Photosynthesis experiment: hill reaction essay sample

Aim of the Experiment: To analyse the effects of light intensity on photosynthesis via the Hill reaction.

Research Question: How does light intensity affect the rate of photosynthesis more precisely the rate of reaction in photosystem II, at the primary acceptor location?

Hypothesis: In the light dependant reactions, an artificial electron acceptor, DCPIP can be substituted for NADP it is very similar to this molecule but changes colour when it is reduced; it changes from blue to colourless. This is because DCPIP is a redox dye. As DCPIP becomes colourless light transmittance is therefore increased and one can use a colorimeter to measure the light acceptance. This means that it is the rate of electron flow in photosynthesis that is being measured. It is also important to note that DCPIP interrupts the passage of electrons from photosystem II to photosystem I, explaining why only photosystem II activity can and is measured.

For this experiment the variables are the following:

Independent variable: Light intensity – Produced from a lamp at various distances.

Dependant variable: Transparency- Light absorbance of the solutions

Controlled variable: Ambient Light – The shutters were closed to stop sunlight from entering the room in which the lab took place.

Method

Material Used:

* Chloroplast solution pre-extracted from spinach

* Diluted DCPIP solution

* Pipette

* Small beaker

* Vernier Colorimeter with 8 plastic cuvettes for the DCPIP-Chloroplast-Buffer solution

* Phosphate reaction buffer

* Lamp with a 40W light bulb

* 1 meter ruler

* A timer

Steps:

1. The colorimeter wavelength was set to 635 nm.

2. A cuvette was filled with the 2 ml of buffer and 1ml of chloroplast solution it was then placed into the colorimeter to calibrate it (Averaging 100% absorbance).

3. A cuvette was filled with DCPIP (1ml + 3 drops) and 2ml of buffer solution and was tested for transmittance percentage.

4. Six other plastic cuvettes were filled with 1 ml of DCPIP solution and 2 ml of reaction buffer solution. 1ml of chloroplast solution was added to these solutions.

5. The six cuvettes were thoroughly shaken and placed alongside a ruler at distances of 15, 30, 45, 60, 75 and 90 cm each.

6. Each cuvette had its light absorbance tested to make sure they all contained a similar solution so as to avoid data collection errors later on in the lab.

7. Turn on the lamp and wait for 2 minutes allowing the Hill reaction to take place.

8. Then, once the time had elapsed, each cuvette was quickly tested by using the colorimeter for its light absorbance.

Processed Data

Table 2. Raw data with the light intensity calculated with the proportionality with 40W as the constant of this relation. Because 6 retakes where made, an average was made with its uncertainty calculated. Even if only 6 values were tested, a correlation coefficient was calculated and was 0. 9953, suggesting a high probability of correlation.

Graph 1. On the graph underneath all the sequential tries have been plotted, showing their resemblance and trend. A disagreement is noticed in the last set of values.

Graph 2. This graph shows the average values plotted and their respective error bar. A trend is easily noticed as the values increase in a logarithmic way, steadily approaching a certain optimum. However the last value (44. 5 Wm-2) does not correspond to anything expected and is an admitted error possibly caused by another limiting value of photosynthesis that has changed, such as temperature.

Conclusion

An undetermined but possibly non-linear regression relation was found; possibly a logarithmic one as Blackman and Wilson’s “ Physiological and ecological studies in the analysis of plant environment” heavily suggests this as it is stated that “ the net assimilation rate during the season of active growth is linearly related to the logarithm of the light intensity”.

However a clear conclusion that has to be made is that light is indeed a limiting and essential factor to the rate of photosynthesis in photosystem II, as it is light that provides photons and excites electrons as soon as it touches the pigment molecule. In isolated chloroplasts, light is required as the name of the observed reaction states, light dependant reaction.

In addition, since DCPIP stopped electrons from passing from photosystem II to I, the primary products of the light dependant reactions, ATP and NADPH2, could not have been required and the Calvin cycle had no resources available to take place. This also allows us to conclude that no CO2 was used to form carbohydrates and that CO2 is only required at a later stage in photosynthesis that was left unmonitored but safely assumed to have been paralysed due to the lack of travelling electrons. ATP was not produced as

electrons did not pass entirely through the electron transport chain in photosystem II and NAPDH2, could not have been formed, as the electrons did not reach photosystem II.

Our second conclusion then is that CO2 is only used at a later stage and that a plant still has the ability to photosynthesize.

Evaluation

Firstly, this lab did not measure the rate of photosynthesis as DCPIP blocked the procedure from occurring early on, so claiming that this lab measured the rate of photosynthesis does not make sense and we should focus on the fact that we are only observing and assessing the first steps of photosynthesis.

The chloroplast solute containing many enzymes and co-enzymes would have had its activity affected by the temperature at which it was stored and the temperature it was subject to during the experiment, therefore a possible source of inaccuracies could have originated from this and more reactions where happening due to high temperature increasing rate of reactions. The chloroplasts could have been kept in a container surrounded by ice cubes during the experiment so as to avoid any incidental reactions.

The chloroplast solute was also subject to light many times before the experiment started and during it (opening the fridge door, low but existing ambient light in the room of the experiment allowing one to see). The precaution of putting aluminium foil to possibly preserve the temperature and stop light entering was a bad idea as once a ray of light entered, a

similar effect to total internal reflection was created. Another non-reflective material such as paper should have been used and then foil could have been placed over the layer of paper for heat transfer concerns.

Additionally the trials taken at 10cm had an exceptionally high measurement. This was highly likely due to the heat radiated by the lamp, causing the rate of reaction to be higher than as if it were only due to light intensity. Instead another limiting factor, temperature was increased. To avoid such problems a thin tank of water could have been placed between the light and the solutions, as water has an exceptionally high specific heat capacity and acts as an effective heat blocker, while still letting light through.

The high measurement for the 10cm trials can also be explained by the arrangement of the cuvettes, which caused each cuvette to cast a shadow upon the next cuvette, removing light and thus meaning that only the first cuvette at 10cm received the intended amount of light. This could have easily been fixed by placing the cuvettes in an alternating fashion, describe in a diagram besides.

The experiment’s quality was also deteriorated by the light pollution emitted by surrounding experiments in the room. The simple solution is simply to do each experiment one at a time.

DCPIP being a blue compound itself when oxidized obscured the light intended for the chloroplasts to react causing the rate of reaction to happen at an increasing rate while the DCPIP reduced, becoming colourless.

Another factor that would have increased the quality of the results would have been to use a lux meter to measure the illuminance at various distances and not have to really on the relationship, which is not the most precise way of measuring light intensity.

Finally more distances should have been tested, as we have no data for 15 to 45 Wm-2. Distances such as 16, 17 , 20 , 22, 25, 27 would have been extremely useful for two reasons. Firstly because we would have data for the large range of light intensity blank and we would have had 12 different measures allowing a proper statistical test to be used. In this case a non-linear regression test would have been the most suited for testing the relationship.