Lab #7: Photosynthesis & Cellular Respiration Lab

OVERVIEW – PHOTOSYNTHESIS

Photosynthesis is the process by which light energy converts inorganic compounds to organic substances with the subsequent release of elemental oxygen. It may very well be the most important biological event sustaining life. Without it, most living things would starve and atmospheric oxygen would become depleted to a level incapable of supporting animal life.

Sunlight powers photosynthesis. Using a prism, the English physicist Sir Isaac Newton demonstrated that white light consists of a variety of colors ranging from red at one end of the **visible spectrum** to violet at the other end. In the mid 1800s, James Clerk Maxwell illustrated that the visible spectrum was a minute portion of a continuous spectrum, or **electromagnetic spectrum**, which includes radio waves, visible light, x-rays, and cosmic rays. Radiations of the spectrum travel in waves measured in nanometers (1nm = 10^{-9}m). Radiations with longer wavelengths (radio waves) have less energy, and those with shorter wavelengths (x-rays) have more energy.

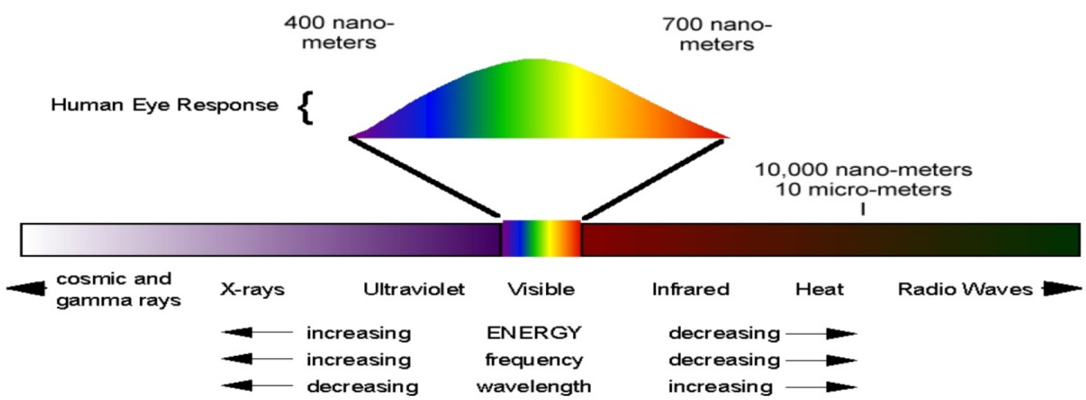

Figure 1. The electromagnetic spectrum

Question: Observe Figure 1. What has more energy, a microwave signal or a gamma ray?

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Question: Observe Figure 1. What has more energy, green light or purple light?

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Question: If you go scuba diving, how do the colors shift with depth? Why?

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For an organism to utilize light energy, it has to be absorbed. In living systems, **pigments** absorb light energy. Some pigments, such as melanin, absorb all wavelengths of light, and they appear black. At the other end of the spectrum, many pigments absorb only certain wavelengths of light and reflect the other wavelengths. The light **absorption spectrum** of a pigment illustrates the wavelengths that are absorbed. For example, green leaves contain the pigment **chlorophyll**, which reflects the green portion of the spectrum.

Chlorophyll is the most important pigments in photosynthesis. Several types of chlorophyll exist in nature. Chlorophyll *a* is the main photosynthetic pigment in some cyanobacteria and in plants. Other pigments important in plants but not involved directly in photosynthesis are called **accessory pigments**. Xanthophyll is a yellowish pigment (e.g. fall leaves), and carotene is an orange pigment (e.g. carrots). Chlorophyll *b* is considered an accessory pigment in plants, broadening the spectrum that can be used in photosynthesis.

Leaves are the most conspicuous part of a plant. They vary tremendously in shape and size, and some large trees have more than 100,000 leaves. One of the major functions of a leaf is as a photosynthetic factory. The internal anatomy of a typical lead is complex. A waxy **cuticle** covers the upper side of the lead, and an **epidermis** completes the upper and lower layers of a typical leaf. Scattered primary throughout the lower epidermis are stomata (singular stoma), which are tiny openings regulated by **guard cells**. The stomata allow the carbon dioxide from the atmosphere to enter the leaf.

Chloroplasts reside within plant cells and serve as the organelles of photosynthesis. A chloroplast consists of two outer membranes that surround a semifluid matrix called the **stroma**. A third membrane system forms a series of flattened sacs called **thylakoids**. In some chloroplasts, the thylakoids become stacked, forming a **granum**. Pigment molecules embedded in the membrane of the thylakoids initiate photosynthesis. Sugars are synthesized in the stroma.

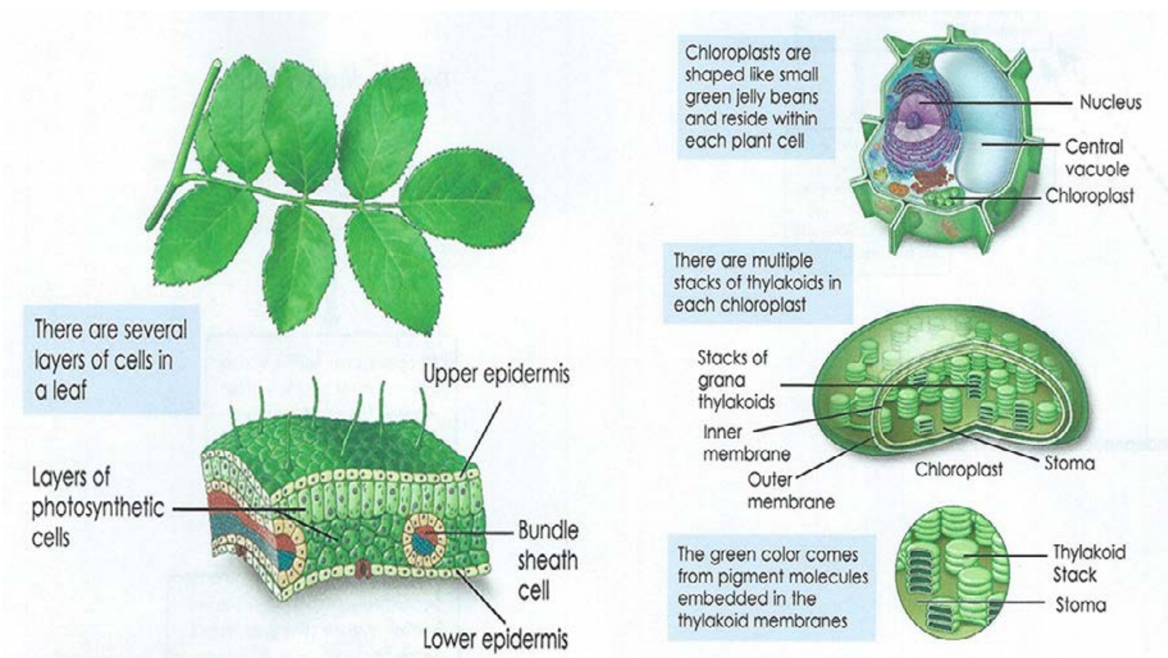

Figure 2. Leaf hierarchy

The overall reaction for photosynthesis is:

$$6CO_2 \quad + \quad 6H_2O \xrightarrow{\hspace{2em}} C_6H_{12}O_6 \quad + \quad 6O_2$$

Carbon dioxide Water Sunlight Glucose Oxygen

This reaction is the result of a series of chemical reactions that are controlled and carried out by specific enzymes. These reactions of photosynthesis are divided into two distinct metabolic pathways:

1. In the **light reaction**, or **light-dependent reactions**, the pigments chlorophyll absorbs light energy from sunlight and produces ATP, the coenzyme NADPH, and oxygen. The light-independent reaction takes place in the thylakoid membrane of the chloroplasts.
2. The **dark reaction**, also known as the **light-independent reaction** or **Calvin Cycle**, takes place in the stroma of the chloroplasts. It is responsible for the fixing of a carbohydrate (glucose).

Nutritionally, two types of organisms exist in our world, autotrophs and heterotrophs. **Autotrophs** (auto means self, troph means feeding) synthesize organic molecules (carbohydrates) from inorganic carbon dioxide. The vast majority of autotrophs are the photosynthetic organisms that you are familiar with – plants, as well as some protists and bacteria. These organisms use light energy to produce carbohydrates. Some bacteria produce their organic carbon compounds chemosynthetically, that is, using chemical energy. By contrast, **heterotrophs** must rely directly or indirectly on autotrophs for their nutritional carbon and metabolic energy. Hetertrophs include animals, fungi, many protists, and most bacteria.

The following experiments will give you a better understanding of the principles of photosynthesis.

Relationship Between Light and Photosynthesis Products

This experiment addresses the hypothesis that light is necessary for photosynthesis to proceed. For the analysis of this experiment, we will take advantage of the Lugol's test for the presence of starch compared to other carbohydrates, such as glucose.

Materials:

1. Sharpie
2. 1000mL beaker
   (filled with distilled H_2O)
3. Boiling beads (do not discard!)
4. Distilled water
5. Hot plate
6. Long forceps
7. 2 petri dishes
8. Hot plate
9. 1000mL
   (filled with ethanol & covered w/ foil)
10. Boiling beads
11. Lugol's solution
12. Light Source
13. Light-grown and dark-grown geranium plants

Procedures:

1. Observe the two geranium plants available. One plant has been growing on bright light for several hours. The other has been kept in the dark for a day or more. Both leaves have an area covered with a piece of foil paper.
2. Write a prediction regarding the presence of starch and the activity of photosynthesis for each condition in the following table.

Table 1. Predictions of the presence of starch and the activity of photosynthesis for two different geranium plant growing conditions.

Geranium Plant Growing Condition	Covered Area	Uncovered Area
Light-Grown Plant		
Dark-Grown Plant		

3. Select a leaf from one of the two geranium plants. **Pigment present in the plants must be removed** before a test for starch can be performed.
   a. Turn on the hot plate and set it to a high setting. Allow the water to come to a boil.
   b. With a sharpie, label on petri dish "light-grown plant" and the other "dark-grown plant."
   c. Remove a leaf from each condition and take it to your station.
   d. Remove the piece of foil paper from both leaves.
   e. Place both leaves in the beaker of boiling water for about a minute. This kills the tissue and breaks down internal membranes (cell wall, plasma membrane, and vacuolar membrane). Make sure to keep track of which leaf was from which growing condition throughout the experiment.

f. Remove the wilted leaves from the water with long forceps and place it on the petri dish.
g. Place the wilted leaves in the beaker of boiling alcohol and keep the beaker covered with foil. Let it sit for about a minute. *This will extract the photosynthetic pigments from the plant tissues*. When the pigments have been extracted, the solution will turn green, and the leaf will appear to be mostly bleached.
h. Remove the leaves from the alcohol with long forceps and dip it back into the boiling water for about 15 seconds to rehydrate the leaves and remove excess alcohol.

4. Test the plant for the presence of starch
    a. Place the processed leaves in two separate petri dishes and pour 2mL of Iodine (Lugol's) solution on top of the leaves.
    b. Let it soak in the iodine solution for about two minutes. Rinse the leaves and petri dishes with water to remove the iodine solution in order to observe the pattern of staining.
    c. Record your results in the given table.

Table 2. The presence of starch and the activity of photosynthesis for two different geranium plant growing conditions.

Geranium Plant Growing Condition	Covered Area	Uncovered Area
Light-Grown Plant		
Dark-Grown Plant		

Question: What does the blue-black coloration of the leaf show?

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Question: Why did the covered area fail to stain?

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Carbon Dioxide Uptake During Photosynthesis

Recall that plants require carbon dioxide to produce carbohydrates. To detect the uptake of carbon dioxide by the plant, you will use a **pH indicator** solution. An indicator is a molecule that changes color depending on pH. In this experiment, we will be using a **phenol red** solution. Phenol red turns **yellow** when adding carbon dioxide to the solution, which makes the solution acidic (pH < 7.0). When phenol red turn **red**, there is a decreased amount of carbon dioxide in the solution, which makes the solution basic (pH >7.0).

In order to see this reaction take place, we will blow through a straw into a solution of phenol red and water to provide our plant with a carbon dioxide source. This results in the following chemical reaction:

$$CO_{2(g)} + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

Carbon Dioxide Water Carbonic Acid Hydrogen Ion Bicarbonate

Materials:

1. 2 large test tubes
2. Test tube rack
3. Lamp
4. 400mL beaker
5. Straw
6. 10-cm of *Elodea*

Procedures:

1. The instructor will have a volunteer from the class assist in the setup of this experiment (one setup per class, placed at the instructor's bench)
2. Fill one test tube with the phenol red solution. This test tube will serve as a control.
3. With a straw, blow into the beaker with the remaining phenol red solution until it turns yellow. This introduces CO_2 to the solution.
4. Transfer the yellow phenol red solution into the second test tube.
5. Transfer a 10 cm strip of *Elodea* into the second test tube.
6. Place both test tubes in front of a bright light for 60-90 minutes. This will ensure that the plant has enough light energy, and ultimately ATP, necessary for the light-independent reaction.

Question: Has the color in either test tube changed? If so, why? Explain.

Question: What is carbon fixation? How is carbon fixation related to this experiment?

OVERVIEW – CELLULAR RESPIRATION

Life is a conquest of energy that all begins with the sun. The radiant energy of sunlight is converted into chemical energy (primarily glucose) through photosynthesis. In turn, the glucose is used to produce **adenosine triphosphate (ATP)**, the energy currency of living systems, through the process of cellular respiration. Plants and animals have a unique evolutionary relationship based upon each using the other's products. As the results of photosynthesis, some bacteria algae, and plants produce oxygen as a waste product. In turn, the oxygen is a vital component in aerobic cellular respiration. The plants then use the CO_2 produced in cellular respiration to ultimately build carbohydrates.

In nature, two major types of cellular respiration have evolved.

1. Some bacteria and fungi undergo **anaerobic cellular respiration**, which occurs in the absence of oxygen. The ancestors of anaerobic organisms first appeared on Earth approximately 3.5 billion years ago. Today, many species of anaerobic organisms abound. Anaerobic bacteria can be found in certain soils, sediments in bodies of water and the gut of some animals. Several species of anaerobic bacteria are responsible for diseases such as gangrene, tetanus, and botulinism.
2. **Aerobic cellular respiration** takes place in the presence of oxygen. The evolution of aerobic cellular respiration began approximately 2.7 billion years ago, and the vast majority of organisms on Earth today are aerobic. The overall reaction of aerobic cellular respiration is the reverse of photosynthesis:

$$\underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Oxygen}}{6O_2} \longrightarrow \underset{\text{Carbon dioxide}}{6CO_2} + \underset{\text{Water}}{6H_2O} + \underset{\text{Chemical Energy}}{ATP}$$

Anaerobic and aerobic respiration both begin with a molecule of glucose produced through photosynthesis. With a few exceptions, the majority of living things undergo glucose metabolism to release the energy from the sun locked within a molecule of glucose. The first step in unlocking the energy within glucose is **glycolysis**. This complex pathway occurs anaerobically in the cytoplasm of a cell. Because glycolysis is common to life on Earth, it arose early in the evolution of life.

The end products of glycolysis are two molecules of ATP, two molecules of NADH, and two molecules of pyruvate. If pyruvate is metabolized in the cytoplasm anaerobically, the process is known as **fermentation**. If the pyruvate is shuttled into the mitochondrion, the process continued aerobically and is known as aerobic cellular respiration.

Two significant types of fermentation reactions occur anaerobically: the **alcoholic fermentation** reaction and **lactate fermentation** reaction. In both reactions, pyruvate is reduced by NADH to form either ethyl alcohol (C_2H_5OH) or lactate ($C_3H_5O_3$). Both reactions produce two molecules of water and only two molecules of ATP. Combined with the two ATPs produced from glycolysis, the net yield of ATP during each fermentation reaction is only four molecules of ATP. Thus, anaerobic organisms have no “energy to spare.” That explains why anaerobic life forms cannot engage in a game of tennis or even “putt” around like a paramecium. Other types of commercially important microbial fermentation process yield acetone and methanol.

Alcoholic Fermentation

Alcoholic fermentation is essential in making wine, beer, and bread. In making bread, the CO_2 produced causes the bread dough to rise. The ethyl alcohol evaporates during baking. Have you ever eaten a slice of pizza or a piece of bread that smells a little of beer? The beer odor is a bit of residual alcohol that has not completely evaporated!

Materials:

1. Half package of yeast
2. 25 mL of 10% glucose
3. Water
4. 250 mL beaker
5. Empty glass bottle (flask)
6. Stirring rod
7. Glass dropper
8. Microscope
9. Microscope slides and coverslips
10. Balloon
11. Hot plate
12. Thermometer
13. Rubber band

Procedures:

1. Obtain the materials to be used in this activity and bring them to your lab station.
2. Add 200mL of tap water to the beaker. Using the hot plate, heat the water to approximately 35°C to 37°C.
3. Carefully add the half package of yeast and the 25mL of 10% glucose to the water and stir. Wash your hands after handling the yeast.
4. Carefully pour the mixture into the flask to approximately the halfway mark.
5. Cover the lip of the flask with a balloon and secure the base of the balloon to the flask with a rubber band.
6. Observe and record what happens to the balloon over the next 30 minutes.
7. Using a glass dropper, take a drop of the mixture from the flask.
8. Prepare a wet mount of this mixture.
9. Observe the mixture using a microscope on low and high-dry power.
10. At the end of 30 minutes, remove the balloon from the glass bottle and waft your hand over the bottle. Smell the contents.
11. Clean your laboratory station, glassware, and hands.
12. Return your materials to the designated area.

13. Sketch your observations in the space below.

Yeast

Question: What happened to the balloon? Why?

Question: Describe the odor of the mixture.

Aerobic Cellular Respiration

Both autotrophs and hetertrophs undergo respiration. Photoautotrophs such as plants utilize the carbohydrates they have produced by photosynthesis to build new cells and maintain cellular machinery. Hetertrophic organisms may obtain materials for respiration in two ways: by digesting plant material or by digesting the tissue of animals that have previously digested plants.

During aerobic respiration, the carbohydrates undergo a series of oxidation-reduction reactions (**redox reactions**). Whenever one substance is **oxidized** (loses electrons), another must be **reduced** (accept or gain those electrons). The **final electron acceptor** in aerobic respiration is oxygen. Tagging along with the electrons as they pass through the electron transport chain are protons (H^+). When the electrons and protons are captured by oxygen, water is formed.

In the following experiment, you will examine aerobic respiration in three sets of seeds.

Seeds contain stored food material, usually in the form of some type of carbohydrates. When a seed germinates, the carbohydrate is broken down by aerobic respiration, liberating the energy (ATP) required for each embryo to grow into a seedling. Two days ago, one set of dry pea seeds were soaked in water to start the germination process. Another set was not soaked (ungerminated) and the last set was boiled. In this experiment, you will compare carbon dioxide production between germinating pea seeds, germinating pea seeds that have been boiled, and ungerminated (dry) pea seeds.

This experiment investigates the hypothesis that germinating seeds produce carbon dioxide from aerobic respiration.

Materials:

1. 3 flasks (labeled germinating, germinating-boiled, and ungerminated).
2. 3 large test tubes (filled with water)
3. 3 large test tubes (filled with phenol red solution)
4. Test tube rack
5. 2 L plastic beaker or container (filled with tap water)
6. Pea seeds (germinated, germinated-boiled, ungerminated)

Procedures:

1. The instructor will have a volunteer from the class assist in the setup of this experiment (one setup per class, placed at the instructor's bench)
2. Collect the appropriate pea seeds and transfer them into the corresponding flask.
3. Immediately insert the large rubber stopper into the glask. Be sure that both the small and large rubber stoppers are placed correctly, so no air can enter or escape the flasks.
4. Insert the glass tubing inside the large test tube that is filled with water as shown in the figure below. This keeps gases from escaping the flask.

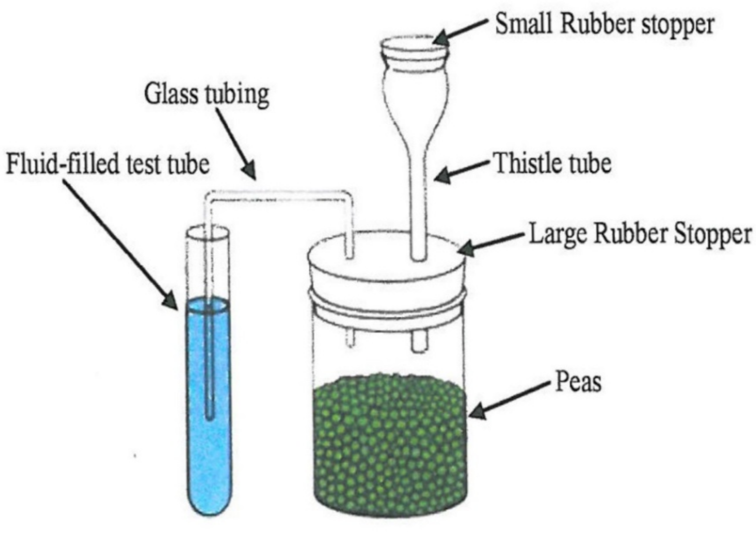

Figure 8.1 Respiration bottle apparatus

5. Set the three respiratory flask apparatus aside for 90 minutes.
6. Make a prediction about carbon dioxide production in each respiratory flask apparatus in the table below.

Table 3. Prediction of aerobic respiration (CO_2 production) of pea seeds

Pea Seeds	Indicator Color? (pink or yellow)	CO_2 production? (CO_2 present or absent)
Germinating		
Germinating-boiled		
Ungerminated (dry)		

7. After 90 minutes, replace the test tubes that are filled with water with the test tubes filled with phenol red.
   a. Phenol red solution, which should appear pink in the stock bottle, will be used to test for the presence of CO_2 within the respiratory flask apparatus. If CO_2 is bubbled through water, carbonic acid forms, causing the solution to turn acidic, and therefore yellow.

$$CO_{2(g)} + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

Carbon Dioxide Water Carbonic Acid Hydrogen Ion Bicarbonate

8. Remove the small rubber stopper on the top of the thistle tube and slowly pour water from the bucket into each thistle tube. The water will force out gases present in the flasks into the phenol red solution. If CO_2 is present, the phenol red solution will turn yellow.
9. Record your observations in the table below.

Table 4. Pea seeds results

Pea Seeds	Indicator Color? (pink or yellow)	CO_2 production? (CO_2 present or absent)
Germinating		
Germinating-boiled		
Ungerminated (dry)		

Question: Which set(s) of seeds produced CO_2 as a result of aerobic respiration? Why?

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Question: Explain the results observed from the germinating-boiled seeds. What does boiling do to the seeds?

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Question: Write a conclusion, accepting or rejecting the hypothesis.

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