Measuring the rate of photosynthesis using a spectrophotometer

Spectrophotometer Lab: Length: 1437 words (4. 1 double-spaced pages) Rating: Red (FREE) – Spectrophotometer Lab Introduction: A spectrophotometer is an instrument that measures the quantitative amounts of light of different wavelengths absorbed and transmitted by a pigment solution (Mitchell, Reece). The spectrophotometer includes a light bulb, a reflector, and a detector. When a sample is in place and the chamber lid is closed, light from the light bulb passes through it.

The detector measures the amount of light transmitted. The white light from the bulb is separated into different wavelengths by reflecting the beam off of a diffraction grating, forming a spectrum. A mirror is used to control the angle at which the light is reflected, so light of a single wavelength can be passed through the sample. A pigment solution (such as spinach and acetone) is exposed to different wavelengths of light to determine the points of the highest absorbance and transmittance percentages. (Hickey, Mary Kay). For this experiment, a Bausch and Lomb Spectrophotometer was used.

Often objects appear colored because of their absorption of light within regions of the visible spectrum. The color of light we can see is composed of the wavelengths that the object did not absorb. Photosynthesis in plants takes place in organelles called chloroplasts. Chloroplasts contain a number of coloured compounds, known as pigments, in their thylakoids, where light dependent reactions occur(Mitchelsl, Reece). Spinach leaves contain chlorophyll a and b and b -carotene as major pigments as well as smaller amounts of other pigments such as xanthophylls. The two forms of chlorophyll are identical except that a methyl group in a is replaced by an aldehyde in b.

In terrestrial plants, the most important pigments are chlorophyll a (blue-green), chlorophyll b (yellow-green), xanthophyll (yellow) and carotene (orange-yellow). (Pavia, D. L.) Each pigment has a characteristic absorption spectrum, showing which wavelengths of visible light it absorbs best.

(Campbell, Neil) In this experiment, by observing the transmittance percentage and absorbance of light when shone at spinach at different wavelengths, we will determine which wavelengths are best absorbed by spinach. Figure 1: [IMAGE] Figure 1: Labelled Diagram of a Bausch and Lomb Spectrophotometer. (Chemical Instrumentation Laboratories) Purpose: To determine the % of absorption and transmission of light from spinach leafs at different wavelengths of energy. Hypothesis: The wavelength with the greatest transmission % will be 550 nm because the spinach leaf reflects green light (hence, why plants are green). It is expected that the highest absorption % will be between 420-450 nm and 650-700 nm.

The reason for this is because Plants absorb blue light and red light the best, and 650-700 nm is the range in which photosystem 1 and 2 absorb light to being the light dependant reactions. Materials: * 6. 1 mL Spinach solution * 2 Cuvettes * Spectrophotometer Variables: Controlled: * Room temperature * Concentration of spinach * Amount of spinach * Type of spectrophotometer * Pressure Dependent: * Transmittance percentage * Absorption Percentage Independent: * Wavelength of light Procedure: 1) The instrument was turned on by rotating the amplifier control (c) in a clock-wise direction. minutes were allowed for the instrument to warm up.

2) A blank cuvette was placed in the sample compartment and the guide mark was aligned on the cuvette at the front of the sample compartment. The lid was closed. It was often checked that the meter needle reads 100% Transmittance when the blank cuvette was in the sample compartment with the lid closed. 3) The amplifier control was adjusted with the sample compartment lid closed until the meter needle read 0 on the % Transmittance scale.

4) The blank cuvette was removed and the ground-up spinach was inserted. The cuvette was placed in the sample compartment. The guide mark on the cuvette was aligned with the guide mark at the front of the sample compartment. The lid was closed. 5) The wavelength was set to zero nm using the wavelength control knob.

6) The transmittance percent and absorption were recorded from the Transmittance/Absorbance scale. 7) It was often checked that the meter needle readss 0 on the % Transmittance scale with the sample compartment empty and the lid closed. 8) Steps 1 – 8 were repeated using the wavelengths of 360 nm to 900 nm, at every 20 nm. Observations: Chart 1: Observations of Transmittance Percentages and Absorbance of spinach solution at wavelengths of 340 nm to 900 nm.

The highest absorbance recorded was 2, and it occurred in the region of wavelengths from 340 to 400 nm (purple). The lowest absorbance was 0. 35 at a wavelength of 700 nm(red). The highest transmittance percentage recorded was 45% at a wavelength of 700 nm. The lowest recorded was 0 at a region of 340 to 400 nm. Chart 1 Wavelength (nm) Colour of Wavelength Transmittance (%) Absorbance 340 Purple 0 2 360 0 2 380 0 2 400 0 2 420 1 1.

9 440 Purple-Blue 1 1. 9 460 1 1. 9 480 Blue 1 1. 500 9 1. 1 520 Green 33 0.

49 540 Green- Yellow 34 0. 48 560 25 0. 6 580 Yellow 12 0. 95 600 8 1. 1 620 Orange 4 1. 4 640 Orange-Red 3 1.

5 660 2 1. 6 680 Red 12 0. 9 700 45 0. 35 720 33 0.

49 740 18 0. 75 760 10 1. 1 780 5 1. 4 800 3 1.

6 820 2 1. 9 840 1. 5 1. 9 860 1 2 880 1 1. 9 900 1 1. 9 Figure 2: [IMAGE] Figure 2: The transmittance percents of light of a spinach sample displays two peaks when graphed: a peak between 500-500 nm and 680-720 nm.

Therefore, the spinach reflected these wavelengths the most. Figure 3: [IMAGE] Figure 3: The absorption spectrum of light of a spinach sample displays two alleys when graphed: a valley between 500-500 nm and 680-720 nm. Therefore, the spinach absorbed these wavelengths the least, and absorbed 340-500 nm and 720-900 nm the most. Absorption Spectrum of Spinach Figure 4: [IMAGE] Figure 4: The wavelength of highest transmittance % coincides with the wavelength of the lowest absorbance.

This relationship is demonstrated by the equation Absorbance = log 100/ Transmittance Percent. Sources of Error: When grinding up the spinach leaves, impurities, such as dust particles, may have entered the substance, changing the transmittance percentage slightly. In addition, the cuvettes may have been scratched, or have had fingerprints on them, despite efforts to keep them clean. This would also alter the transmittance percentage.

Also, there may have been human errors of misreading the spectrophotometer, making our results have a small degree of uncertainty. Finally, when turning the knobs to the desired wavelength, the numbers may have been slightly off. Discussion/Conclusions: In conclusion, chlorophyll inside of an intact chloroplast that has absorbed light gives different results when the chloroplast is not intact. Campbell, Neil) When inside of a thylakoid membrane, chlorophyll acts in a photosystem. In a photosystem, the energy gained from the absorbed light is transferred to an electron transport chain.

(Campbell, Neil) However, when the spinach was ground up, the chloroplasts were broken up. Therefore, the photosystems were not functioning, and the chlorophyll fluoresces since there were no electron acceptors to prevent the excited electrons from dropping back down to ground state. Initially, it was hypothesized that the wavelength with the greatest transmission % would be 550 nm. However, by analyzing the graph, it becomes apparent that although there was a peak in the graph in the region of 550 nm, the region of 700 nm contained the highest transmittance percentage.

This unexpected peak can be accounted for by the fact that when the thylakoids were mashed up, the photosytems, which are best at absorbing wavelengths of light at 680 nm and 700 nm, were disabled. Therefore, the energy from the absorbed light that would normally be transferred by the photosystems to the electron transport chain was fluoresced, resulting in a higher transmittance percentage. In addition it was expected that the highest absorption % would be between 420-450 nm and 650-700 nm. 650-700 nm is the range in which photosystem 1 and 2 absorb light to being the light dependant reactions. It was true that for the purple and blue light, absorbance was very high. Also, as expected, absorbance in the green region was very low, since green light is the least effective wavelength that can be used in photosynthesis.

(Campbell, Neil) However, the unexpected low occurred in the 650-700 nm region, where absorbance was expected to be high. The rise in the absorbance graph in the range of 550 nm to 600 nm can be explained by the presence of xanthophyll (which absorbs yellow wavelengths) and carotene (which absorbs yellow wavelengths orange-yellow), which are also present in spinach. This can be explained in the same way the unexpected high in the 650-700 nm range for transmittance percentage: the photosystems were disabled, and therefore that range of light was fluoresced. A comparison of the two graphs shows a relationship between ransmittance percentage and absorbance. When the transmittance percentage increases, the absorbance tends to decrease. This relationship is represented by the equation Absorbance = log 100/ Transmittance Percent.

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