Pigments and photosynthesis

ASSIGN BUSTER

Lab Four: Plant Pigments and Photosynthesis

Part A

Table 4. 1:

Distance Moved by Pigments Band (millimetres)

Band Number	Distance (mm)	Band Colour
1.	15	Yellow
2.	35	Yellow
3.	73	Green
4.	172	Olive Green
5.	–	–

Distance Solvent Front Moved 180 (mm)

Table 4. 2:

. 083334= R_f for Carotene (yellow to yellow orange)

. 194445= R_f for Xanthophyll (yellow)

. 405556= R_f for Chlorophyll a (bright green to blue green)

. 955556= R_f for Chlorophyll b (yellow green to olive green)

Analysis

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What factors are involved in the separation of the pigments?

The factors that are involved in the separation of the pigments are the pigments solubility, the formation of the intermolecular bonds, and the size of each individual pigment particle. Since capillary action is the method by which the solvent moves up the strip of paper, the attraction of the molecules to the paper and to each other molecule is essentially determined by those factors.

Would you expect the R value of a pigment to be the same if a different solvent were used? Explain.

No, because in different solvents, the solubility of the pigments would be different causing the R_f value to be different. In different solvents, the solvent rate would be affected, and since the rate is different, the distance travelled would also be affected, causing the R_f value to also be different.

What type of chlorophyll does the reaction centre contain? What are the roles of the other pigments?

Chlorophyll a is contained in the reaction centre. Because it is the primary photosynthetic pigments in plants, other chlorophyll a molecules, chloroplast b, and the carotenoids (carotenes and xanthophylls) capture light energy and transfer it to the chlorophyll a at the reaction centre. (College Board, 46)

Part B

Purpose

The purpose of this lab is to measure the effect of various conditions of chloroplast on the rate of photosynthesis or percentage of light transmittance. By using unboiled chloroplast in light, unboiled chloroplast in

dark, and boiled chloroplast in light, DPIP was placed into each cuvette and a colorimeter was used to measure the rate of light transmittance.

Since DPIP is the electron acceptor, as there is more light present, the DPIP absorbs more elections thus reducing the DPIP. Eventually the reduction causes the DPIP to change colour from a deep blue to a clear or opaque colour.

Variables

Independent Variable

- The independent variable in this lab is the different forms/conditions of the chloroplast. These include boiled chloroplast in light, unboiled chloroplast in light, and unboiled chloroplast in dark.

Dependent Variable

- The dependent variable in this lab is the rate/ level of light transmittance over a period of time measured by the colorimeter. From this data we can determine the rate of photosynthesis because as the DPIP becomes excited and reduced by the electrons, the colour changes indicating the rate of photosynthesis.

Control Variable

- The control variables in this lab includes the type of cuvette, size of cuvette, type of buffer used, amount of phosphate buffer used (1mL), and the time intervals (min) used to measure the % or level of transmittance in the colorimeter.

Measurement

- To measure the dependent variable, in this lab, a colorimeter and DPIP was used to determine the level of light transmittance. As the electron

acceptor, DPIP was placed in each cuvette. Later after a certain interval of time, each was placed into a colorimeter which determined the level of light transmittance. As electrons were accepted, the DPIP became excited and reduced causing the color in the cuvette to also change, thus affecting the level of light transmittance as measured by the colorimeter.

Hypothesis

Since photosynthesis is the process by which plants, bacteria, and other autotrophic organisms obtain energy to produce sugars, the right conditions and the right environment are necessary in order to carry out this complex process. Based on prior knowledge and information from this lab, cuvette 3 will have the highest percent of light transmittance and the highest rate of photosynthesis.

Since photosynthesis requires light and functional chloroplast to absorb and produce sugars, without either one, the process is interrupted and cannot function properly. Unboiled chloroplast will have a higher percent of light transmittance than boiled chloroplast because of the impact temperature has on the proteins/enzymes of the chloroplast. As high temperatures, like the boiling point, the heat generated will denature the enzymes/proteins thus reducing its effect on photosynthesis.

Without functional chloroplast to absorb the energy from the light, the electrons will not be bumped to a higher energy level and will not be able to reduce DPIP. Of the two cuvettes with unboiled chloroplast, the cuvette place in front of the light will have a higher percent of light transmittance than the

cuvette placed in the dark because with light, energy can be absorbed, DPIP can be reduced, ATP can be created, and photosynthesis can be carried out.

Similar to functional chloroplast, light is another essential component of photosynthesis, without light photosynthesis cannot occur. Therefore, the cuvette placed in the dark may have functional chloroplast but without light to provide the necessary energy, the reaction will either occur very slowly or not at all. Finally, the cuvette with no chloroplast will not photosynthesize at all, because without chloroplast to absorb the energy from the light, the solution will not carry out photosynthesis.

Procedures

First, a beaker of water was positioned between the samples and the light source which was to be the heat sink. Next, an ice bath was created to preserve the phosphate buffer and chloroplast by filling an ice bucket with ice. Then, before the cuvettes could be used, they had to be cleaned out with lint free tissue to ensure the light transmittance goes smoothly and uninterrupted.

Before anymore is done with each cuvette, both boiled and unboiled chloroplast were obtained in pipettes and place in the ice bath inverted. Next, of the five cuvettes labelled 1 to 5, cuvette 2 had a foil container constructed for the sake of keeping light out of the solution. Each cuvette then received the corresponding amount of phosphate buffer, distilled water, and DPIP. The colorimeter was then set up by starting up the computer program that would read the colorimeter and was linked accordingly.

The first cuvette received three drops of unboiled chloroplast, and then shaken up and placed in the slot of the colorimeter. The first solution would be the first calibration point of reference for the colorimeter at zero percent light transmittance. Following the setting of the first calibration point, the second calibration point was also set. In cuvette 2, three drops of unboiled chloroplast was added and immediately timed with a stopwatch and the light transmittance was recorded.

The same cuvette was encased with the foiled created earlier and then placed in the light. Cuvette 3 also received three drops of unboiled chloroplast at which the time and the light transmittance was also recorded. Right afterwards, the cuvette returned to the light. Cuvette 4 received three drops of boiled chloroplast at which the time and the light transmittance was also recorded. Just like cuvette 3, cuvette 4 was returned to the light. Curette 5 the control would receive no chloroplast but still has the time and light transmittance recorded. The light transmittance for each would continue to be recorded at an interval of every five minutes (5 minutes, 10 minutes, 15 minutes) following the same procedure until all data had been collected.

Conclusion

The process of photosynthesis is described as the conversion of light energy to chemical energy that is stored in glucose and other organic compounds. Essential to the development of plants and animals, light from the sun or from an artificial source is necessary for this process to occur and to carry out its benefits. Having performed this lab, the results obtained supports this concept and it also supports my hypothesis.

After gathering all the data, cuvette 3 did have the highest percentage of light transmittance and the fastest rate of photosynthesis. Because of the unboiled chloroplast in the cuvette absorbing the light and a light source available to provide energy to reduce the DPIP, the conditions were right for photosynthesis to occur.

In cuvette 3, photosynthesis did occur because when the light shined on the unboiled chloroplast, the electrons were excited and moved to a higher energy level.

This energy was then used to produce ATP and to reduce DPIP causing the solution to change colour creating a higher and faster rate of photosynthesis/light transmittance. This cuvette essentially showed that light and chloroplast are needed in order to carry out photosynthesis. Although the graph may show the rate of photosynthesis slowing down, the reason why the curve begins to slow down and level off is not because of photosynthesis but because as the process of photosynthesis occurs, the DPIP will begin to be used up causing the reaction to slow down and level off.

Cuvette 2 showed different results in that no photosynthesis occurred because there was no light present for the chloroplast to absorb and to reduce the DPIP. Photosynthesis requires light but without out light, photosynthesis could not occur causing essentially no change in the cuvette. The data table and graph does show that there were some change in the rate of photosynthesis but that occurred because since we had to take the cuvette out of the aluminium sleeve to place in the colorimeter, the DPIP broke down because of the brief exposure to the light.

However, overall, the data shows that because there was no light present, photosynthesis could not occur causing no change. Cuvette 4 also showed little increase or change in the percentage of light transmittance because since the cuvette had boiled chloroplast, the high temperatures denatured the proteins/enzymes found in the chloroplast rendering them ineffective. Because the light could not be absorbed by the chloroplast, photosynthesis could not occur or it occurred at a very slow pace.

Similar to cuvette 2, the date table and graph also shows that there were change in the percentage of light transmittance in cuvette 4 but because the DPIP was exposed to the light, the DPIP did break down causing a slight change in the rate of light transmittance. Essentially, this cuvette showed that chloroplast in addition to light is required for photosynthesis.

Cuvette 5 also showed no change in the percentage of light transmittance because without the presence of chloroplast, the light could not be absorbed to excite the elections and to reduce the DPIP. Without the functions of chloroplast, photosynthesis could not occur because the DPIP would not be reduced and ATP would not be created. Any fluctuations in the data or graph for cuvette 5 could be explained by human or data error.

Analysis

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What is the function of DPIP in this experiment?

The function of the DPIP in this experiment is to act as the electron acceptor, replacing the usual NADP found in plants. When the light shines on the active chloroplasts, the electrons are excited, which causes them to jump to a

higher energy level thus reducing the DPIP. As the DPIP is reduced, the colour changes from deep blue to colourless, which affects the rate and level of light transmittance when measured by the colorimeter.

What molecule found in the chloroplasts does DPIP " replace" in this experiment?

DPIP in this experiment " replaces" the electron acceptor NADP

What is the source of the electrons that will reduce DPIP?

When the light shines on the chloroplast, the light provides enough energy to bump the electrons to a higher energy level thus reducing the DPIP. The source of the electrons can also come from the photolysis of water.

What was measured with the spectrophotometer in this experiment?

The spectrophotometer in this experiment is used to measure the percentage/level of light transmittance through the cuvette based on the amount of photosynthetic activity.

What is the effect of darkness on the reduction of DPIP? Explain.

Because there is not an absence of light shining on the chloroplast, the DPIP could not be reduced because there was no or not enough energy to excite the electrons and move them to a higher energy level in order to reduce the DPIP.

What is the effect of boiling the chloroplasts on the subsequent reduction of DPIP? Explain.

Similar to the effects of darkness, by boiling the chloroplast, the proteins were denatured by the high temperatures which caused the process of photosynthesis to be slowed down and inhibited. Because the chloroplast

could not absorb light and perform its job, the DPIP could not be reduced which reduced the percentage/level of transmittance.

What reasons can you give for the difference in the percentage of transmittance between the live chloroplast that were incubated in the light and those that were leapt in the dark?

Because light is essential for photosynthesis, the chloroplast placed in light was able to reduce DPIP and perform photosynthesis. As the chloroplast absorbed the light, the energy absorbed, pushed the electrons to a higher energy level which caused the DPIP to reduce.

As the DPIP reduced, the colours changed and the rate of light transmittance was higher. In the dark chloroplast, however, because there is no energy source for the chloroplast to use and since the DPIP could not be reduced due to the lack of light energy, the percentages of light transmittance were lower.

Identify the function of each of the cuvettes

Cuvette 1: Cuvette 1 was used to measure how the absence of DPIP and chloroplast affected the percentage of light transmittance. This cuvette was also used to calibrate the colorimeter.

Cuvette 2: Cuvette 2 was used to measure how the lack of light and unboiled chloroplast affected the percentage of light transmittance. It essentially showed how important light was to the process of photosynthesis.

Cuvette 3: Cuvette 3 was used to measure how light and unboiled chloroplast affected the percentage of light transmittance. It essentially

showed how light and active chloroplasts are needed to carry out the process of photosynthesis.

Cuvette 4: Cuvette 4 was used to measure how light and boiled chloroplast affected the percentage of light transmittance. It essentially showed how the denatured proteins in the chloroplast prevented the light to be absorbed and the process of photosynthesis to be carried out.

Cuvette 5: Cuvette 5 is the control of the experiment and is used to show how the availability of light but absence of chloroplast will prevent the process of photosynthesis from being performed and its effect on the percentage of light transmitted.