Yeast Immobilised in Alginate Balls

Studies of respiration

Immobilising cells in small balls of calcium alginate has become a significant industrial procedure. For example, immobilised algae are being trialled for biofuel production and immobilised yeast are being used for brewing and alcohol production. “Immobilised cells” are unicellular algae or yeasts entrapped in a gel-like substance, calcium alginate. The cells remain viable and metabolically active.

Yeast is a useful model organism for studies of eukaryotic cell growth and biochemical and molecular processes. *Saccharomyces cerevisiae*, bakers and brewer’s yeast, respires anaerobically. Factors regulating the rate of respiration can be investigated. Anaerobic respiration can be represented with the equation

GLUCOSE → ETHANOL + CARBON DIOXIDE

When cells are bathed in solution, the carbon dioxide released from respiration produces carbonic acid. When the solution contains a pH indicator, the acidic change can be detected as a colour change.

Anaerobic respiration can be investigated in yeast immobilised in alginate balls. These notes outline the procedure. Once you have mastered the technique you can explore your own questions about respiration in yeast, such as the effects of temperature, different sugars or conduct a search for inhibitors.

Materials

- 1.5% sodium alginate solution
- Saccharomyces cerevisiae, Baker’s yeast
- 2% $CaCl_2$ in beaker
- Tea strainer
- Tap water (tap or squirt bottle)
- Spatula or forceps
- Screw capped bottle for storage
- Petri dishes
- 0.1M HCl
- 0.1M NaOH
- Phosphate buffered saline (PBS) with phenol red (1mL of 0.1% phenol red stock solution per 100mL PBS) [*PBS recipe is on page 5*]
- 3 Transfer (bulb) pipettes 2-5mL
- 10mL pipette or measuring cylinder
- 1ml pipette
- 1 % glucose solution (1g glucose dissolved in 100mL PBS with phenol red)

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

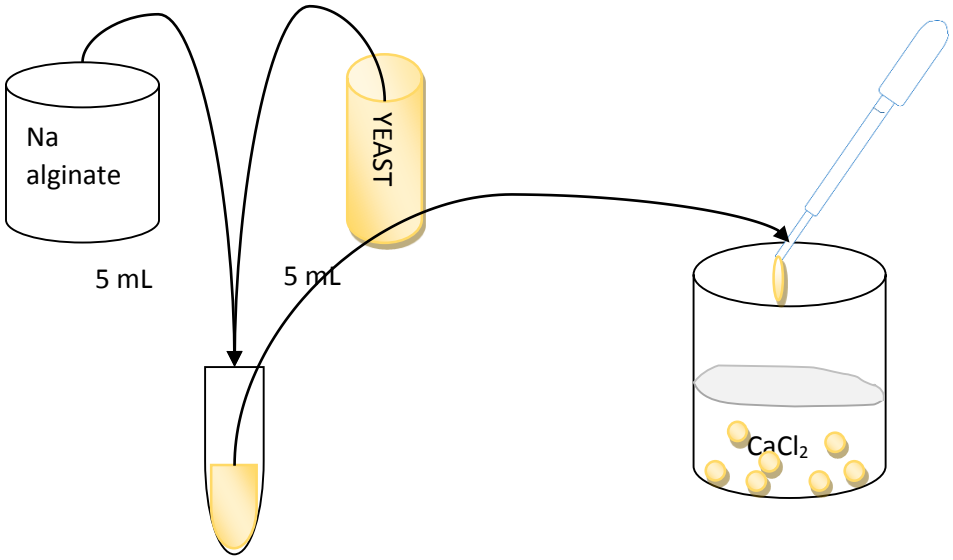
- Dissolve 2.0g calcium chloride ($CaCl_2$) in 100mL dH_2O
- Approximate volumes to use: 20mL for preparing up to 40 alginate balls (about 3mL of alginate – cell mix)
- Have a beaker with 20-50mL of 2% $CaCl_2$ ready before you start mixing the yeast and alginate

Prepare PBS with 0.001% phenol red indicator

- Phenol red indicator stock solution (0.1%)
- Add 1mL per 100mL PBS. Phenol red is yellow when acidic (<pH 6.2), red between pH 6.2 and 8.2, pink when basic (> pH8.2).

Immobilising Saccharomyces cerevisiae baker's yeast in alginate balls (for 10mL)

a) Bring dry baker's yeast to room temperature
b) Make a suspension with a small spatula, approx. 0.25g yeast in 10mL tap water. Mix until uniform suspension (this is likely to vary with different yeast sources)
c) To 5mL of yeast suspension add 5mL warm (37°C) Na-alginate (use a cut-tip transfer pipette for the viscous alginate)
d) Gently mix the yeast 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
a) Add the yeast-alginate mix drop-wise into a beaker with 30-50mL of 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
e) Transfer the balls to a tea strainer and rinse thoroughly with tap water to get rid of the $CaCl_2$
f) Keep the balls of immobilised yeast in tap water.
g) Surplus yeast balls can be stored in the refrigerator (not the freezer) for a few days for further experiments.



Yeast can be a bit tricky because a high density changes the pH very quickly. Buffered solutions such as PBS, phosphate buffered saline, can be used for experiments with alginate balls as long as they do not contain calcium chelating agents such as EDTA. Buffers with EDTA make the alginate disintegrate. However, much longer incubation times are needed to see a significant colour change in the pH indicator.

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).

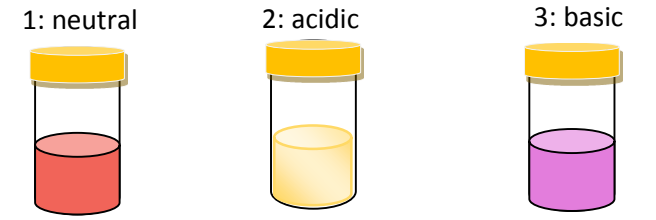
Phenol red colour range		
Yellow ----------	Red ----------	Pink
<pH6.2	pH 6.2-8.2	>pH 8.2

First, test that the PBS-phenol red solution you will use for the yeast balls do show these colour changes.

Tube 1: 10mL PBS–phenol red

Tube 2: 10mL PBS –phenol red + 1-2 drops 0.1M hydrochloric acid (HCl, an acid)

Tube 3: 10mL PBS –phenol red + 1-2 drops 0.1M 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.

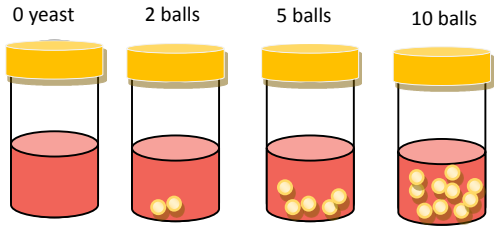
Detecting respiration via a pH change

When cells respire they release CO_2. In solution CO_2 forms carbonic acid, detected as an acidic pH change. With a pH indicator in the medium, direct visual evidence of respiration is possible.

Test the method for detecting respiration with different cell density

This part of the procedure would be best done by technical staff for your school conditions as part of checking that a result with 10 yeast balls (or more) will work for students.

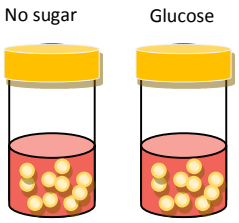
Record the colour change over 30-60 min or longer to find a good number of yeast balls for testing.
It is best to initially leave the tubes undisturbed – the first indication of colour change is immediately around the yeast balls.



Testing the effect of glucose on yeast respiration

Method

Tube	Yeast-alginate balls	PBS – Phenol red	1% glucose solution
1	10 or as determined above	10 mL	0
2	10 or as determined above	9 mL	1 mL



Incubate at room temperature.
Observe and record any changes every 30 minutes.
With this preparation, a change in colour around the alginate balls is usually evident in 30minutes. The tubes can be left at room temperature for 2-4 hours for an obvious colour change (acidic-yellow) or overnight.

Possible Student Investigations using this method

- Greater precision of pH change with a pH meter or pH test strips
- Different species of yeast – available from brewing shops
- Respiration at different temperatures
- Ability to use different sugars (yeast can use most sugars, but most yeast cannot metabolise lactose)
- Factors that increase or decrease the rate, e.g. ions (magnesium, iron,...) (be aware that most chemical inhibitors of respiration will be toxins).
- Gas conditions e.g. high concentration of CO_2, or removal of O_2.

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.		
Saccharomyces cerevisiae	Bakers yeast, supermarket	**inexpensive**
Phenol red stock solution	Southern Biological **SI13 - Phenol red, 0.1% 100mL**	**$12.90**

Phosphate buffered saline

Reagent	Amount to add for 1L	Final concentration (millimolar, mM)
NaCl	8g	137 mM
KCl	0.2g	2.7 mM
Na_2HPO_4	1.44g	10 mM
KH_2PO_4	0.24g	1.8 mM

- Dissolve the above reagents in 800mL dH_2O.
- Adjust the pH to approximately pH 7.4 with HCl
- Add dH_2O up to 1L

Reference

http://www.eurovolvox.org/Protocols/PDFs/ImmobilisedYeast2.1_UK_eng.pdf