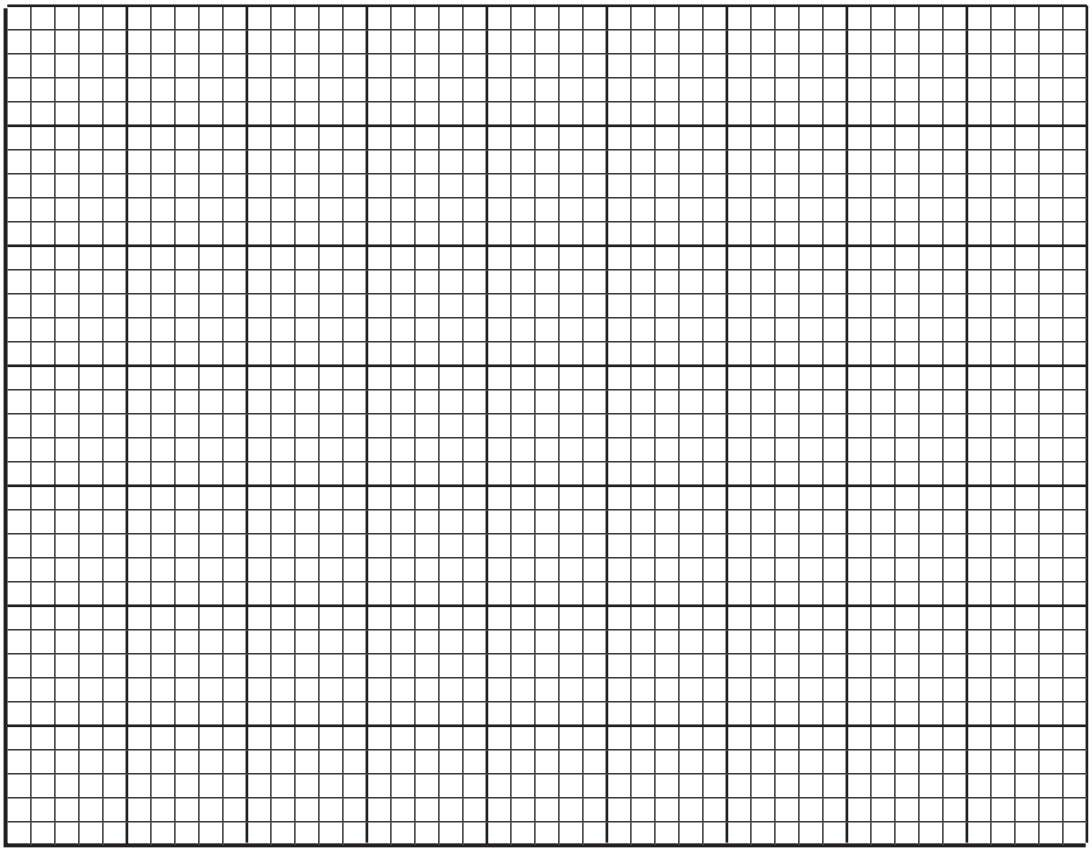
a. Label the independent variable (horizontal x-axis).
b. Label the dependent variable (vertical y-axis).
c. Title the Graphs
d. $ET_{50light}$ is the point at which 50% of leaf disks are floating (the median). Find the $ET_{50light}$ value of each trial (if applicable).
e. ET_{50resp} is the time for 50% to sink after the leaf disks were transferred to the dark conditions. Determine the ET_{50resp} value.
f. Because respiration occurs in both the light and dark, the rate of photosynthesis (ET_{ps}) is the sum of the rate in the light plus the respiration rate.

$$1/ET_{50ps} = 1/ET_{50light} + 1/ET_{50resp}$$

What is the rate of photosynthesis for your experiment?



Data Collection and Analysis



Study Questions

1. What is the function of the sodium bicarbonate in this experiment?
2. Explain the process that causes the leaf disks to rise.
3. Explain the process that causes the leaf disks to sink.
4. What is the purpose of using water/soap solution for one of the trials?
5. What is the effect of darkness on photosynthesis? Explain.
6. How does light intensity affect the rate of photosynthesis?