Separation of Pigments by Paper Chromatography and the Effects of Light on Respiration and Photosynthesis in *Spinacia Oleracea*

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**Introduction**

Plants and specific types of bacteria gain their energy via a process known as photosynthesi*s.* The equation of photosynthesis is; 6CO2 + 6H2O + light energy → C6H12O6 + 6O2 (Simms, 2017). In order for this process to occur, there must also be pigment molecules present to absorb photons of light (Simms, 2017). Photosynthetically active radiation, also known as PAR, is the exact range of wavelengths that an organism is able to utilize in order to execute photosynthesis (Simms, 2017). According to Simms (2017), most photosynthetic organisms have a PAR ranging from 400 to 700 nanometers. PAR can be affected by environmental and seasonal conditions. The wavelengths of light that are absorbed and reflected by the organism are determined by the pigments present in the organism (Simms, 2017).

According to Simms (2017), *Spinacia Oleracea* is known to contain chlorophyll *a* and *b* pigments. Simms (2017) notes that electrons that are in chlorophyll *a* and *b* are “excited” after light has been absorbed, so they move to a higher energy level. Simultaneously, photolysis is occurring which causes oxygen to be released from the *Spinacia Oleracea* as well as two electrons and two protons. The energized electrons continue to go through several “electron acceptors in the thylakoid membrane of the chloroplast” (Simms, 2017) as adenosine triphosphate (ATP) s created and nicotinamide adenine dinucleotide phosphate, also known as NADP+, is converted to NADPH. This is the first stage of photosynthesis and is considered a light dependent reaction (Simms, 2017). This stage also allows glucose (C6H12O6) to be produced by utilizing both ATP and carbon dioxide, also known as CO2, from the atmosphere (Simms, 2017).

In contrast, Simms (2017) writes that cellular respiration is close to the complete opposite of photosynthesis: C6H12O6 + 6O2 → 6CO2 + 6H2O + energy. Cellular respiration is a process that is used by all living organisms in order to catabolize glucose in order to release ATP, which is one of the main sources of energy in a living organism (Simms, 2017). First, glycolysis occurs, which is the process of all 6-carbon glucoses being split directly in half to form two 3-carbon pyruvate molecules (Simms, 2017). The two pyruvate molecules are then moved into the mitochondria which is where a CO2 molecule is removed from both molecules. This process, named pyruvate oxidation, does not produce ATP (Simms, 2017). However, NAD, which is the electron carrier, is reduced to NADH (Simms, 2017). Following this step, the acetyl CoA molecules that were formed after CO2 molecules were initially removed enters the Krebs cycle. In this cycle, each acetyl CoA molecule binds with a 4-carbon oxaloacetate in order to create a 6-carbon sugar citrate (Simms, 2017). Over time, this citrate is converted back to oxaloacetate which releases 2 molecules of CO2 as well as 3 NADH, 2 FADH2, and 1 ATP per Acetyl CoA (Simms, 2017).

*Experiment 1: Separation of plant pigments in Spinacia Oleracea by paper chromatography*

The goal of the first experiment was to examine the separation of plant pigments in *Spinacia Oleracea* by paper chromatography. This experiment was used to show the diverse pigments present in a sample of *Spinacia Oleracea* by separating each pigment from the next. The hypothesis for experiment one was that chlorophyll would be present in the sample of *Spinacia Oleracea* because all plants have chloroplasts which contain the chlorophyll pigment. If chlorophyll *a* and/or *b* was present in the sample, then a strip of green and/or blue green would appear on the paper. Additionally, the calculated Rf value for those pigments would be similar to the calculated Rf created by the controls for chlorophyll *a* and *b* (made by lab instructor). The Rf value represents how much each pigment sticks to the chromatography paper (Simms, 2017). A low Rf value means the pigment is more polar and moves slowly through the paper because it absorbs well. In contrast, a high Rf value means the pigment is less polar and will move quickly through the paper. There were not any independent variables for this experiment since nothing was manipulated to show a relationship. Instead, this experiment was used to determine a measurement and pigment types. The dependent experiment was how far each pigment moved from the origin as well as what pigments showed up. The control variables included temperature, light exposure, appropriate pigmented controls (determined by lab instructor), time, and paper type.

*Experiment 2: Determining the rate of respiration and photosynthesis in Spinacia Oleracea under varying light conditions*

The goal of the second experiment was to determine the rate of respiration and photosynthesis in *Spinacia Oleracea* under varying light conditions. The hypothesis for this experiment was that the rate of O2 production and CO2 consumption would be the greatest under strongest white light conditions and weakest under dark/black light conditions because white light allows for the plant to absorb light energy thus powering the process of photosynthesis similar to what happens when plants are exposed to sunlight. If the sample of *Spinacia Oleracea* was put under the greatest white light conditions, then there would be a high rate of CO2 production and O2 consumption. The independent variables of this experiment included the wattage and color of light. The dependent variables of this experiment included the rate of consumption/production of CO2 and O2. Control variables for this experiment were measuring time of CO2 and O2 production/consumption, type of species used, and equilibration time.

**Materials and Methods**

*Experiment 1*

For the first experiment, 1*Spinacia Olercea* sample which was one Spinach leaf was added to a mortar to be grinded. 5 milliliters of 80% acetone was added while grinding continued. Then, a dot was drawn on a piece of chromatography paper 3 centimeters from the end of the paper, and the plant extract solution was painted over the mark 15 times allowing time to dry in between each paint stroke. The chromatography paper was inserted into 10 milliliters of chromatography solvent with the painted end towards but not touching the solution. It was left to sit while pigments and solvent moved up the piece of paper. Once the solvent reaches the top, the strip of paper was removed, and the solvent front was marked with a pencil. The solvent front is where the solvent ended up reaching on the chromatography paper. Once the strip dried completely, distance from the initial plant stripe to the pigment was measured, and the Rf was calculated by dividing the distance moved by the pigment from origin by the distance from pigment origin to solvent front. Based on the colors present on the chromatography paper, the pigments present in the sample of *Spinacia Olercea* were determined.

*Experiment 2*

*White light vs. dark light*

LabQuest and Logger Pro software were used in conjunction in order to measure O2 and CO2 levels in a small chamber. First, the default duration time was set to 600 seconds in order to ensure each test would collect CO2 levels and O2 levels for the same amount of time. 4 medium sized Spinach leaves were placed into the chamber. Then, the O2 gas sensor was placed into the chamber vertically, and the CO2 gas sensor was placed in the same chamber horizontally. The chamber was first placed under dark light conditions for four minutes in order to equilibrate. Next, CO2 and O2 levels were collected for a total of 600 seconds (10 minutes). A graph was created for both CO2 level and O2 level changes throughout the duration of 600 seconds. A linear regression line was calculated using the LoggerPro software, which provided the slope (rate of change) for each graph. The leaves and gas sensors were removed from the chamber in order to prepare for a new data collection. The same Spinach leaves were used but a clean chamber was used for the next data collection. Data was collected after the chamber was placed over a 9 wattage white light for a 4-minute equilibration time. Data collection time was 600 seconds again. The slopes of both levels of gasses were recorded in Table 1.1. The exact values of O2 and CO2 in the chamber were recorded every 50 seconds in Figure 1.1, 1.2, 1.3, and 1.4

*White light vs. black light*

For this portion of the experiment, new Spinach leaves were used. The exact same steps were repeated for this part of the experiment except, first the chamber of Spinach leaves and gas sensors was placed over a 7 watt white light and then a 7 watt black light. The slopes of both levels of gasses were recorded in Table 1.2. The exact values of O2 and CO2 in the chamber were recorded every 50 seconds in Figure 1.5, 1.6, 1.7, and 1.8.

*White light vs. white light*

Again, the exact same steps were repeated with more fresh Spinach leaves for this part of the experiment except the chamber of Spinach leaves and gas sensors were placed over a 9 watt white light and then a 7 watt white light. The slopes of both levels of gasses were recorded in Table 1.3. The exact values of O2 and CO2 in the chamber were recorded every 50 seconds in Figure 1.9, 1.10, 1.11, and 1.12.

**Results**

*Experiment 1*

The results for experiment 1 were surprising as a total of 5 pigments showed up as being present in the sample of *Spinacia Olercea.* The colors that were present included a yellow-green, green, blue-green, orange, and yellow. In order of the listed colors, the corresponding pigments are xanthophyll, chlorophylls *a* and carotene, and lutein. The corresponding Rf values were 0.192, 0.277, 0.346,0.485, and 1.0.

Table 1.1 Effect of dark light vs. white light on CO2 and O2 rate of production/consumption

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Treatment | Light Bulb (watts) | | Equilibration Time (mins) | CO2 – Rate of  Production/  Consumption  (ppt/sec) | O2 – Rate of Production/  Consumption (ppt/sec) |
| Dark | | N/A | 4 | 0.0005046 | -0.0008657 |
| White Light | | 9 | 4 | -0.0007367 | 0.008818 |

Table 1.1 illustrates the effects of dark light and white light on the rate of production and consumption of CO2 and O2 in *Spinacia Oleracea*. Positive numbers mean either CO2 or O2 was being produced. Negative numbers mean either CO2 or O2 was being consumed. When *Spinacia Oleracea* was under dark light after an equilibration time of 4 minutes, the rate of production of CO2 was 0.0005046 ppt per second. The rate of consumption for O2 under dark light was 0.0008657. The rate of consumption of CO2 under white light (7 watts) was 0.0007367 ppt per second while the rate of production of O2 was 0.008818 ppt per second.

Table 1.2 Effect of white light vs. black light on CO2 and O2 rate of production/consumption

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Treatment | Light Bulb (watts) | | Equilibration Time (mins) | CO2 – Rate of  Production/  Consumption  (ppt/sec) | O2 – Rate of Production/  Consumption (ppt/sec) |
| White Light | | 7 | 4 | -0.0006616 | -0.001939 |
| Black Light | | 7 | 4 | 0.00006145 | 0.0005428 |

Table 1.2 illustrates the effect of white light and black light on CO2 and O2 production or consumption in *Spinacia Oleracea* The rate of consumption of CO2 was 0.0006616 ppt per second under white light (7 watts), which means that the sample of *Spinacia Oleracea* absorbed about 0.0007 ppt of CO2 per second from the air in the chamber. The rate of consumption of 02 was 0.001939 ppt per second which means that the sample also absorbed about 0.002 ppt of O2 per second from the chamber’s air. Under black light (7 watts), the rate of production of CO2 was 0.00006145 ppt per second, and the rate of production of O2 was 0.0005428 ppt per second.

Table 1.3 Effect of white light with 9 minutes to equilibrate vs. white light with 7 minutes to equilibrate

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Treatment | Light Bulb (watts) | | Equilibration Time (mins) | CO2 – Rate of  Production/  Consumption  (ppt/sec) | O2 – Rate of Production/  Consumption (ppt/sec) |
| White Light | | 9 | 4 | -0.0004720 | 0.004385 |
| White Light | | 7 | 4 | -0.0006616 | -0.001939 |

Table 1.3 illustrates the effect of a 9 watt white light and a 7 watt white light on the rate of production and consumption of CO2 and O2 in *Spinacia Oleracea*. The rate of consumption of CO2 from the chamber’s air under a 9 watt white light was 0.0004720 ppt per second, and the rate of production of O2 into the chamber’s air was 0.004385 ppt per second. Under the 7 watt white light, the rate of consumption of CO2 from the chamber’s air was 0.0006616 ppt per second, and the rate of consumption of O2 from the chamber’s air was 0.001939 ppt per second.

*White light vs. dark light*

Figure 1.1. Effect of white light on CO2 levels in Spinacia Olercea. CO2 levels, also called ppt, were measured. The duration was 10 minutes (600 seconds).

There was a steady rate of consumption of CO2 under the white light. CO2 levels started at 0.739 ppt in the chamber and ended at 0.280 ppt in the chamber after 10 minutes (600 seconds). The sample of *Spinacia Oleracea* was able to take in about 0.5 ppt of CO2 under these conditions.

Figure 1.2. Effect of white light on O2 levels in Spinacia Oleracea. O2 levels, also called ppt, were measured. The duration was 10 minutes (600 seconds).

There was a steady and strong rate of production of O2 levels. O2 levels started at 197.4 ppt in the chamber and ended at 202.3 ppt in the chamber after 10 minutes (600 seconds). The sample of *Spinacia Oleracea* was able to produce about 5.0 ppt of O2. This light condition ended up producing the greatest increase of O2 levels within the chamber.

Figure 1.3. Effect of dark light on CO2 levels in Spinacia Oleracea. CO2 levels, also known as ppt, were measured. The duration was 10 minutes (600 seconds).

There was a steady but slow rate of production of CO2. CO2 levels started at 0.934 ppt in the chamber and ended at 1.250 ppt in the chamber after 10 minutes (600 seconds). The sample was able to produce about 0.3 ppt of CO2.

Figure 1.4. Effect of dark light on O2 levels in Spinacia Oleracea. O2 levels, also known as ppt, were measured. The duration was 10 minutes (600 seconds).

There was a steady rate of consumption of O2. The O2 level started at 197.5 ppt in the chamber and ended at 196.7 ppt in the chamber after 10 minutes (600 seconds). The sample was able to absorb almost 1 ppt of O2.

Figure 1.5. Effect of white light on CO2 levels in Spinacia Oleracea. CO2 levels, also known as ppt, were measured. The duration was 10 minutes (600 seconds).

There was a steady rate of consumption of CO2. CO2 levels started at 0.683 ppt in the chamber and ended at 0.292 ppt in the chamber. The sample was able to absorb about 0.4 ppt of CO2.

*White light vs. black light*

Figure 1.6. Effect of dark white light on O2 levels in Spinacia Oleracea. O2 levels, also known as ppt, were measured. The duration was 10 minutes (600 seconds).

There was a fairly steady rate of consumption of O2. O2 levels started at 200.4 ppt in the chamber and ended at 198.9 ppt in the chamber. The sample absorbed about 1.5 ppt of O2 from the air in the chamber.

Figure 1.7. Effect of black light on CO2 levels in Spinacia Oleracea. CO2 levels, also known as ppt, were measured. The duration was 10 minutes (600 seconds).

There was a very slow but steady rate of production of CO2. CO2 levels started at 0.200 ppt in the chamber and ended at 0.240 ppt in the chamber. The sample was only able to produce 0.04 ppt of CO2

Figure 1.8. Effect of black light on O2 levels in Spinacia Oleracea. O2 levels, also known as ppt, were measured. The duration was 10 minutes (600 seconds)

There was a somewhat consistent rate of production of O2. O2 levels started at 198.8 ppt in the chamber and ended at 199.1 ppt in the chamber. The sample was only able to produce about 1 ppt of O2.

*7 watt white light vs. 9 watt white light*

Figure 1.9. Effect of 7 watt white light on CO2 levels in Spinacia Oleracea. CO2 levels, also known as ppt, were measured. The duration was 10 minutes (600 seconds).

There was a steady rate of consumption of CO2. CO2 levels started at 0.683 ppt in the chamber and ended at 0.292 ppt in the chamber. The sample was able to absorb about 0.4 ppt of CO2.

Figure 1.10. Effect of 7 watt white light on O2 levels in Spinacia Oleracea. O2 levels, also known as ppt, were measured. The duration was 10 minutes (600 seconds).

There was a fairly strong and somewhat steady rate of consumption of O2. O2 levels started at 200.4 ppt in the chamber and ended at 198.9 ppt in the chamber. The sample was able to absorb 1.5 ppt of O2.

Figure 1.11. Effect of 9 watt white light on CO2 levels in Spinacia Oleracea. CO2 levels, also known as ppt, were measured. The duration was 10 minutes (600 seconds).

There was a fairly steady rate of consumption of CO2. CO2 levels started at 0.531 ppt in the chamber and ended at 0.166 ppt in the chamber. The sample was able to consume about 0.4 ppt of CO2.

Figure 1.12. Effect of 9 watt white light on O2 levels in Spinacia Oleracea. O2 levels, also known as ppt, were measured. The duration was 10 minutes (600 seconds).

There was a strong and steady rate of consumption of O2. O2 levels started at 199.5 ppt in the chamber and ended at 201.9 ppt in the chamber. The sample was able to produce about 2.5 ppt of O2.

**Discussion**

*Experiment 1*

The hypothesis for experiment one was that chlorophyll would be present in the sample of *Spinacia Oleracea* because all plants have chloroplasts which contain the chlorophyll pigment. If chlorophyll *a* and/or *b* was present in the sample, then a strip of green and/or blue green would appear on the paper. Additionally, the calculated Rf value for those pigments would be similar to the calculated Rf created by the controls for chlorophyll *a* and *b* (made by lab instructor). Results from experiment 1 did support the hypothesis and prediction. A green and blue-green color appeared on the chromatography paper which indicates that both chlorophyll *a* and *b* were present in the sample of *Spinacia Olercea.* Additionally, 3 other pigments were present including xanthophyll, carotene, and lutein. These are known as accessory pigments and are less prominent than chlorophyll *a* and *b.*

A study conducted in 2013 by Johnston et al. had the goal of introducing and reinforcing common organic techniques such as solvent extraction, column chromatography, and thin layer chromatography in order to identify and isolate pigments from spinach leaves. Johnson et al. (2013) performed the experiment by first boiling the spinach leaves in water, drying the leaves, extracting plant pigments using acetone, and using column chromatography for separation of pigments. Johnson et al. (2013) found 3 prominent pigments in there sample of spinach leaves including carotene, xanthophyll, and chlorophyll *a.* The results of this experiment were consistent with the results of the current experiment as the current experiment found all 3 of those pigments to be present.

*Experiment 2*

The hypothesis for this experiment was that the rate of O2 production and CO2 consumption would be the greatest under strongest white light conditions and weakest under dark/black light conditions because white light allows for the plant to absorb light energy thus powering the process of photosynthesis similar to what happens when plants are exposed to sunlight. If the sample of *Spinacia Oleracea* was put under the greatest white light conditions, then there would be a high rate of CO2 production and O2 consumption. The generated hypothesis for this experiment was supported by the results and data. The highest rate of O2 production was under the first 9 watt white light condition after being exposed to the dark light. The rate was 0.008818 O2 ppt per second. The closest result to that was under the last 9 watt white light with a rate of 0.004385 O2 ppt per second. Additionally, the highest rate of CO2 consumption was under the first 9 watt white light condition after being exposed to the dark light with a rate of 0.007367 CO2 ppt per second. In conclusion, this means that the sample of *Spinacia Oleracea* was able to undergo photosynthesis, taking in CO2 from the environment and releasing 02 into the environment, best under the 9 watt white light conditions. This is because the 9 watt white light condition was the most similar to sunlight, which is how plants obtain energy in their natural environment in order to undergo photosynthesis. It is also important to note that the rate of cellular respiration was greatest under the first dark light condition with a rate of producing CO2 at 0.005046 ppt per second. This is because cellular respiration is a light-independent process unlike photosynthesis which needs light in order to proceed with the process. By recording data every 50 seconds in this experiment, the rate of both photosynthesis and cellular respiration were able to be calculated after a 10-minute period of time. That was the goal of this experiment, so in conclusion, this experiment was successful in meeting the initial goal.

A 2014 study conducted by McLaughlin, Xu, Rastetter, and Griffin had the goal of predicting ecosystem carbon balance and analyzing the importance of long-term thermal acclimation potential and effects of light (white light) on respiration. Relevant findings included respiratory inhibition in the light which is similar to the findings of the current study. The current study found that when the sample was exposed to light, the rate of photosynthesis was high, but the rate of respiration was not nearly as high.

A limit to this experiment is that no colored lights were utilized in order to determine exactly what light condition is best for photosynthesis. A study conducted by Yu et al. (2017) looked at quantifying and comparing how varying light quality influences biomass, photosynthetic capacity, photosynthetic pigment content, chlorophyll fluorescence, and ultrastructure of stomata/chloroplasts. The results of this study found that red light was the most beneficial for all aspects of the seedling’s growth including the rate of photosynthesis. For future studies, it would be interesting to compare rate of photosynthesis under white light, black light, dark light, red light, green light, blue light, etc. This would provide a greater understanding of the effects of light conditions on photosynthesis. However, a comparison between the study by Yu et al. (2017) and the current study is that both studies found the lowest rate of photosynthesis under a form of dark light conditions. The only difference is that Yu et al. (2017) did not use dark light and instead used black light. These findings of Yu et al. (2017) support the results of the current study because dark light conditions produced the lowest rate of photosynthesis.

References

Johnston, A., Scaggs, J., Mallory, C., Haskett, A., Warner, D., Brown, E., . . . McDougal, O. M. (2013). A green approach to separate spinach pigments by column chromatography. Journal of Chemical Education, 90(6), 796-798.

McLaughlin, B. C., Xu, C., Rastetter, E. B., & Griffin, K. L. (2014). Predicting ecosystem carbon balance in a warming arctic: The importance of long‐term thermal acclimation potential and inhibitory effects of light on respiration. Global Change Biology, 20(6), 1901-1912. 10.1111/gcb.12549

Simms, T. M. (2017). Respiration & Photosynthesis in Spinach (*Spinacia Olercea*) leaves handout for Biology 105 labs, retrieved from https://mnsu.learn.minnstate.edu/d2l/le/content/3971185/viewContent/34002853/View

Yu, W., Liu, Y., Song, L., Jacobs, D. F., Du, X., Ying, Y., . . . Wu, J. (2017). Effect of differential light quality on morphology, photosynthesis, and antioxidant enzyme activity in camptotheca acuminata seedlings. Journal of Plant Growth Regulation, 36(1), 148-160. 10.1007/s00344-016-9625-y