Algae Immobilised in Alginate Balls

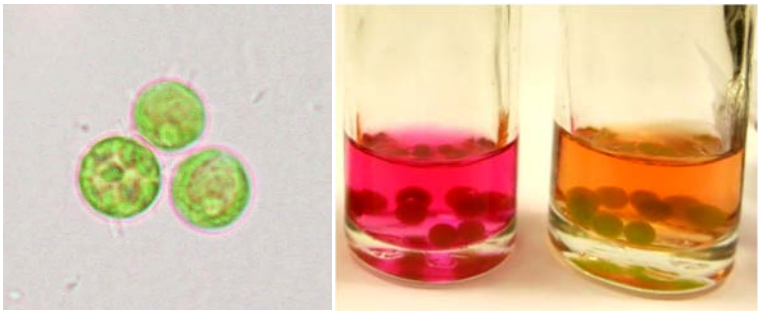
Studies of photosynthesis and respiration

Immobilising cells in small balls or beads of calcium alginate is a significant industrial procedure. For example, immobilised algae are being trialled for biofuel production, immobilised yeast are being used for champagne production, and specific species of bacteria have been immobilised and used for cleaning drinking water.

Sodium alginate is a viscous liquid when dissolved at 1.5-4%. To entrap algae in the balls, equal volumes of cell suspension and sodium alginate are mixed and added drop-wise to calcium chloride solution. Calcium ions link the alginate monomers together to make a gel-like polymer of calcium alginate. Live cells are thus trapped and immobilised in the small balls and can be used for experiments or other biological processes. Unicellular, non-motile algae such as *Chlorella* are suitable readily available species.

In this practical you will prepare alginate balls with algae and conduct an experiment to test the conditions in which the algae conduct photosynthesis and respiration is possible using immobilised algae.

Figure 1 Chlorella viewed under the light microscope (1000x magnification)(left);Algae in alginate balls during experiment (right)



Materials

To make 10mL algae-alginate mix

- 1.5% sodium alginate solution.
    - Different batches of alginate vary. These notes describe starting with 1.5% sodium alginate mixed 1:1 with the cell suspension.
- 5mL healthy culture of *Chlorella* or *Scenedesmus*
- 2% $CaCl_2$; 50mL in a 100mL beaker
- Transfer pipette
- 10mL syringe (optional)
- Tea strainer
- Tap water (tap or squirt bottle)
- Spatula or forceps
- Screw capped bottle for storage
- Petri dishes
- pH indicator – phenol red (0.001% phenol red: 1mL stock solution per 100mL tap water) or hydrogen carbonate indicator
- Sample bottles, beakers or tubes for experimental set up
- 0.1M HCl and 0.1M NaOH
- 10mL pipette or measuring cylinder

Methods

Prepare 1.5% sodium alginate

a) Measure 100mL dH_2O into a clean glass bottle (rinse the bottle thoroughly with dH_2O prior to making this solution to ensure no calcium ions are present)
b) Weigh 1.5g Na-alginate powder, add it to the water
c) Stir with magnetic stirrer overnight. It forms a thick viscous solution.
d) Keep the solution at 37°C for preparing the alginate balls.

The solution can be kept at room temperature for a few days and warmed and stirred again prior to making more alginate balls.

Prepare 2% calcium chloride

- Dissolve 2.0g calcium chloride ($CaCl_2$) in 100mL dH_2O
- Approximate volumes to use: 20mL for preparing up to 40 alginate balls (about 3mL of alginate – cell mix)
- Have a beaker with 20-50mL of 2% $CaCl_2$ ready before you start mixing the algae and alginate

Prepare water with 0.001% phenol red indicator

- Phenol red indicator stock solution (0.1%)
- Add 1mL per 100mL tap water. It may appear a slightly yellowy red. Phenol red is yellow when acidic (<pH 6.2), red between pH 6.2 and 8.2, pink when basic (> pH8.2).

Immobilising algae in alginate balls (for 10mL)

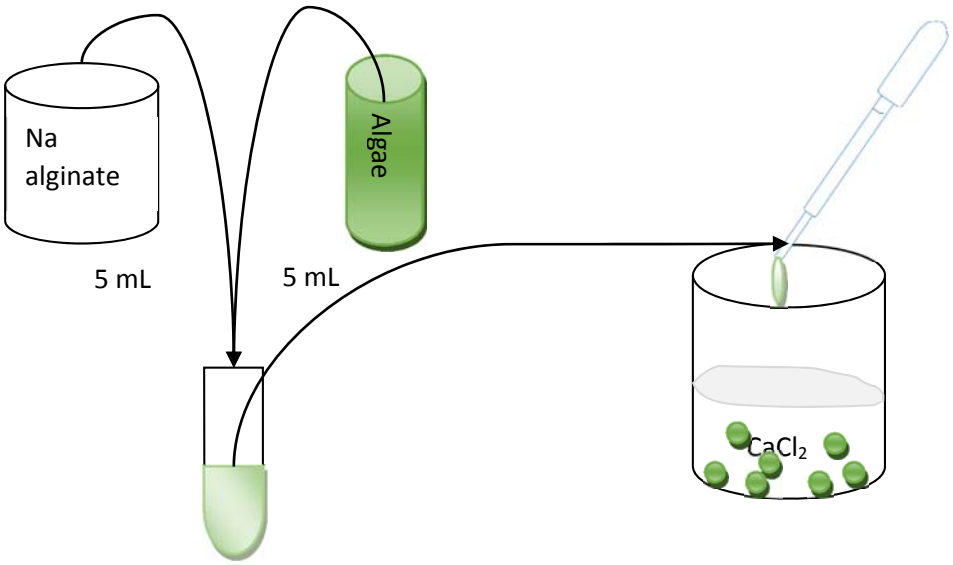
a) Grow or purchase a fresh culture of small non-motile unicellular algae such as *Chlorella*
b) If the culture is at a very high density (a dark green when mixed, you can use 5mL directly from the culture. Otherwise concentrate the cells by letting them settle. For example, let 10ml or more of algae culture settle so the cells are at the bottom of a tube. Remove culture medium to leave 5mL remaining. If you have a centrifuge, spin them for 2 min at a fairly gentle speed (e.g. 500 -1000 rpm) to bring them to the bottom of a tube.
c) To 5mL algae add 5mL warm 37°C Na-alginate (use a cut-tip transfer pipette for the viscous alginate)
d) Gently mix the algae and alginate to a uniform mixture – if using small volumes in a tube, mix with the cut-tip transfer pipette. If using larger volumes in a beaker, swirl until mixed uniformly.
e) Add the algae-alginate mix drop wise into a beaker with 2% $CaCl_2$, swirl the $CaCl_2$ solution as you add the drops. As soon as the mixture hits the $CaCl_2$ the alginate solidifies into little balls. Continue until all the mix is used.
    - Use a cut-tip transfer pipette for small volumes; need to practice controlling the bulb to get uniform drops
    - Alternatively use a syringe for volumes of 5-10 mL; though controlling the plunger is not necessarily any easier than using a bulb pipette
    - For large volumes use a burette set at a constant fairly slow drip rate
f) Transfer the balls to a tea strainer and rinse thoroughly with tap water to get rid of the $CaCl_2$
g) Keep the balls of immobilised algae in tap water.
h) Surplus algae balls can be stored in the refrigerator (not the freezer) for a few days for further experiments.

HOW many balls do you get?

Example: 1.5mL algae + 1.5mL alginate, using a cut-tip transfer pipette, gave 40 balls.

That's enough for students to set up 4 tubes with 10 balls per tube.

THE SUPPLY OF ALGAE IS A LIMITING FACTOR FOR THIS PROCEDURE



Testing the effect of pH on colour of phenol red

Testing the pH indicator colour change. The indicator phenol red usually shows the colour changes from yellow (acidic), red (neutral) to pink (basic).

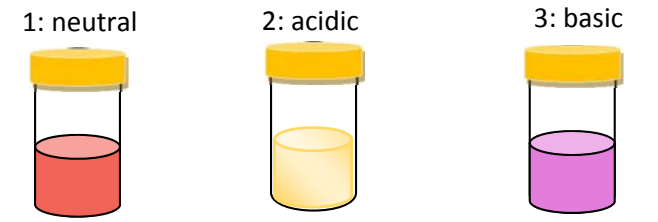
Phenol red colour range		
Yellow ----------	Red ----------	Pink
<pH6.2	pH 6.2-8.2	>pH 8.2

First, test that the water or solutions you will use for the yeast ball do show these colour changes.

Tube 1: 10mL tap water or other solution to be used in tests

Tube 2: 10mL tap water or other solution to be used in tests + 1-2 drops hydrochloric acid (HCl, an acid)

Tube 3: 10mL tap water or other solution to be used in tests + 1-2 drops sodium hydroxide (NaOH, a base)



If a different pH indicator, such as universal indicator, is used you need to perform the same type of test to establish the colour changes to be observed when experimenting with cells.

Photosynthesis and respiration experiments with immobilised algae

Step 1 Place 10 algae balls in each of 2 tubes or beakers

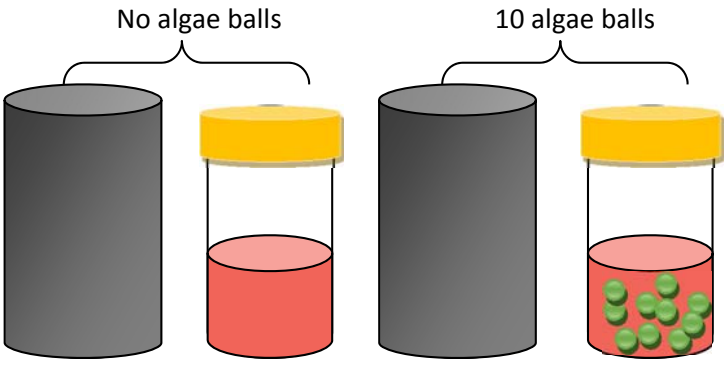
Step 2 Add 10mL tap water with phenol red pH indicator

Step 3 Add 10 algae balls to two of the tubes

Step 4 Incubate 2 tubes (one with algae balls, one without) in the dark

Step 5 Incubate 2 tubes (one with algae balls, one without) in a bright cool light source

Step 6 Record the colour of the phenol red at appropriate time intervals



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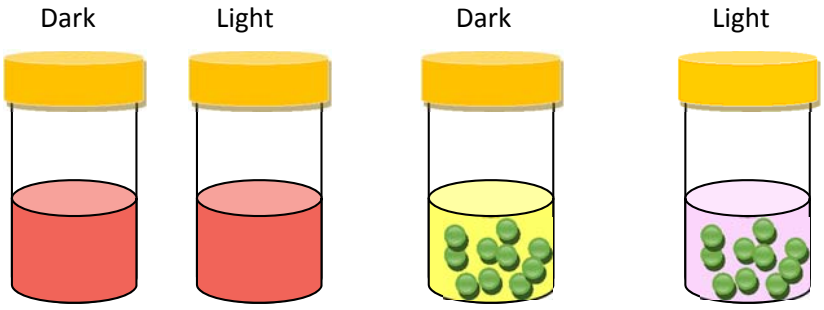
Make predictions based on the equations of respirations and photosynthesis. To make predictions you need to know that:

- When CO_2 is released from cells and dissolves in water, carbonic acid is produced.
- When cells absorb CO_2 , the surrounding water becomes less acidic, or basic

Expected result of the experiment described



No colour change

No colour change

YELLOW

Cells respire, release CO_2, medium goes acidic - yellow

PINK

Cells photosynthesise, take in CO_2, medium goes basic - pink

Possible student investigations using this method

Photosynthesis in different light intensity
Photosynthesis in different wavelength (colour) of light

pH Indicators

NOTE: here we have used phenol red pH indicator for acidic and basic (respiration and photosynthesis) colour changes. Hydrogen carbonate indicator would be better for more detailed analysis of rates of photosynthesis. This is due to a greater range of colours in the range pH 7.6-9.2.

Figure 7 Buffer solutions ranging from pH 7.6 to 9.2 (at intervals of 0.2). 9 cm^3 of each buffer solution was mixed with 1 cm^3 of stock hydrogencarbonate indicator.

Sourcing materials

Item	Supplier	Cost in 2015
Sodium alginate	Haines Educational: Sodium alginate TECHNICAL 250g	
	Science Supply: 1560/250G Sodium alginate Technical SL117/100G Sodium alginate LR	 $73.60 $73.00
	Southern Biological **MC35.1 - Sodium alginate, LR grade 100g**	**$34.50**
Preparations of sodium alginate vary. Test the product for solubility, appropriate concentration for handling and gelling ability before conducting student practicals.		
Chlorella live culture	Southern Biological 10mL, **L1.20 - Chlorella, live** 100mL **L1.20/100 - Chlorella, live, 100mL**	 **$18.80** **$90**
Chlorella culture medium	Southern Biological **CM3 - Chlorella culture medium** Download the medium instruction sheet	**$18.50**
Phenol red stock solution	Southern Biological **SI13 - Phenol red, 0.1% 100mL**	**$12.90**

Growing a *Chlorella* culture

Purchase a 10mL live culture of Chlorella and a bottle of Chlorella culture medium. Download and following the culture instruction sheet from the supplier. Briefly: the initial culture is subcultured approximately 1/10 (1 mL Chlorella culture per 10mL medium) and placed in a warm light position for several weeks. Subculture to maintain the growth. Use sterile glassware for subculturing to help prevent bacterial contamination.

References

Science and plants for schools http://www.saps.org.uk/
http://www.saps.org.uk/attachments/article/123/School%20Science%20Review%20of%20Photosynthesis%20Kit.pdf
Methods for growing large quantities of algae can be found in this reference or through the SAPS web site.