Edexcel A Biology A-Level

Core Practical 11

Investigate photosynthesis using isolated chloroplasts (the Hill reaction).

Dehydrogenase is an **enzyme** found in plant chloroplasts that is crucial to the **light dependent stage** of photosynthesis. In the light dependent stage, **electrons** are accepted by **NADP.** This reaction was discovered in 1938 by Robin Hill and thus is often called the Hill reaction. **Dehydrogenase** catalyses this reaction. When a **redox indicator dye** is present, such as **DCPIP (**which turns from **blue to colourless** when it is reduced), electrons are accepted by this instead.

Equipment

- Leaf sample
- Scissors
- Mortar and pestle (cold)
- Nylon mesh
- Filter funnel
- Centrifuge
- Centrifuge tubes
- Ice-water-salt bath
- Glass rod
- Measuring cylinder
- Beaker
- Pipettes
- Bench lamp
- Buffer
- Isolation medium
- DCPIP solution

Method

1. **Remove stalks** from leaf samples. Cut into small sections. Grind sample using a pestle and mortar and place into a **chilled isolation solution**.
2. Place several layers of muslin cloth into funnel and wet with isolation medium to filter sample into a beaker.
3. Suspend the beaker in an **ice water bath** to keep sample chilled.
4. Transfer to centrifuge tubes and **centrifuge at high speed for 10 minutes**. This will **separate chloroplasts** into the **pellet**.
5. Remove **supernatant** and add pellet to fresh isolation medium.
6. Store isolation solution on ice.
7. Set the colorimeter to the **red filter**. **Zero** using a cuvette containing **chloroplast extract** and **distilled water.**

8. Place test tube in rack **30cm** from light source and add **DCPIP**. Immediately take a sample and add to cuvette.
9. Measure the **absorbance** of the sample using the colorimeter
10. Take a sample and measure its absorbance **every 2 minutes for 10 minutes**.
11. Repeat for different distances from lamp **up to 100 cm.** This will vary the **light intensity**.

NB: This experiment should be done in a **darkened room** to make results more reliable. The sample should not be put too close to the lamp as **temperature** may affect results.

Risk Assessment

Hazard	Risk	Safety Precaution	In emergency	Risk Level
DCPIP	Irritant to skin and eyes; may cause staining	Wear eye protection	Wash from skin/eyes immediately using cold water	Low
Biohazard	Allergies; soil bacteria; contamination	Wash hands after use	Seek assistance	Low
Lamps	Temporary damage to eyes	Do not look directly at lamp	Wait for afterimage to disappear; seek appropriate assistance if needed	Low
Electrical appliances	Liquids near electrical appliances	Do not touch lamp/wires with wet hands; keep liquids away from lamp/wires	Seek assistance	Low

Graph

- Plot a graph of **absorbance** against **time** for each distance from the light.

Conclusion

- As the light intensity **decreases**, the rate of photosynthesis also **decreases**. This is because the lowered light intensity will **slow the rate of photoionisation** of the chlorophyll pigment, so the overall rate of the light dependent reaction will be slower.
- This means that **less electrons** are released by the chlorophyll, hence the **DCPIP accepts less electrons**. This means that it will **take longer to turn from blue to colourless**.
- When the DCPIP is **blue**, the **absorbance is higher**. The rate at which the absorbance decreases can therefore be used to determine the **activity of the dehydrogenase enzyme.** A **higher rate of decrease**, shown by a **steep gradient** on the graph, indicates that the **dehydrogenase is highly active**.