Using leaf discs to investigate photosynthesis – advice for teachers and laboratory technicians

There are three practical activities associated with this resource.

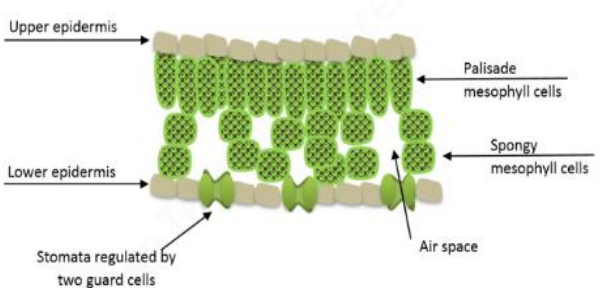
- **Part A:** Aim – to explore a method that uses leaf discs to measure rate of photosynthesis
- **Part B:** Aim – to measure the effect of light colour (wavelength) on the rate of photosynthesis
- **Part C:** Aim – to measure the effect of carbon dioxide concentration on the rate of photosynthesis

These practical activities can be used to support assessment of Unit 3 outcome 2.

Unit 3 Outcome 2	**SAC task type**	**Relevant key science skills you may choose to assess**
Analyse the structure and regulation of biochemical pathways in photosynthesis and cellular respiration, and evaluate how biotechnology can be used to solve problems related to the regulation of biochemical pathways.	Comparison and evaluation of biological concepts, methodologies and methods, and findings from three student practical activities	• Develop aims and questions, formulate hypotheses and make predictions • Plan and conduct investigations • Generate, collate and record data • Analyse and evaluate data and investigation methods • Construct evidence-based arguments and draw conclusions • Analyse and evaluate and communicate scientific ideas

The practical activities can be used in many ways. You can choose to focus on different key science skills for each practical activity providing an opportunity for students to achieve a satisfactory completion of the outcome, or to practice skills in preparation for a SAC task. You may gradually provide less scaffolding for each practical, so they move towards autonomous design, analysis and evalutation. You can focus in on areas to develop a SAC task to measure levels of achievement. You may choose to provide different students with part B and part C. The practical activities are designed to support students using a logbook.

Background on the leaf disc method to measure rate of photosynthesis



This method employs the principal of changes in buoyancy of leaf tissue when the leaf is producing O_2 by photosynthesis. Students punch out small discs from leaves and use a syringe containing sodium bicarbonate solution to force out the gasses trapped in the spongy mesophyll layers of the leaf making them sink in solution and, at the same time, infiltrating the tissue with bicarbonate solution to provide a source of carbon for photosynthesis. When exposed to light, photosynthesis occurs, and oxygen is produced and trapped in the intercellular spaces within the leaf tissue making them buoyant and float. The rate of disc floatation is an indirect measure of the rate of photosynthesis, providing a simple quantitative assay for the school laboratory.

The procedure does not require any expensive equipment; however, a strong light source or light bank may be required if you are unable to do your experiment on a sunny day outside or near brightly lit windows.

The method can be used to perform a variety of controlled experiments to measure the effect of one factor on the rate of photosynthesis. Factors include light intensity, light colour (wavelength), carbon dioxide concentration, pH, and chemical inhibitors, such as those found in weed killer.

Lab preparation:

Reagents for preparation of 1 Litre of 0.2% sodium bicarbonate solution with detergent

- ☐ 2 g sodium bicarbonate ($NaHCO_3$) – or supermarket grade Baking soda.
- ☐ Dishwashing or laundry detergent: The detergent acts as a wetting agent. Wetting agents or surfactants lower the surface tension between two liquids or a liquid and solid. Their hydrophobic and hydrophilic components bring hydrophobic surfaces, such as leaves, in contact with water. The detergent also helps prevent the leaf discs from sticking to the sides of the syringe.
- ☐ Distilled H_2O or tap water.

Method: Dissolve 2g baking soda in 1L dH2O with 4-5 drops detergent added.

NOTE: Adapt this method to make 0.4%, 0.6% and 0.8% sodium bicarbonate solutions (used in part C – controlled experiment for measuring the effect of carbon dioxide concentration on rate of photosynthesis)

Materials required for the whole class:

- ☐ Strong source of light
- ☐ Fresh leaves* (e.g. spinach or baby spinach, ivy)
- ☐ 10mL syringes

***Plant material**: Summary of key points about plant choice from our own tests and other references

- the plant must be fresh and in good health
- use smooth rather than hairy leaves
- use thin rather than thick leaves
- we have found fresh spinach and baby spinach sealed in bags from the supermarket work very well.
- plants reported by others to work well: radish cotyledons, 'fast plant' cotyledons, ivy, clover, geranium.
- apparently, some leaves, like watercress, just don't work
- plants do not need to be de-starched
- run a sinking-floating trial on the chosen leaves before asking students to conduct their experiment.

Materials (per student group to conduct an experiment with 4 different light conditions)

- ☐ 10mL syringe (as many as possible – at least 1/pair of students)
- ☐ 200mL of 0.2% solution of sodium bicarbonate (with 4-5 drops detergent added per litre)
- ☐ hole punch
- ☐ Fresh leaves* (enough material for 50 discs made with the hole punch)
- ☐ stopwatch or timer
- ☐ 5 x 50mL beakers, clear plastic bottles or clear plastic cups
- ☐ red, green, blue and colourless cellophane or lighting filters

To conduct an experiment measuring effect of different carbon dioxide concentrations swap cellophane for:

- ☐ 200ml of each of the following sodium bicarbonate solutions - 0.2%, 0.4%, 0.6% and 0.8%

Procedure

Technical note: students work in groups of 4-6. For a well-controlled experiment, they need to coordinate carefully so all discs are set up at the same time so the experiment is carried out under same sunlight conditions. Disc infiltration is the slow part so using a 10mL (rather than 5mL) syringe or more than one syringe per group, to prepare more discs at once, will speed up the process. Beware squirting syringes. Use an eraser or rubber bung instead of finger to help create the vacuum. Wear safety glasses.

Cut leaf discs

1. Collect fresh leaves
2. Using a hole punch, punch out 50 or more discs avoiding the veins and bruised areas

Infiltrate leaf discs with bicarbonate solution. The student practical illustrates the procedure.

1. Remove the syringe plunger and place the leaf discs into the syringe barrel (10 – 20 discs per 10mL syringe). Replace the plunger being careful not to crush the leaf discs. Push on the plungeruntil only a small volume of air and leaf discs remain in the barrel.
2. Pull sodium bicarbonate solution into the syringe to 3/4 fill the syringe. Tap the syringe tosuspend the leaf discs in the solution.
3. Hold a finger or rubber stopper/eraser against the syringe-opening to prevent air and liquid escaping. Press the plunger forcefully **or** draw back on the plunger to create a vacuum. Hold this vacuum for about 10 seconds. While holding the vacuum, swirl the leaf discs to suspend them in the solution. Release the vacuum. The bicarbonate solution will infiltrate the air spaces in the leaf tissue, causing the disks to sink. You may need to repeat this procedure 2-3 times to get all discs to sink. Perform this procedure for all leaf discs required for your experiment.
4. Rinse the syringe thoroughly and prepare **10 control infiltrate** leaf discs with water with a drop of detergent (no bicarbonate). *Choice of an appropriate control would be a good discussion point for students prior to conducting the experiment.* Place these 10 discs in a container and add bicarbonate solution to a 3cm depth.
5. Pour all the infiltrated discs and solution into a Petri dish. Distribute them into your experimental containers (clear plastic cups, bottles or small glass beakers); 10 discs per container.
6. Add bicarbonate solution to a depth of 3 cm. Use the same depth for each container.

Technical notes:

- *Pushing the syringe against a rubber stopper/eraser works well (rather than against a finger).*
- *If you overload the syringe with discs it is harder to get all the discs to sink. 10 discs per 10mL syringe works well.*
- *If discs do not sink after about 3 evacuations, it may be because there is not enough detergent in the solution. Add a 2-3 more drops of detergent to the sodium bicarbonate solution and try again.*
- *If the solution turns very green, this is a sign of tissue damage and the discs may not give a reliable result*
- *Large quantities of discs can be evacuated in a side-arm flask attached to a vacuum pump.*

Continuing with the method for part A - C:

Part A – Explore a method that uses leaf discs to measure rate of photosynthesis

- Place the 2 containers (0.2% sodium bicarbonate & water) under the light source and start the timer. At the end of each minute, record the number of floating disks. Then swirl the disks to dislodge any that are stuck against the sides of the cups. Continue until all the disks are floating

Part B – measure the effect of light colour on the rate of photosynthesis

- Prepare 4 lots of discs infiltrated with 0.2% sodium bicarbonate solution as described in steps 1-6
- Wrap or cover the containers in different coloured cellophane and secure with elastic band
- Place all 4 containers under the light source and start the timer. At the end of each minute, record the number of floating disks. Then swirl the disks to dislodge any that are stuck against the sides of the cups. Continue until all the disks are floating.

Part C – measure the effect of carbon dioxide concentration on the rate of photosynthesis

- Prepare 4 lots of infiltrated discs – one with 0.2%, one with 0.4%, one with 0.6%, and one with 0.8% sodium bicarbonate solution as described in steps 1-6
- Set up 4 beakers each containing a different concentration of the sodium bicarbonate solution (0%, 0.2%, 0.4%, 0.6% and 0.8%). Add the infiltrated leaf discs (match the sodium bicarbonate concentrations in the disc and beaker)
- Place all 4 containers under the light source and start the timer. At the end of each minute, record the number of floating disks. Then swirl the disks to dislodge any that are stuck against the sides of the cups. Continue until all the disks are floating

Generate, collate and record data

Notes to teachers: data below was retrieved from a demonstrationusing grape ivy leaf discs published at *https://cpb-us-e1.wpmucdn.com/blogs.cornell.edu/dist/3/1009/files/2015/09/Floating-Leaf-Disk-Brad-Williamson.pdf*

WHAT IS ET_{50}? The point at which 50% of the leaf discs are floating (the **median**) is the point of reference for this procedure.

It is called ET_{50} (**E**xtrapolated **T**ime for **50**% of discs to float)

Graph the results: x-axis is time (mins) and y-axis is number of discs floating

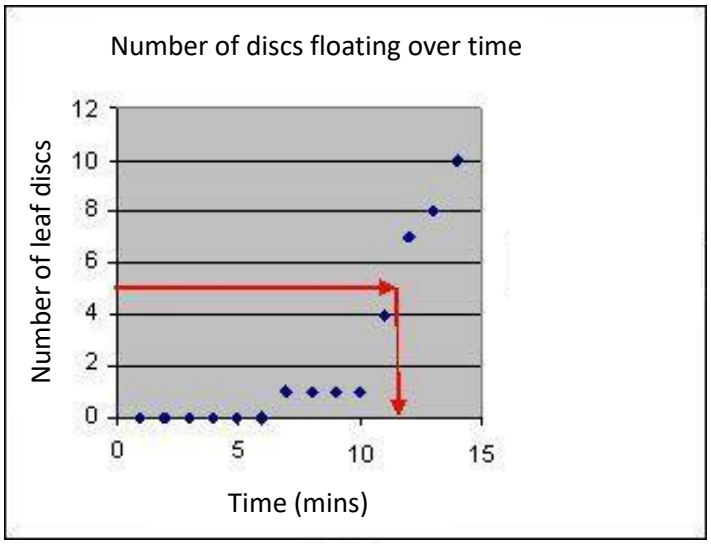


Table 1	
Minutes	Disks
1	0
2	0
3	0
4	0
5	0
6	0
7	1
8	1
9	1
10	1
11	4
12	7
13	8
14	10

Extrapolate from the graph the time where 50% of the leaf discs are floating: approximately 11.5 minutes. Using the 50% pointprovides a greater degree of reliability and repeatability for this procedure. This point is referred to as the ET_{50}.

The ET_{50} decreases as the rate of photosynthesis increases (it is an inverse relationship).

It is easier to understand a positive relationship between the value and the parameter being measured (rate of photosynthesis), therefore we calculate the inverse of ET_{50} (that is $1/ET_{50}$)

Analyse and evaluate data and investigation methods

Example : Experimental analysis for the effect of light wavelength on the rate of photosynthesis:

1) Measure the floating time for each of the four colours of light
2) Graph the results
3) Extrapolate from the graphs to find the ET_{50}, i.e.
   i) read from the graph the time at which half of the discs were floating
   ii) record this ET_{50} value
4) Calculate $1/ET_{50}$
5) Graph the final result Rate of Photosynthesis v Colour of Light

References: This practical is based on information from Science and Plants in Schools, University of Reading, UK http://www.saps.org.uk/secondary/teaching-resources/284-investigating-the-behaviour-of-leaf-discs-

All tables and graphs can be adapted for Part C