BIO LAB:
ALGAL BALLS

TEACHER

LEVEL: Year 11-12 | TOPIC: CELLS | TIME REQUIREMENT: 90 mins

CURRICULUM ALIGNMENT

- *In eukaryotic cells, specialised organelles facilitate biochemical processes of photosynthesis, cellular respiration, the synthesis of complex molecules (including carbohydrates, proteins, lipids and other biomacromolecules), and the removal of cellular products and wastes (ACSBL049)*
- *Cells require inputs of suitable forms of energy, including light energy or chemical energy in complex molecules, and matter, including gases, simple nutrients, ions, and removal of wastes, to survive (ACSBL044)*
- *The cell membrane separates the cell from its surroundings and controls the exchange of materials, including gases, nutrients and wastes, between the cell and its environment (ACSBL045)*
- *Photosynthesis is a biochemical process that in plant cells occurs in the chloroplast and that uses light energy to synthesise organic compounds. The overall process can be represented as a balanced chemical equation (ACSBL052)*

BACKGROUND

During photosynthesis, radiant energy is converted into organic substances that can be stored within the plant to support growth, reproduction, and metabolism. Plants metabolise the sugars that form as a result of sunlight, Carbon Dioxide, and water reacting. Reliably studying photosynthesis first hand can be difficult and require precise setting up. Algal ball photosynthesis kits offer an easier, more accurate method of studying photosynthesis that is perfect for use within the classroom. Algal balls are stored in small vials filled with Hydrogen Carbonate indicator solution. When exposed to light, the encapsulated algae absorb the CO_2 from the solution they are stored in during the process of photosynthesis. As a result of the lowered CO_2 levels, the pH of the solution will rise. When no light is available, respiration will dominate and the pH will decrease through the release of CO_2. Changes in the levels of carbonic acid can be monitored via the colour changes that occur due to the hydrogen carbonate indicator.

In this practical, students have the opportunity to tangibly observe photosynthesis. They are tasked with observing how the colour changes from yellow/orange to purple when the algal balls and solution are placed under a light source as photosynthesis takes up dissolved CO_2 and the pH rises. Additionally, students create a light filter to test whether the wavelength of light affects their photosynthetic rate. This is a great practical to introduce students to the basic principles of photosynthesis in a fun, simple and interactive way.

TEACHER TIP

If you are providing students with the already prepared 7mL vials with the algal balls and indicator, it may be a good idea to expose them to light for half an hour or so beforehand to start photosynthesis.

MATERIALS

- Algal Ball Photosynthesis Kit

or

- 60 algal balls
- 4 × 7 mL empty dram vials
- 40 mL (approx.) hydrogen carbonate indicator
- Plastic pipette

and

- Light source
- Set of pH standards or colour chart
- Red, purple and green cellophane
- Strainer
- Spoon
- Disposable gloves (optional)

SAFETY PRECAUTIONS

- Wear appropriate personal protective equipment (PPE).
- Know and follow all regulatory guidelines for the disposal of laboratory wastes.
- Avoid direct contact with any culture.
- Wash and dry your hands thoroughly before and after any experiment.

SOUTHERN Biological

PREPARATION - BY LAB TECHNICIAN

Making Algal Balls (If making your own algal balls)

1. To make a 3% solution of sodium alginate, add 3g sodium alginate to 100mL of distilled water in an Erlenmyer flask. Stir continuously for at least 4 hours, or overnight using a magnetic stirrer. Do not apply any heat.
2. To make 500mL of 2% calcium chloride, add 10g of calcium chloride to 500mL of distilled water and stir to dissolve.
3. To prepare chlorella culture: Once your chlorella culture has grown enough to be a bright to darkish-green, siphon out as much of the denser areas into a measuring cylinder. Leave the culture to rest overnight to allow the algae to settle to the bottom of the cylinder. After this time, there should be a dark "plug" of dense algae at the bottom.
4. To prepare chlorella alginate solution, take note of the volume of the plug, then carefully remove the supernatant until it accounts for 2/3 of the total volume in the measuring cylinder, with 1/3 dense alginate. For example, if you have 10mL of chlorella down the bottom, remove all but 20mL of supernatant so that you are left with 30mL in total. Keep the supernatant aside. If the mixture is too thick later to drop easily through the syringe, then you can use this to thin it out a little. Mix the remaining chlorella and supernatant in the measuring cylinder and add to an equal volume of your sodium alginate, e.g., 30mL of chlorella and 30mL of alginate to make 60mL altogether.
5. To set up retort stand apparatus, attach a 30mL syringe barrel to the retort stand clamp so that the outlet is pointing downwards. Position the syringe barrel over a beaker of calcium chloride solution.
6. To make algal balls, transfer your algae/alginate mix to the syringe, and allow it to drop through into the solution. Check the rate of the drops and the shape of the balls as they fall to the bottom.
    - If the rate is slower than a drop or two per second, add a small amount of the supernatant.
    - If the balls flatten on entering the liquid, the syringe is too high and should be lowered.
    - If the mixture comes through the syringe is too low, you may end up with "sausages" of algae rather than balls.
7. Leave the algal balls in solution for 5-10 minutes then rinse with distilled water. If you do not plan to use immediately, store in a cool place away from direct sunlight (but not total darkness) and use within 2 weeks.
8. If you are using a 10x concentrate hydrogen carbonate indicator, add 10 mL of the indicator to 90mL of distilled water to dilute.

Algal Ball Culture Care (if you purchase the algal balls)

1. The algal balls will arrive stored in a container of distilled water.
2. The Chlorella within the balls is alive and therefore needs light to stay active. We recommend that you leave the balls in the container that they arrive in, and that you place this vial in a well lit area until required. Do not place in direct sunlight, nor expose to hot light sources, as the heat will be detrimental.
3. It will also help to loosen the lid of the container to allow air access.
4. Use the algal balls as early as you can; otherwise, they are best used within 2 weeks.

Vial Preparation

1. Rinse the vials for your experiment using hydrogen carbonate indicator solution. To do this, add approximately 1mL of hydrogen carbonate indicator solution to a vial, replace the cap on the vial and shake.
2. Next, pour the rinse liquid into the second vial and repeat until you have rinsed all required vials. Discard the rinse liquid from the last vial.

METHOD - STUDENT PRACTICAL

1. Separate the algal balls from the surrounding liquid using the strainer. To do this, pour the algal balls into a strainer over a small beaker.
2. Use the spoon to place an equal number of balls into each dram vial.
3. Using a plastic pipette, fill all the vials with the hydrogen carbonate indicator. Make sure the caps are secured.
4. Keep one vial to act as your control.
5. Wrap a different piece of coloured cellophane around the other three vials.
6. Place each vial approximately 10 cm away from your light source. Make sure the vials do not get hot.
7. Copy the results table into your logbook and complete column A by comparing the colour of the hydrogen carbonate indicator to the set of pH standard solutions. You can use the image below (Figure 1) as a guide if standards are not available, however comparing the colour of the liquid in your vials to actual pH standard solutions will provide the most accurate results.
8. After 40 minutes complete column B of the results table by comparing the colour of the hydrogen carbonate indicator to the set of standard reference.
9. Complete the final column of the results table by subtracting the amount in column A from the amount in column B.

Figure 1: Set of pH standard solutions

OBSERVATION AND RESULTS

After exposing the vial to a light source, the colour in the solution should change from yellow/orange to purple as photosynthesis absorbs the CO_2 and the pH rises. The pH of vials wrapped red, blue or purple cellophane will experience a greater rise than vials wrapped orange, yellow or green. This is due to the way the algae absorb light - we observe them as green because that is the colour that bounces off them out of the spectrum that makes up white light and so that is the colour that is not used in photosynthesis. Some change should be expected by the end of the class, however if it is too slight to notice you can return at the end of the day or the next day to check your vials.

Cellophane colour	Colour of solution before exposure (A)	Colour of solution after exposure (B)	Colour change (B – A)
None (control)	7.6	9.2	1.6
Red	7.6	8.8	1.2
Purple	7.6	8.6	1
Green	7.6	8.4	0.8

Table 1: Colour change after exposuure

INVESTIGATION

- Carbon dioxide dissolved in water forms carbonic acid. Hydrogen carbonate indicator is used to measure the acidity of a system. The pH of the system is low (yellow) when there is a lot of dissolved CO_2. As CO_2 is removed, the pH rises and the colour becomes purple. Use this information to construct a bar graph of CO_2 changes as a function of wavelength.
- Why do we include a control in the experiment? What does this control represent in term of light wavelength?
    - The control represents all of the wavelengths of light, as it is not obscured by a colour filter. This control is used to compare the level of photosynthesis that takes place in the vials. All three vials are placed under the same conditions, to see whether the colour filters slow down photosynthesis or block usable wavelengths entirely.
- Describe the process that is happening in the vials with regards to photosynthesis and respiration.
    - Both photosynthesis and respiration are happening all of the time, with photosynthesis dominating when light is available and respiration dominating in the absence of light. During photosynthesis, carbon dioxide and water plus the energy from light synthesise glucose and oxygen. As carbon dioxide is used up, carbonic acid in the water is converted to carbon dioxide, raising the pH of the water.
    - During respiration, glucose is broken down along with oxygen to make carbon dioxide and water. As carbon dioxide enters the water, excess molecules are converted to carbonic acid, lowering the pH of the water.

EXTENSION EXERCISES

Light availability is another factor that affects photosynthesis. Design an experiment to test how distance from light effects the rate of photosynthesis.