From photons to food - illuminating

The wonders and importance of the process of photosynthesis are presented to learners at many different levels and in several different contexts within *Curriculum for Excellence* [1]. Learners embarking on the study of photosynthesis at H*igher* level will already understand the importance of the process of photosynthesis to life on Earth.

Ideas about the global importance of photosynthesis in terms of the gas balance of the atmosphere and world food production may also be familiar to them. They will know that, in the presence of light energy, green leaves will synthesise carbohydrate and that this process requires the presence of water and carbon dioxide and that oxygen is a product. From their study of photosynthesis at National 5 level, they will also already know that the simple 'equation' often given for photosynthesis is a summary of a complex process and that photosynthesis involves two main stages each with multiple steps.

Carrying out the Hill reaction, which is a suggested learning activity in the new *NQ (CfE) Higher Biology* [2], is an excellent context in which learners can begin to engage with the more complex biochemistry of photosynthesis required at Higher level. Interestingly it also provides a historical perspective on the development of our understanding of photosynthesis.

A vital clue to understanding the mechanism of photosynthesis was the discovery that the oxygen released by plants during photosynthesis is derived from water and not from carbon dioxide. This had been predicted in the 1930s by CB van Niel, a Dutch-American microbiologist, researching photosynthesis in bacteria. Working at Cambridge a short time later, Robert Hill was able to demonstrate that, in the presence of light and a suitable electron acceptor, isolated chloroplasts will generate oxygen even if no carbon dioxide is present, thus confirming that the oxygen which is generated during the light dependent stage of photosynthesis comes from water rather than from carbon dioxide.

$$2H_2O + 2A \longrightarrow 2AH_2 + O_2$$

Here 'A' represents an artificial electron acceptor. During what has come to be known as the Hill reaction, 2, 6-dichlorophenol-indophenol (DCPIP), which is blue in its oxidised form and colourless when it is reduced (DCPIPH2), is the electron acceptor.

$$DCPIP + 2H^+ + 2e^- \longrightarrow DCPIPH_2$$

$$2H_2O + 2DCPIP \xrightarrow[\text{Chlorophyll}]{\text{light}} 2DCPIPH_2 + O_2$$

Hill concluded that:

- When leaf extract was illuminated, a non-biological electron acceptor (dye) became colourless and oxygen was evolved
- In the dark neither reduction of the dye nor evolution of oxygen took place.
- Oxygen was neither required nor was it reduced under these conditions i.e. oxygen evolution did not involve carbon dioxide.

Hill was able to hypothesise that water had been split into oxygen and hydrogen.

Following Hill's discoveries it has been shown that the electrons and hydrogen produced by the splitting of water in chloroplasts are accepted by the coenzyme NADP.

$$2H_2O + 2NADP^+ \xrightarrow{\text{light}} 2NADPH + 2H^+ + O_2$$

For learners at Higher level the light dependent reactions of photosynthesis may be summarised as:

- Light energy is absorbed by chlorophyll and other pigments creating excited electrons.
- High energy electrons are transferred through an electron transfer chain releasing energy and generating ATP.
- Energy is also used to split water (photolysis) into oxygen which is released and hydrogen which is accepted by NADP to form NADPH.

The following protocol describes a method which might be used in the classroom to demonstrate the Hill reaction.

photosynthesis

Demonstrating the Hill reaction

Equipment

- Fresh spinach leaves
- Scissors
- 5 x test tubes and parafilm covers
- 1 x 100 cm^3 beaker
- 1 x large beaker
- 1 x microcentrifuge tube
- 1 x foam tube holder

- 1 x 10 cm^3 syringe
- 5 x 1 cm^3 disposable pipettes
- Mortar and pestle
- 1 x filter funnel
- 1 x Bijou bottle
- Muslin
- Aluminium foil

- Stopwatch
- Bench lamp
- Crushed ice
- Centrifuge
- Ice bath
- Water bath set at 800° C
- DCPIP solution
- Buffer solution

DCPIP (dichlorophenolindophenol) **Solution:** 0.01 g DCPIP in 100 cm^3 of buffer solution.
Buffer solution: 2.8 g anhydrous disodium hydrogen phosphate (Na_2HPO_4), 6.4 g potassium dihydrogen phosphate (KH_2PO_4), 102.8 g sucrose, potassium chloride, 1 litre water.

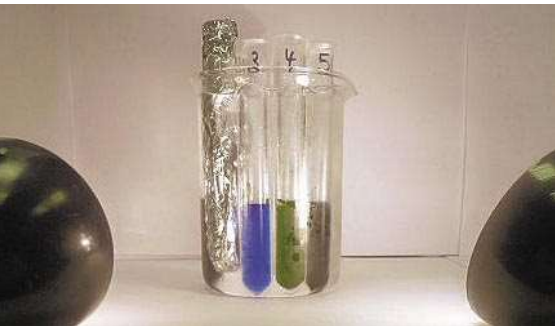

Figure 3

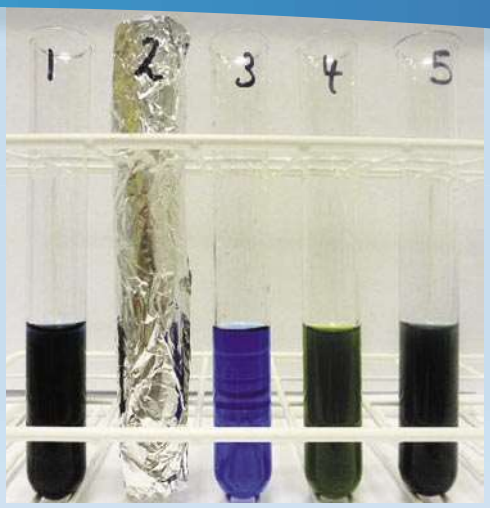

Figure 1

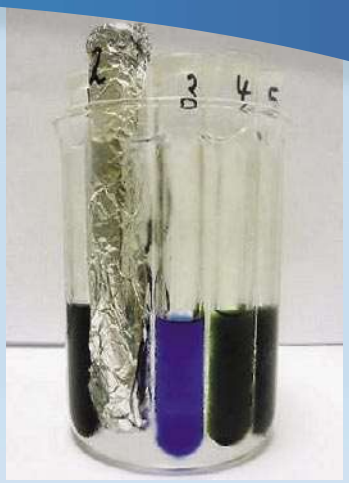

Figure 2

Method

Note:
It is essential that all solutions, tubes and other equipment are chilled beforehand and kept in melting ice until the DCPIP reduction stage.

1) Chop 3 fresh spinach leaves (excluding stems) into a mortar. Add 10 cm^3 of buffer solution and grind with the pestle.
2) Quickly strain the mixture through muslin held in a filter funnel over a small beaker. Squeeze the liquid through the muslin by gathering the corners and twisting them. Discard the solid contents.
3) Fill the microcentrifuge tube with the suspension. Label the tube with your initials. Centrifuge the suspension for 10 minutes.
4) While the centrifuge is running, prepare 5 test tubes for the next stage as follows:
   - Label the test tubes 1-5.
   - Cover test tube 2 with aluminium foil to exclude light.
   - Place all 5 tubes in a large beaker of iced water.
   - Using the syringe, add 3 cm^3 buffer solution to tubes 1, 2, 4 and 5. Add 4 cm^3 buffer solution to tube 3.
   - Using a clean pipette, add 2 cm^3 DCPIP to tubes 1, 2 and 3.
   - Using a clean pipette add 2 cm^3 distilled water to tube 4.
5) Once the 10 minute centrifuge run is complete, pour off the supernatant taking care not to lose the pellet. Discard the supernatant.
6) Place 5 cm^3 buffer solution in the Bijou bottle. Using a pipette add a little of this buffer solution to the pellet in the microcentrifuge tube and mix to re-suspend the pellet. It is important that the re-suspension is done thoroughly. Add this re-suspended mixture to the buffer solution in the Bijou bottle and mix well to produce an appropriate volume of chloroplast suspension.
7) Using a fresh pipette, add 1 cm^3 chloroplast suspension to tube 5. Cover the tube with parafilm and place it in the water bath for 2 minutes. Allow to cool. Add 2 cm^3 DCPIP. Place the tube in the beaker of iced water.
8) Now add 1 cm^3 chloroplast suspension to tubes 1, 2 and 4.
9) Cover all 5 tubes with parafilm, shake each gently and note the starting colour of the contents [Figure 1].
10) Replace the tubes in the beaker of iced water ensuring that they are lying against the inner surface of the beaker [Figure 2].
11) Place the beaker in bright light [Figure 3] and examine the contents of the tubes for disappearance of the blue colour after 5 minutes, 10 minutes and 20 minutes [Figure 4].

Figure 4 *- This photograph, taken in natural sunlight, shows the resulting colours in the test tubes after 20 minutes.*

A detailed protocol for this activity can be found on the SSERC website.

Table 1 provides a summary of the contents and treatment of each test tube.

What might learners conclude from the appearance of the contents of each tube?

- Test tube 1 demonstrates that in the presence of light and a chloroplast suspension DCPIP loses its blue colour indicating that it has been reduced.
- Test tube 2 demonstrates that the reactions in test tube 1 will not proceed without the presence of light.
- Test tube 3 demonstrates that without the 'reducing agent' supplied by the chloroplasts DCPIP will not be reduced.
- Test tube 4 shows that the colour of the chloroplast suspension and buffer solution remains stable - the buffer solution does not change the colour of the chloroplast suspension
- Test tube 5 demonstrates that the presence of intact chloroplasts is required for the reduction of the DCPIP in this scenario.

Like Hill, learners with support might reasonably conclude that in bright light a reaction happens in chloroplasts which results in water being split into oxygen and hydrogen. The reduction of DCPIP is evidence that hydrogen from the photolysis of water has been produced. This provides a context in which to discuss, the role of chlorophyll and electron transfer in the capture of solar energy and the production of ATP and NADPH in the light dependent reactions of photosynthesis.

	Tube1	Tube 2	Tube 3	Tube 4	Tube 5
Covered with aluminium foil	No	Yes	No	No	No
Buffer solution	3 cm^3	3 cm^3	4 cm^3	3 cm^3	3 cm^3
DCPIP	2 cm^3	2 cm^3	2 cm^3		2 cm^3 *
Distilled water					2cm^3
Chloroplast suspension	1 cm^3	1 cm^3		1 cm^3	1 cm^3
Heated to 800° C for 2 minutes **	No	No	No	No	Yes

** Add DCPIP to tube 5 after heating buffer and chloroplast suspension mixture.*
*** Heat tube 5 before adding chloroplast suspension to tubes 1, 2 and 4.*

Table 1

In the *NQ Higher Biology (2002)* photosynthesis is located in the unit called Cell Biology. Here the emphasis is on both photosynthesis and respiration as major 'biochemical conversions' taking place within cells [3]. In the new *NQ (CfE) Higher Biology (2014)* the study of photosynthesis is positioned at the beginning of Unit 3, *Sustainability and Interdependence* [4]. While knowledge of the biochemical conversions involved in photo-synthesis is still required, there is a shift in emphasis that reflects current biological research into understanding photosynthetic processes in the context of of world food production:

"Food production is an area of vital importance for biological research. An understanding of photosynthesis lies at its core. Studies should focus on the energy-gathering process and the transfer of high-energy electrons through an electron transfer chain to generate ATP. The action of RuBisCo as part of the Calvin cycle should be included as this is the carbohydrate-forming stage." [5]

SSERC has developed a discussion activity [Figure 5] which might also spark the interest of learners to do some follow-up research of their own into the role of RuBisCo in carbohydrate production [6], C3 and C4 photosynthesis and the genetic modification of food crops [7, 8] especially 'Turbo-charged Rice' [9].

Figure 5 *- Discussion cards, Let's Talk Genetic dilemmas.*

Curriculum links

Higher Biology Unit 3 - *Sustainability and Interdependence*

Mandatory Course - *key area (b) (i) Photosynthesis*

Absorbed energy excites electrons in the pigment molecule. Transfer of these high-energy electrons through electron transport chains releases energy to generate ATP by ATP synthase. Energy is also used for photolysis, in which water is split into oxygen, which is evolved, and hydrogen, which is transferred to the coenzyme NADP....

The enzyme RuBisCO fixes carbon dioxide by attaching it to ribulose bisphosphate (RuBP) in the Calvin cycle. The intermediate produced is phosphorylated by ATP and combined with hydrogen from NADPH to form glyceraldehyde-3-phosphate (G3P). G3P is used to regenerate RuBP and for the synthesis of sugars. These sugars may be synthesised into starch or cellulose or pass to other biosynthetic pathways to form a variety of metabolites.

References

[1] *Curriculum for Excellence* Science Experiences and Outcomes are available at http://www.educationscotland.gov.uk/myexperiencesandoutcomes/sciences (accessed August 29th 2014).
[2] Course and Unit Support Notes for the *new NQ (CfE) Higher Biology* are available via the SQA website www.sqa.org.uk/ (accessed August 29th 2014).
[3] Arrangements documents for the *NQ Higher Biology* are available at http://www.sqa.org.uk/sqa/39306.html (accessed August 29th 2014).
[4] Course and Unit Support Notes for the *new NQ (CfE) Higher Biology* are available via the SQA website www.sqa.org.uk/ (accessed August 29th 2014).
[5] Course Support Notes for the *new NQ (CfE) Higher Biology* (Sustainability and Interdependence, Introduction), http://www.sqa.org.uk/files_ccc/CfE_CourseUnitSupportNotes_Higher_Sciences_Biology.pdf
[6] Available at http://www.sserc.org.uk/index.php/biology-2/biology-resources/higher-biology-revised/sustainability-a-interdependence/3747-the-science-of-food-production-2 (accessed August 29th 2014).
[7] Student support material, SAPS available at http://www.saps.org.uk/students/further-reading/1266.
[8] http://www.saps.org.uk/secondary/teaching-resources/828-genetic-engineering-and-photosynthesis.
[9] International Rice Research Institute, http://irri.org/news/media-releases/rice-of-the-future-gets-financial-boost.