**Name:**

**Lab Partner(s):**

Photosynthesis in Leaf Discs

# I. Introduction:

## Background

Photosynthesis is when “the chloroplasts in plants and other photosynthetic organisms capture light energy that has travelled 150 million kilometers from the sun and convert it to chemical energy stored in sugar and other organic molecules” (Reece 185). This process is how all life is sustained. Plants and other autotrophs, or organisms that produce their own food, are at the bottom of the food chain for they do not eat other organisms. In any level of the food chain the source of the food can be traced back to autotrophs. Therefore, photosynthesis is vital to all life (185-186).

The process of converting the sun’s energy and carbon dioxide ($CO_2$) and water ($H_2O$) into glucose ($C_6H_{12}O_6$) and oxygen ($O_2$) is complex and occurs in the chloroplast of plant cells. In leaves there are many layers of cells, one of which is the mesophyll layer. This layer contains carbons dioxide and oxygen gas. The gas located in this part of the leaf is what makes leaves buoyant when placed in water (Goodwin). In mesophyll cells there are chloroplasts. The anatomy of a chloroplast is vital to its function. The stroma or the “dense fluid” (Reece 187) that is inside the two membranes of the chloroplast holds the the thylakoids in place. Thylakoids are membranes that separate the thylakoid space from the stroma. Many thylakoids stacked are called a granum. The thylakoid membranes hold chlorophyll, “the green pigment that gives leaves their color” (187).

There are two types of chlorophyll in the thylakoid space, chlorophyll *a*, “the key light-capturing pigment that participates directly in the light reaction” (191) and chlorophyll *b*, an “accessory pigment” (191). Plants are green due to the chlorophyll in them. Chlorophyll *a*, especially, absorbs blue-violet, and red lights the best and green is absorbed the least which results in its reflection. Chlorophyll *b* adds to the spectrum of absorption (191-192).

The process of photosynthesis involves two subprocesses, the light reaction and the Calvin cycle. The light reaction is a process in which ATP and NADPH are synthesized. There are two photosystems that are vital to the linear electron flow of the light reaction. Photosystem II comes before Photosystem I. (195). The reaction begins with a photon of light striking “one of the pigment molecules in a light-harvesting complex of PS II, boosting one if it electrons to a higher energy level” (195). The energy that is passed among the molecules eventually reaches P680, a pair of chlorophyll *a* molecules which transport the electron to the primary electron acceptor. A water molecule is also split two $H^+$ ions, two electrons and one oxygen atom. The electrons travel through

P680 and also to the primary electron acceptor. The $H^+$ molecules are released into the thylakoid space and the oxygen atom joins with another that was created in the split of a different $H_2O$ molecule. The oxygen, a product of photosynthesis, is released into the environment at this point (195-196).

The electrons collected by the primary acceptor are then passed along an electron transport chain which synthesises ATP from ADP and a phosphate. This process is similar to that of cellular respiration and through the concentration of $H^+$ molecules, chemiosmosis generates a gradient that creates ATP. The electrons pass through the chain and are accepted in PS I by another pair of chlorophyll *a* molecules called P700. As this occurs a photon strikes the pigment molecules in PS I and the energy is passed among the molecules until it reaches P700. The electron is then passed up to the primary acceptor which turns P700 into $P700^+$. The pair is ready to accept more electrons and that is where the electrons from the electron transport chain are accepted and are again passed on. At this point the electrons travel through another electron transport chain but ATP is not synthesized. The electrons pass through ferredoxin, a protein known as Fd, and “the enzyme $NADP^+$ reductase catalyzes that transfer of electrons from Fd to $NADP^+$” (196). It takes two electrons to make NADPH and removes one $H^+$ from the stroma. The ATP and NADPH both travel from the light reaction to the Calvin cycle (195-196).

The Calvin cycle is the second process in photosynthesis. This process “is anabolic” meaning that it builds “carbohydrates from smaller molecules” (199) and takes in energy to do so. There are three steps to the Calvin cycle and the first is carbon fixation. A molecule of carbon dioxide ($CO_2$) is taken n and attached to ribulose bisphosphate or RuBP. This synthesis is catalyzed through the protein rubisco. The six carbon product is unstable and therefore breaks into two, 3 carbon molecules known as 3-phosphoglycerate. Two of the 3-phosphoglycerate are created with each $CO_2$ molecule (199).

In reduction, the second step of the Calvin cycle, 3-phosphoglycerate becomes 1,3-biphosphoglycerate due to a phosphate joining from an ATP molecule. The molecule is then reduced by NADPH which results in the loss of a phosphate which creates glyceraldehyde 3-phosphate. There are 6 of these molecules present after 3 $CO_2$ molecules are used and one glyceraldehyde 3-phosphate or G3P is released as the other 5 continue to the phase of regeneration. The G3P molecule is a vital sugar. 1 G3P make from 3 $CO_2$ molecules and two G3P molecules make 1 glucose molecule (199-200).

In the regeneration of RuBP the 5 molecules of G3P are rearranged to create 3 molecules of RuBP. In this rearrangement 3 ATPs are used but the cycle is again ready to accept more $CO_2$ molecules and begin again (200). The glucose produced by photosynthesis is a sugar that is easily usable by the cell. It is made in plants but is consumed by all organisms and is broken down into energy by cellular respiration.

Like all plants, baby spinach leaves have chloroplasts in their cells which conduct photosynthesis. The two reactions, the light reaction and the Calvin cycle depend on each other. The NADPH and ATP produced by the light reaction are used by the Calvin cycle and are given back to the light reaction as $NADP^+$ and ADP. The two phases work in a cycle. When baby spinach has its air sucked from it through the vacuum created by the syringe, the gases leave the mesophyll area and are replaced with the solution. The soap in the solution allows for the hydrophobic exterior of the leaf to be broken down in order for the solution to enter the leaf ("Investigation 5: Photosynthesis"). If the aqueous solution has carbon dioxide, like baking soda ("Sodium Bicarbonate") does than the leaves will have the products needed to perform photosynthesis and replenish the gases that allow them to float. The color of the light allows for certain amounts of energy to be consumed by the chlorophyll. If the light was all green very little to no energy would be absorbed while if the light was blue-violet or red, there would be plenty of energy to absorb. White light is all the colors on the color spectrum and as seen with the action spectrum for photosynthesis, green light provides the least amount of energy and therefore there is the least amount of photosynthesis occurring (192).

### Objectives:

The objectives of the lab were to not only explore the factors that affect photosynthesis but also to relate the parts of the chloroplast to the functions they serve. For example the chlorophyll in the thylakoid space was examined through the light and the air held in the mesophyll was tampered with to see the results. The overall goal was to further understand photosynthesis.

## Research Question and Hypothesis:

### Research Question:

How does the addition of sodium bicarbonate impact the rate of photosynthesis in spinach leaf discs?

### Hypothesis & Justification:

The leaf discs will rise at a faster rate if in a solution with sodium bicarbonate because it adds $CO_2$ to the environment. Photosynthesis uses carbon dioxide as a reactant in the Calvin cycle and therefore without it there will be no glucose produced. The purpose of photosynthesis is to synthesize glucose and without it or the Calvin cycle there would be no photosynthesis.

### Independent and Dependent Variable Identification:

**Independent:** Concentration of Sodium Bicarbonate

**Dependent:** Number of spinach discs rising over the course of 20 minutes

### Research Question:

How does blue light effect the rate of photosynthesis?

Hypothesis & Justification:

The leaf discs will rise at a faster rate if under blue light. Blue light is ideal for plants because chlorophyll does not take in green light. White light has green in it but with blue light all of the energy can be taken in by the plant and therefore the rate will be higher when the plant is under a blue light.

### Independent and Dependent Variable Identification:

**Independent:** Color of the light source

**Dependent:** Number of spinach discs rising over the course of 20 minutes

# II. Methods:

## Materials:

- Baking soda ($NaHCO_3$) (sodium bicarbonate)
- Liquid soap
- Plastic syringes
- Plastic Pipette
- Leaves (spinach)
- Hole punch
- 2 Glass Beakers (500 mL)
- 1 Glass Beaker (100 mL)
- Timer
- White Light Source
- Blue Light Source
- Deionized water
- Glass stirrer
- Large Beaker (800 mL)

## Procedure:

Baby Spinach leaves were collected and using a hole punch, 20 discs were punched out of the leaf from in between the veins on the leaf. While this was being done, a group member prepared a solution. For the solution, 300mL of deionized water was measured out into the large glass beaker and a pinch of baking soda was added. The pinch was about 3 grams so that the ratio of 100 mL of water to 1g of baking soda was followed. In a smaller glass beaker, a diluted solution was made. It consisted of 70 mL of deionized water that was added to the glass and was followed by the addition of 3 drops of dish soap. The solution was carefully combined to avoid the creation of suds. One drop of the soap solution was drawn up using a plastic pipette and one drop was dispensed into the 300mL bicarbonate solution. This was then swirled carefully using the glass stirrer to avoid the creation of bubbles. Then, 10 of the 20 leaf discs were placed into the syringe after the plunger had been removed. After the leaves were added to the tube the plunger was replaced and a small amount of the soap and bicarbonate solution was drawn up. The air was then pushed out of the tubes being careful not to crush the leaves in the process. A vacuum was then created by placing a finger on the tip of the syringe to block air from entering. The plunger was pulled in order to pull air out of the leaves. The vacuum was then released so that the solution entered the discs. The disks then sunk and the process was repeated until all the disks had fallen. When the disks became stuck to the sides of the barrel, the side of the syringe was tapped to dislodge them. After all the leaves sunk the plunger was removed and the disks and the solution was added back into the large beaker with the soap and bicarbonate solution. At this point all the disks should have fallen to the bottom of the beaker and if they did not they were placed again in the syringe and the same process was repeated until it was certain that the disc fell to the bottom. A large beaker with water was then placed in between the light source and the bicarbonate and soap solution to prevent the extreme heat from reaching the leaves. A timer was started for 20 minutes and the number of leaves that floated was recorded at one minute intervals for 20 minutes or until all the leaves floated. The solution was occasionally stirred to dislodge disks that were stuck to each other or the bottom of the beaker. After 20 minutes the timer was stopped and the beaker was placed to the side. Another bicarbonate solution was made in a large beaker but the soap was admitted. The syringe was cleaned with DI water and then the same process of creating a vacuum was performed with the 10 leaf discs that were placed to the side. This was set up with the glass of water in between the beaker and the light source and ran for 20 minutes on a timer with the leaves floating being recorded every minute or until all 10 were floating.

Following the end of the test without the addition of baking soda, the original test was ran again. 10 leaf discs were punched out of a piece of baby spinach and then were manipulated with the vacuum test. The same procedure involving the plastic syringe was followed with this test. The fallen disks were then added to the original large beaker of the bicarbonate and soap solution. The original leaves were removed before the addition of the 10 new. The light source was changed to a blue light and the large water beaker was placed in between the light and the solution to protect the leaves from extreme heat. The experiment ran for 20 minutes with recordings being taken every minute.

**Bicarbonate and Soap with White Light: vs Water with White Light**

Constants: the type of plant, the materials, the time tested, the light source

Control: spinach leaves in plain water

**Bicarbonate and Soap with Blue Light vs Bicarbonate and Soap with White Light:**

Constants: the type of plant, the materials, the time tested, the solution

Control: the spinach disks in a bicarbonate and soap solution under white light

# III. Results:

## Quantitative Data:

**Table 1:**

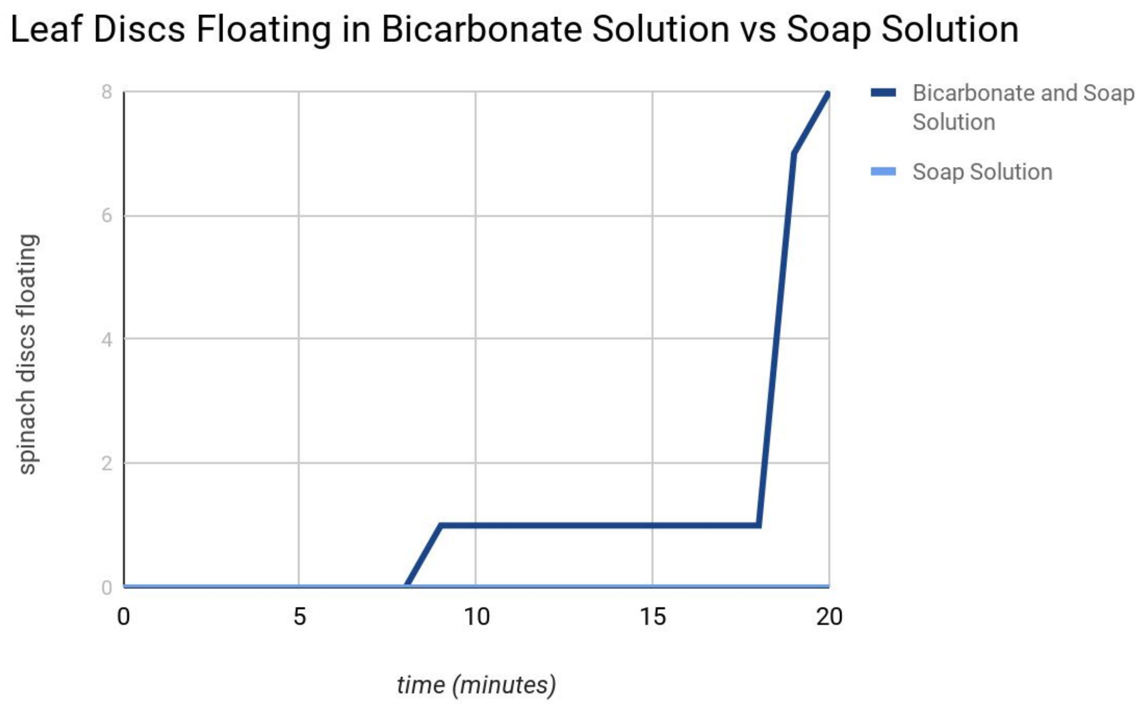
| Time (minutes) | # of leaves floating in solution with baking soda and soap | # of leaves floating in water |
|---|---|---|
| 0 | 0 | 0 |
| 1 | 0 | 0 |
| 2 | 0 | 0 |
| 3 | 0 | 0 |
| 4 | 0 | 0 |
| 5 | 0 | 0 |
| 6 | 0 | 0 |
| 7 | 0 | 0 |
| 8 | 0 | 0 |
| 9 | 1 | 0 |
| 10 | 1 | 0 |
| 11 | 1 | 0 |

| | | |
|---|---|---|
| 12 | 1 | 0 |
| 13 | 1 | 0 |
| 14 | 1* | 0 |
| 15 | 1* | 0 |
| 16 | 1* | 0 |
| 17 | 1* | 0 |
| 18 | 1* | 0 |
| 19 | 7 | 0 |
| 20 | 8 | 0 |

Number of spinach discs floating in a baking soda and soap solution vs the number of spinach discs floating in a plain water solution over the course of 20 minutes.

*numbers that affected by the fire drill and lack of data taken in that time

**Figure 1:**




Number of leaf discs floating in a bicarbonate and soap solution vs the number of leaf discs floating in water throughout 20 minutes

**Table 2:**

| Time (minutes) | # of leaves floating with blue light | # of leaves floating with white light |
|---|---|---|
| 0 | 0 | 0 |
| 1 | 0 | 0 |
| 2 | 0 | 0 |
| 3 | 0 | 0 |
| 4 | 0 | 0 |
| 5 | 0 | 0 |
| 6 | 0 | 0 |
| 7 | 0 | 0 |
| 8 | 0 | 0 |

| | | |
|---|---|---|
| 9 | 0 | 1 |
| 10 | 0 | 1 |
| 11 | 0 | 1 |
| 12 | 0 | 1 |
| 13 | 0 | 1 |
| 14 | 0 | 1* |
| 15 | 0 | 1* |
| 16 | 0 | 1* |
| 17 | 0 | 1* |
| 18 | 0 | 1* |
| 19 | 1 | 7 |
| 20 | 1 | 8 |

Number of leaf discs floating under a blue light vs a white light in a bicarbonate and soap solution for 20 minutes

*numbers that affected by the fire drill and lack of data taken in that time

**Figure 2:**

Number of leaf discs floating under a blue light in a bicarbonate and soap solution vs the number of leaf discs floating under a white light in a bicarbonate and soap solution over 20 minutes

### Calculations & Statistical Analysis:

Slope Formula= $\frac{y_2 - y_1}{x_2 - x_1}$

**Line of best fit:**

**Leaf Disks in Bicarbonate and Soap Solution under white light:**

$\frac{8-0}{20-0} = \frac{8}{20} = 0.4$ leaves per minute

$y = 0.4x$

**Leaf Disks in Water under white light:**

$\frac{0-0}{20-0} = \frac{0}{20} = 0$ leaves per minute

$y = 0$

**Leaf Disks in Bicarbonate and Soap Solution under blue light:**

$\frac{1-0}{20-0} = \frac{1}{20} = 0.05$ leaves per minute

$y = 0.05x$

# IV. Discussion:

### Conclusions:

Autotrophs need carbon dioxide in order to perform photosynthesis. In the first test, the test regarding baking soda or carbon dioxide, the spinach disks required the carbon dioxide in order to perform photosynthesis. Without baking soda the leaves had no gasses in their mesophyll. The vacuum had sucked out all of the air and replaced it with water. The lack of carbon dioxide prevented the Calvin cycle from supplying the light reaction with $NADP^+$ and ADP. Without one half of photosynthesis working, the entire process shuts down. The Calvin cycle begins with the input of $CO_2$ and without that vital molecule no glucose can be made. The Calvin cycle consumes the NADPH and ATP produced by the light reaction. The Calvin cycle uses the electrons from the molecules to rearrange the carbon structure. Without the molecules available with electrons the Calvin cycle cannot create glucose. When the spinach disks were placed in water the rate of disks floating per minute was 0. When the spinach disks were in a sodium bicarbonate solution they rose at a rate of 0.4 disks per minute. The difference after twenty minutes was 8 disks in the presence of $CO_2$ and 0 disks with lacking it. As the disks in the bicarbonate solution performed photosynthesis the oxygen built up in the mesophyll which lowered the density of the disk. After enough oxygen built up the disk was able to float.

In the presence of different light sources, white light works better than blue light. Both tests involved disks in an environment with carbon dioxide and therefore photosynthesis ran. The light did impact the rate of photosynthesis despite the availability of reactants because of the energy it provides. The light provides the chlorophyll in the photosystems with energy to transfer electrons. Chlorophyll absorbs colors best on the extremes of the spectrum such as purple and red. The white light is comprised of all the colors. The chlorophyll takes in energy from all but green and therefore reflects the green color that spinach is. The energy available to the plant is greater under white light. The rate of reaction was 0.4 disks per minute. While the blue light could all be absorbed by the plant because it was not green, it did not carry enough energy to solely power the light reaction at the same rate as the culmination of many colors. The rate of reaction was 0.05 disks per minute. It was higher than the disks that lacked baking soda but that was due to a lack of reactants. After 20 minutes there were 8 disks floating in the bicarbonate solution under the white light and 1 under the blue light.

In nature the sun provides white light. It is the natural energy that plants need in order to produce their own sugars. The sun provides every color like the white light and therefore plants have optimal rates in their own environments. Through evolution the autotrophic organisms have adapted to receive optimal energy from what is provided. The air is also filled with carbon dioxide. It is a product of cellular respiration in all organisms. The ideal environment in the tests proved to be the leaves in a solution that held both water and carbon dioxide and was in the presence of white light.

## Experimental Evaluation:

This lab was impacted by the fire alarm. In the middle of the first test at about 14 minutes in the fire alarm rang and the school was evacuated. The data was unable to be collected during the time the school was vacant. During this period of about 5 minutes the leaves went without stirring and therefore some were stuck to the bottom and would have risen at an earlier time.

If this lab was to be performed again, it should include different tests with different colored lights. With more time the amount of baking soda could also have become a variable. Further testing the light would provide an action spectrum for photosynthesis. The variation of baking soda could have proved whether photosynthesis occurs at a fixed rate, has a peak rate, or is directly proportional in rate with the amount of reactants available. The tests that were performed did open up the concept of photosynthesis but there are still unanswered questions that could be explored through furthering the lab.

## V. References:

Works Cited

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