## The importance of photosynthesis



To understand the importance of photosynthesis, research is conducted to help determine which wavelength of light and light intensity the chloroplast would generate the fastest photosynthetic reaction rate of photosynthesis. In plants, photosynthesis takes place in the chloroplast. The chloroplast absorbs the light energy to convert to chemical energy such as ATP AND NADPH. Photosynthesis is the process of converting carbon dioxide to organic compounds, such as simple sugar, using the energy from sunlight (Smith, A. L.). The chemical reaction equation of photosynthesis is as followed: 6 CO2 + 6 H2O + Light Energy → C6H12O6 + 6 O2

There are a number of limiting factors on the rate of reaction for photosynthesis. However, the purpose for this lab experiment is to measure the light intensity and the wavelength. Light is a form of energy known as electromagnetic energy, also called the electromagnetic radiation (Campbell 190). The most important segment of the visible light is the narrow band range from 380 nm to 750 nm (Campbell 190). Pigments are substance that absorbs the visible light; however, it may be also reflected or transmitted. Different pigments absorb light of different wavelengths (Campbell 190). Light emits a wavelength, the distance between the crest of electromagnetic waves, is inversely related to the amount of the energy: the shorter the wavelength, the greater the energy of each photon of that light (Campbell 190). Chloroplast contains pigment chlorophyll that absorbs the light energy from the sunlight and drive synthesis of organic molecules (Campbell 186). In addition, plants also use other pigment, such as carotenes and xanthophyll, to absorb different wavelength of the light. Chlorophyll absorbs violent-blue and red light while transmitting and reflecting green light, which

gives leaf its color. Violent-blue and red light are the most effective color of the light spectrum to conduct photosynthesis, whereas green light is the least effective color (Campbell 192).

Photosynthesis starts when the chlorophyll molecules are excited by the absorption of light. The chlorophyll molecules are organized along with other small organic molecules and proteins into photosystem (Campbell 193). The photosystem are composed of a protein complex called a reaction-center complex that is surrounded by several light-harvesting complexes, which contain various pigments that are embedded to the protein. In Photosystem II, light travels through the chloroplast and strikes a pigment molecule in the light harvesting complex. This excites the electron to a higher energy level and fall back down to ground state. As the electron falls back down to its ground state, it stimulate a nearby pigment until this process reaches the reaction center called the P680, a pair of chlorophyll  $\alpha$  molecules in the Photosystem II reaction -center complex. The electron is then transfer to the primary electron-acceptor. As the P680 loses its electron, it is replace by the enzyme catalyzes the splitting of water molecules into two hydrogen ions and  $\frac{1}{2}$  of oxygen in the thylakoids space. The oxygen atom immediately combines with another oxygen atom, forming O2, which was generated from the splitting of another water molecule. The excited electron from the primary electron-acceptor in PS II passes through the electron transport chain to the chlorophyll  $\alpha$  molecules, which is called P700, located in PS I. In the meantime, light energy travels through the light harvesting complex into the P700, which excited the electron, transferring the electron to PS I primary electron-acceptor. These electrons are passed on through electron

acceptors that donate the electron to NADP+. The energy release drive the transfer of electron in an oxidation-reduction mechanism in which NADP+ is reduced to NADPH. Involving a redox reaction, oxidation is the loss of electrons from a substance, whereas reduction is the addition of electrons to a substance. The excess of energy from the oxidation-reduction process provides energy for the synthesis of ATP, which generates a proton gradient across the chloroplast membrane that is used in chemiosmosis. Overall, the light reactions are steps of photosynthesis to convert light energy to chemical energy, such as ATP and NADPH, in order to produce pieces of sugar in the Calvin cycle.

In this study, we first separate and identify pigments within plants cells by a process called chromatography. We will also study how several factors quantitatively affect the rate of photosynthesis. The factor that was tested includes the light intensity and wavelength. Thus, we can determine the effectiveness of the different pigments to absorb light to different wavelength and light intensities. The hypotheses are formed as follows:

H1: Violent-blue and red light would have a faster photosynthetic rate compared to green light.

H2: Light intensity is directly correlated with rate of photosynthesis.

H3: Carbon dioxide is directly proportional to the amount of carbon present in the atmosphere.

## Materials and methods

Chromatography is to separate and identify pigment within the plant cell which spinach leaves was use to conduct this experiment. Using a paper chromatography of 14 cm wide by 16cm tall, a pencil line of 2 cm is drawn from the bottom edge of the paper. Then apply the plant extract along the line to within 1 cm of each edge. Allowing the extract to dry each time, this process is repeated 10 times or more to ensure the pigment are on the chromatography. The paper chromatography is stapled into a cylinder at the bare edges and place into chromatography jar that contain a 15 ml solvent of petroleum ether-acetone. The chromatography jar is set under a ventedhood with the jar covered. This will allow the atmosphere inside to be saturated with the solvent. The solvent will move up the paper chromatography and carry the pigments along. Each pigment will move at different rate along the paper. The discrete pigment band will be formed from the front, which is the leading edge of the solvent, to the origin where the pigments were added to the paper. To determine the distance of each discrete pigment band, Rf ratio is used. The Rf is the ratio of the distance a band travels to the distance the front traveled (lab manual). The Rf equation is as follow:

After the pigments are separated, each band will be pooled with other group and eluted into 10 cc of acetone. The unknown pigments from the each band are placed in a cuvette and place in a spectrophotometer. Four cuvettes were obtained and label as band 1, 2, 3, and 4. A spectrophotometer is used to measure the percent of each wavelength of light absorbed by the pigment (Campbell 190). Each band is measure at specific wavelength ranging from

400 nm to 680 nm. Thus, each of the bands is identify according to its pigment by comparing its wavelength to the known standard wavelength.

To determine at which wavelength of light and at which light intensity the chloroplast would generate the fastest photosynthetic reaction rate of photosynthesis, the floating leaf disk assay is use for this experiment. The wavelength of red, green, and blue light is test to determine the rate of photosynthesis. In addition, the effect of light intensity is determined by the distance of light (white) from the leaves. For each trail, a 0. 2% of 300 ml sodium bicarbonate solution (baking soda) is use as an alternate dissolved source of carbon dioxide for photosynthesis by using 1/8 of a teaspoon of baking in a 300 ml of water (lab manual). Then a hole-punch is use to cut out 10 or more uniform leaf disks (avoid major veins). The air space of the leaf disks is infiltrates with the sodium bicarbonate solution, which the solution will cause the leaf disk to sink due to its increase in density. Infiltration of the leaf disks with sodium bicarbonate is as followed:

Remove the plunger and place the leaf disk into the syringe barrel.

Replace the plunger and slowly push air out while being careful not to crush the leaf.

With a small volume of sodium bicarbonate solution into the syringe. Tap syringe to suspend the leaf disks in the solution.

While holding a finger over the syringe opening, draw back the plunger to create a vacuum for 10 seconds. In addition, swirl the leaf disks to suspend

them in the solution. This procedure may be repeated 2-3 times in order to get the leaf disk to sink.

After the leaf disks sink, pour the disk and the solution into a clear cup or beaker. A constant volume of bicarbonate solution is added and should be the same depth for each trail. Place the cup or beaker under the light source and start the timer. Each minute is to record the number of floating disk. In addition, dislodge any disks stuck against the sides of the cup by swirling the disks. Continue until all of the leaf disks are floating.

In addition, the presence of CO2 is measured.