



**Prepare One Enzyme
Immobilisation and Examine
Its Application**

**Biology – Leaving Cert
Experiments**

Materials/Equipment

Sachet of yeast (without CaSO_4)

Sodium alginate

Calcium chloride powder

Sucrose

Distilled water

Glucose test strips

Hot water ($30^\circ\text{C} - 40^\circ\text{C}$)

Graduated cylinders (100 cm^3)

Beakers (100 cm^3)

Beakers (500 cm^3)

Separating funnels

3 Thermometers

2 Retort stands

Electronic balance

Weigh boats

Syringe (20 cm^3)

Sieve

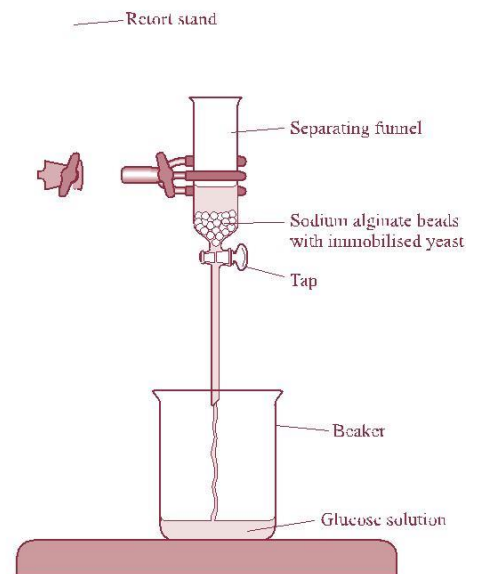
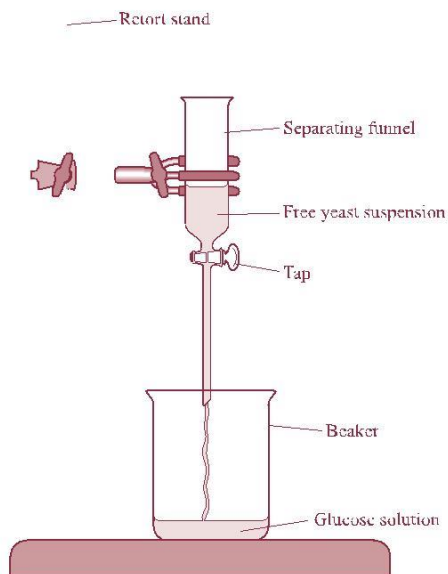
Labels

3 Spatulas

Wash bottle

Timer

Glass rods



Procedure

1. Familiarise yourself with all procedures before starting.

Preparation of the immobilised enzyme

2. Add 0.4 g of sodium alginate to 10 cm³ of distilled water in a 100 cm³ beaker. Mix thoroughly.
3. Mix 2 g of yeast in 10 cm³ of distilled water in a 100 cm³ beaker.
4. Prepare 100 cm³ of a 1.4% w/v calcium chloride solution in the large beaker.
5. Add the yeast suspension to the sodium alginate solution and mix thoroughly with a glass rod.
6. Draw all of the mixture into a 20 cm³ syringe.
7. From a height of 10 cm release the mixture from the syringe, one drop at a time, into the calcium chloride solution. Beads containing yeast cells will form.
8. Leave the beads to harden for at least 10 minutes.
9. Filter the beads through a sieve and rinse with distilled water.

Application of the immobilised enzyme – production of glucose from sucrose

10. Mix another 2 g of yeast in 10 cm³ of distilled water.
11. Pour this yeast suspension into a separating funnel labelled 'Free yeast' as shown in the diagram.
12. Pour the beads into another separating funnel labelled 'Immobilised yeast'.
13. Prepare 100 cm³ of 1% w/v sucrose solution with water warmed to about 40 °C.
14. Pour 50 cm³ of the sucrose solution into the yeast in each of the separating funnels.
15. Using glucose test strips, immediately test samples from each funnel for glucose.
16. Repeat the test at two-minute intervals until glucose appears in both.
17. Record result.
18. Run off the remaining product from each funnel into the beakers as shown in the diagram.
19. Compare the turbidity of the solutions from both funnels.
20. Replicate the investigation or cross reference your results with other groups.

Result

Time (minutes)	Free yeast – presence of glucose	Immobilised yeast – presence of glucose
0		
2		
4		
6		
8		
10		

////////////////////////////////////	Free yeast	Immobilised yeast
Turbidity of solution		

Conclusion/Comment

Skill Attainment

Prepare One Enzyme Immobilisation and Examine Its Application

Following instructions

Familiarise yourself with all procedures before starting

Follow instructions step by step

Listen to the teacher's instructions

Correct manipulation of apparatus

Use the balance

Use a graduated cylinder to measure volumes Prepare solutions and mixtures

Draw the mixture of alginate solution and yeast suspension into the syringe Release the mixture drop by drop into the calcium chloride solution Transfer the beads to the separating funnel

Filter the beads

Rinse the beads with distilled

water Set up the separating

funnels Warm water to about 40

°C

Use a thermometer

Pour 50 cm³ of sucrose solution into each

funnel Use glucose test strips to test for

glucose Repeat at two-minute intervals using the timer

Observation

Note the clarity of both sucrose solutions

See beads forming

Observe any colour change using glucose test strips

Compare the turbidity of both end products

Recording

Write up the procedure

Tabulate the results

Record the time

Interpretation

Draw reasonable conclusions from your observations and results

Application

Become aware of any other application(s) of what you learned in this activity

Organisation

Exercise caution for your personal safety and for the safety of others Work in an organised and efficient manner

Label as appropriate

Work as part of a group or team

Clean up after the practical activity

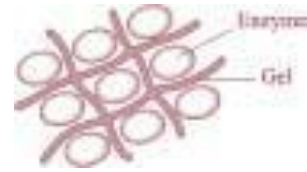
Background information

Alginates

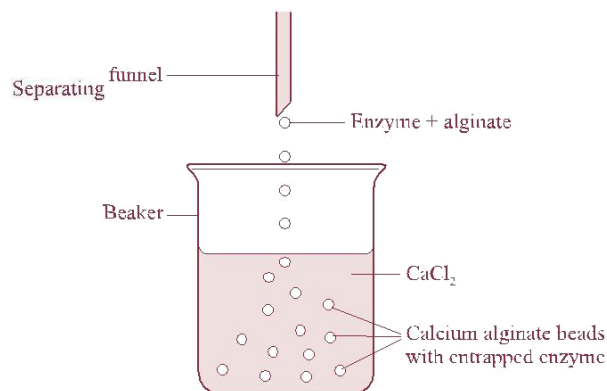
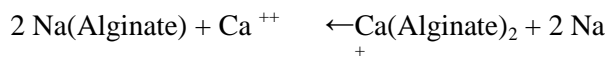
Most alginates are produced by brown algae (Phaeophyceae, mainly *Laminaria spp.*) whereas agars are produced from red algae (Rhodophyceae).

Immobilised enzymes

An immobilised enzyme is one that has been made insoluble or held in place so that it can be reused many times. Once immobilised, an enzyme's stability is increased, possibly because its ability to change shape is reduced. One method of immobilisation is an entrapment method, in which the enzyme is



enclosed within a gel (e.g. alginate beads). The openings in the encapsulating material have to be of sufficient size to permit the substrate or reactant to pass through to the enzyme without allowing the enzyme to leak out. The most common entrapment method is the formation of calcium alginate beads. The enzyme is mixed with sodium alginate, an acidic polysaccharide, and the mixture is dropped into a solution of calcium chloride. The calcium ions replace the sodium ions and cross-link the polysaccharide. The result is the production of insoluble calcium alginate beads containing trapped enzymes. Calcium alginate gel entrapment is a suitable method for this activity because it is simple to prepare and allows high enzyme activity and stability.



Advance preparation

- Prepare the hot water required to make the sucrose solution.

Helpful hints

- Sodium alginate is not readily soluble in water. Sodium alginate solution is best prepared by adding the powder to agitated water, rather than vice versa to avoid the formation of clumps.
- If left to stand, sodium alginate will absorb water and will dissolve more readily.
- Beads may be stored under distilled water for two to three days until ready to use.
- To avoid beads blocking the separating funnel, a plastic disposable pipette or straw may be inserted into the funnel before adding the beads.
- When using glucose test strips make sure to read instructions carefully as the procedure varies depending on the brand used.
- Only use yeast which does not contain calcium sulfate.
- For a better visual display, calcium chloride solution may be transferred to a clear graduated cylinder before adding yeast/alginate mixture.
- Cut glucose test strips in two, lengthwise, to make the supply last longer.
- Use separate weigh boats for the different chemicals to avoid calcium coming in contact with the yeast and alginate.
- Separating funnels with Teflon taps, rather than glass taps, are preferable as they are less likely to stick.
- Use wide-topped separating funnels as it is easier to introduce beads.