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Immobilised yeast

Immobilisation of yeast in calcium alginate beads

Aim

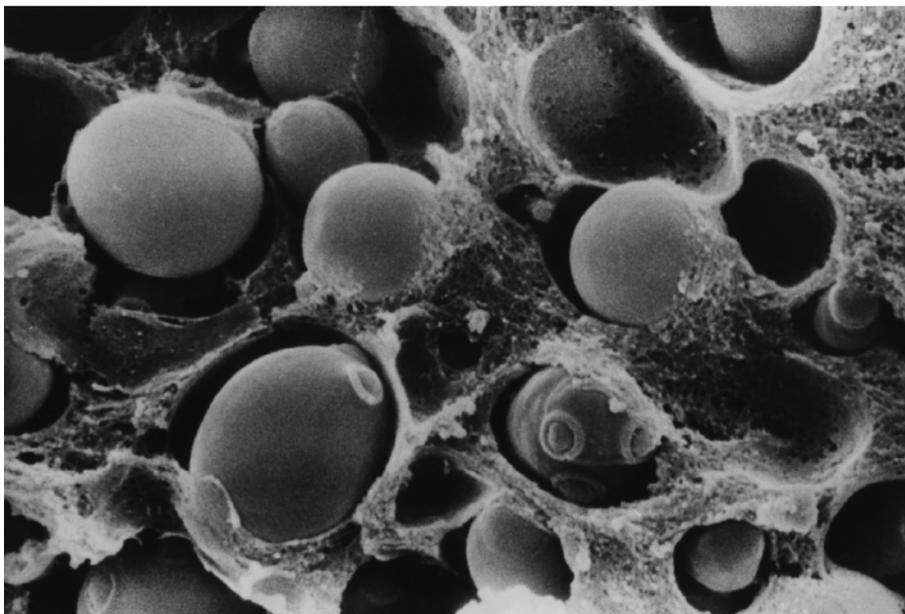
To provide an introduction to the techniques of cell immobilisation and the quantitative study of fermentation.

Introduction

Entrapment within calcium alginate is the most widely-used technique for immobilising cells. It is especially suited to living cells as it tends not to damage them. Applications of this versatile method include immobilisation of cells in bioreactors, entrapment of plant protoplasts and plant embryos ('artificial seeds') for micropropagation, immobilisation of hybridomas for the production of monoclonal antibodies, and the entrapment of enzymes and drugs (see table).

The cells or enzymes to be entrapped are first mixed with a solution of sodium alginate. This is then dripped into a solution containing multivalent cations (usually Ca^{2+}). The droplets form spheres as they fall, entrapping the cells in a three-dimensional lattice of ionically cross-linked alginate.

PHOTOGRAPH COURTESY DUNCAN CASSON



Electron micrograph of yeast cells immobilised in calcium alginate. Bud scars are visible on some of the cells.

Cells	Product or purpose	Cells	Product or purpose
Bacteria		Algae	
<i>Erwinia rhapontici</i>	Isomaltulose	<i>Botryococcus braunii</i>	Hydrocarbons
<i>Pseudomonas denitrificans</i>	Cleaning of drinking water	Plant cells	
<i>Zymomonas mobilis</i>	Ethanol	<i>Chatharanthus roseus</i>	Alkaloids for cancer therapy
Cyanobacteria		Various species	Artificial seeds
<i>Anabena</i> sp.	Ammonia	Plant protoplasts	Cell handling, microscopy
Fungi		Mammalian cells	
<i>Kluyveromyces bulgaricus</i>	Hydrolysis of lactose	Hybridomas	Monoclonal antibodies
<i>Saccharomyces cerevisiae</i>	Ethanol	Islets of Langerhans	Insulin/implantation
<i>Saccharomyces bajanus</i>	Champagne production	Fibroblasts or lymphomas	Interferons (α or β)

Examples of uses of alginate-immobilised cells. After Smidsrød and Skjåk-Bræk (1990).

Equipment and materials

Needed by each person or group

Equipment

- 10 mL plastic syringe (without a needle)
- Small beakers or disposable plastic cups, 2
- 250 mL conical flask
- Bung to fit flask, bored to take a fermentation lock
- Fermentation lock
- Tea strainer
- Glass stirring rod

Materials

- 4% sodium alginate solution, 25 mL
- 1.5% calcium chloride solution, 100 mL
- Bakers' yeast, dried, 2.5 g
- 8% sucrose solution, 150 mL
- Universal indicator solution, 1 mL, diluted with 1 mL of distilled or deionised water

Note

All solutions must be made up using distilled or deionised water. Sodium alginate is not readily soluble, and requires both warm water and stirring to dissolve it.

For measuring carbon dioxide evolved (optional)

Equipment

- 250 mL conical flask
- Bung, to fit conical flask, bored and fitted with a delivery tube
- 100 mL measuring cylinder
- 500 mL beaker

Materials

- 13% sodium chloride solution, about 1 litre (ordinary table salt is adequate)

Procedure

- 1 Mix the dried yeast with 25 mL of distilled water in a small beaker. Cover and leave to rehydrate for 10 minutes at room temperature.
- 2 Add 25 ml of sodium alginate solution to the yeast suspension. Stir well.
- 3 Draw up some of the yeast/alginate mixture into a syringe. Add it, a drop at a time, to the calcium chloride solution.

Fig. 1

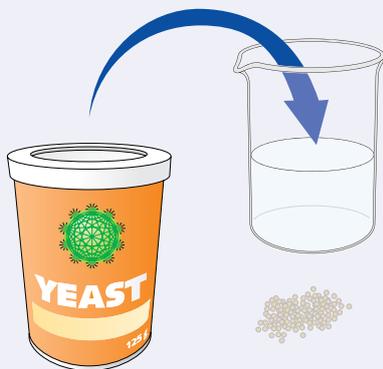


Fig. 2

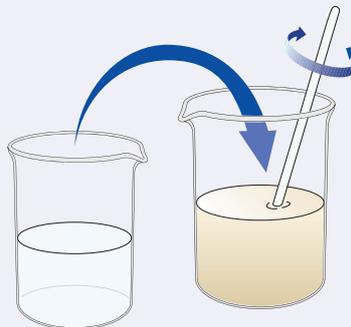


Fig. 3



- 4 Leave the immobilised yeast cell beads to harden in the calcium chloride solution for 5–10 minutes. *The alginate will be ionically cross-linked by the calcium ions.*
- 5 Separate the beads from the solution using the tea strainer.
- 6 Place the beads in a sugar solution in a conical flask. Stopper a flask with a bung that has been fitted with a fermentation lock. *If universal indicator is added to the fermentation lock, the indicator will change colour as carbon dioxide is produced.*

Fig. 4

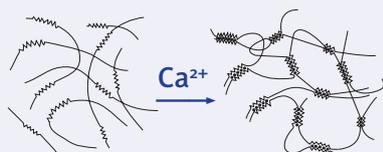
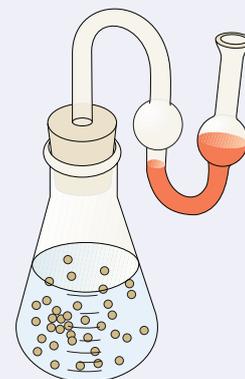


Fig. 5



Fig. 6



OPTIONAL Measuring carbon dioxide production

- 7 Stopper the flask with a bung to which a delivery tube has been fitted.
- 8 Maintain the flask at 21–25 °C. Collect the gas that is produced over a 13% solution of sodium chloride. *Carbon dioxide will not dissolve in this solution.*
- 9 Record the volume of gas that has been collected at convenient intervals. Plot the results on a graph, showing the volume of gas evolved against time.

Fig. 7

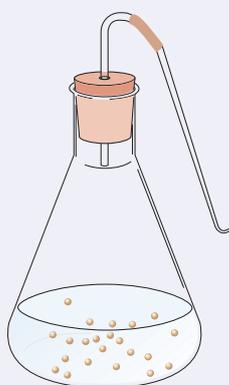


Fig. 8

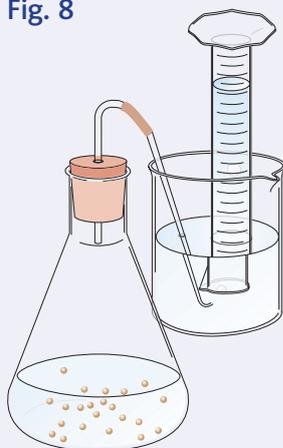
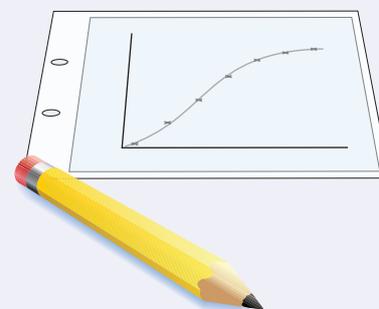


Fig. 9

**Safety guidelines**

The build-up of gas within glass vessels could be dangerous. Ensure that the flasks are adequately vented.

Preparation and timing

Immobilised yeast cells can be prepared in 10–15 minutes. The sodium alginate takes some time to dissolve, so the solution is best prepared before the lesson. If you wish to store sodium alginate solution for more than a few days, it is advisable to autoclave it. To prevent excessive depolymerisation of the alginate chains, however, it is advisable to raise the pH to 7–8 before autoclaving.

Troubleshooting

Because sodium alginate is difficult to dissolve, it may help to leave the alginate to dissolve overnight. If you wish to try additional activities using buffer solutions, please note that any containing phosphate, citrate or EDTA should be avoided, as these will cause the alginate matrix to dissolve (50 mM sodium citrate or phosphate buffer at pH 7 can be used to recover cells from the beads).

Additional investigations

- 1 Ordinary bakers' yeast, *Saccharomyces cerevisiae*, is unable to ferment the sugar lactose. The enzyme β -galactosidase breaks down lactose to glucose and galactose. Yeast that is co-immobilised with this enzyme is able to grow in a medium that contains lactose. Of the two sugars formed by enzyme action, glucose is used preferentially. Once supplies of this sugar have been exhausted, the yeast adjusts its metabolism and the other breakdown product of lactose, galactose, is utilised. The activity of the yeast is readily-monitored simply by measuring the volume of gas (carbon dioxide) evolved during the fermentation.
- 2 Different sugar solutions, incubation temperatures or types of yeast *e.g.*, wine-makers' or bakers' yeast, may be compared.

Other sources of information

Publications

Smidsrød, O. and Skjåk-Bræk, G. (1990) Alginate as an immobilization matrix for cells. *Trends in Biotechnology* **8** (3) 71–78.

Practical fermentation: A guide for schools and colleges by John Schollar and Bene Watmore (1999) Society for General Microbiology, Reading. ISBN: 0 9536 8380 x.

Two 20-page booklets describing 14 practical investigations of fermentation. The Teacher's guide includes ideas for extension activities and specimen results. The booklets may be downloaded from: <http://www.ncbe.reading.ac.uk/NCBE/PROTOCOLS/fermentation.html>

Immobilized biocatalysts: An introduction by Winnifred Hartmayer (1988) Springer-Verlag, Heidelberg. ISBN: 0387 188088.

A useful handbook with numerous practical protocols.

Immobilised enzymes and cells: a practical approach by Jonathan Woodward [Ed] (1985) Oxford University Press, Oxford. ISBN: 0 947946 21 7.

An academic laboratory manual describing methods of immobilising enzymes and cells.

Web site

EIBE Unit 1: Microbes and molecules

<http://www.eibe.info>

Suppliers

Sodium alginate may be purchased from school chemical suppliers. It is also used in food production, so may be available from food industry suppliers.

Acknowledgement



This practical protocol was adapted from a practical protocol by Dean Madden, that was first published in *EIBE Unit 1: Microbes and molecules* (see link above). The *Volvox* project is funded under the Sixth Framework Programme of the European Commission.