Practical 9 - S(e) Producing a model industrial immobilised enzyme column

This practical focuses on: **Defining the problem**

You will be developing other assessed skills throughout the practical.

Intended learning outcomes

By the end of this practical and its write-up you should be able to:

- Set up an effective working model of an immobilised enzyme column.
- Understand the usefulness of immobilised enzymes in biotechnology.
- Identify variables that should be controlled.
- Experience relevant methods, including the use of a control.

Safety Information

	You should wear eye protection throughout this practical.
	Calcium chloride is an **irritant**.
	All enzymes including sucrase enzyme should be assumed to be **harmful**.

Background information

- In industry (e.g. the confectionary industry), enzymes are used on a large scale.
- It is very costly to use enzymes only once, but most enzymes are only commercially available in liquid or dehydrated forms and once they have been used in solution it is very difficult and time consuming to separate them from the product.
- To allow their re-use, enzymes may be immobilised. One way of immobilising enzymes is to 'stick' the enzyme molecule to an alginate bead.
- In industry these immobilised enzymes are used in large columns. The substrate enters at the top of the column and the product collected at the bottom.
- Sodium alginate (used to produce alginate beads) will turn from liquid to solid when immersed in calcium chloride.
- Sucrase is an enzyme which breaks down sucrose into glucose and fructose
- The presence of glucose can be tested using Benedict's reagent.

You will produce a model of an immobilised enzyme column.

- Read the information above.
- List the variables that should be controlled, using your knowledge of enzymes.
- Describe ways in which each variable may be controlled.
- Suggest a suitable control experiment to prove your model is working correctly.

Method

Immobilise enzymes and prepare column

1 Put 4 cm^3 of 1% sucrase solution into a beaker.

2 Add 6 cm^3 of sodium alginate to the sucrase. Use a measuring cylinder to obtain the correct volume of sodium alginate as it is very viscose and will not be easily expelled from a pipette).

3 Stir the sucrose / sodium alginate mixture with a glass rod for at least 2 minutes.

4 Put 50 cm^3 of calcium chloride into a clean beaker.

5 Using a dropping pipette, transfer the sucrase / sodium alginate mixture *one drop at a time* into the calcium chloride. The drops of alginate mixture will form solid beads when immersed in calcium chloride. These beads have the enzyme (sucrase) on them.

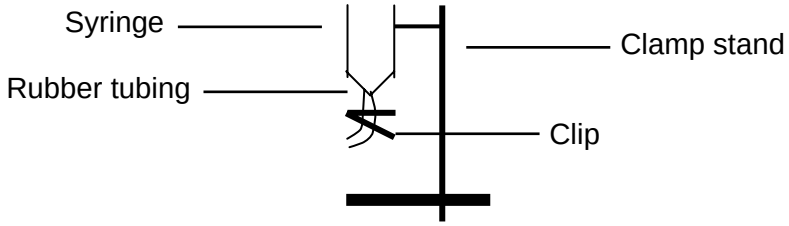
6 Fix a 15 cm^3 syringe to a clamp stand and place a small piece of muslin in the bottom to prevent the nozzle from becoming blocked.

7 Attach a piece of rubber tubing to the nozzle of the syringe and seal it with a clip.

8 Pour the contents of the calcium chloride beaker through the syringe, allowing the liquid to drain away by opening the clip on the rubber tubing. Your column will now be full of immobilised enzyme beads.

9 Rinse the beads by passing water through the syringe and allowing it to drain away.

Diagram



Test your immobilised enzymes

1 Close the clip on the rubber tubing, then pour enough 1M sucrose into the column to fill it.

2 After 5 minutes, open the clip on the rubber tubing and collect the liquid in a clean boiling tube. This is your 'product'.

3 Test the 'product' you have just collected for the presence of glucose, using Benedict's reagent. If you have clinistix or other glucose testing strips use these to estimate the glucose concentration.

4 (Optional) Repeat the whole procedure, omitting the addition of sucrase – this is a *control* experiment.

Write-up

1 Evaluate the procedure, considering the following:

- The limitations of the apparatus you used e.g. the size of the syringe (column), the size of the dropping pipette and therefore the size of the beads produced.
- The effectiveness of the system – was it efficient? Would it be possible to re-use the enzyme? Was a pure product obtained?

2 Explain what happened during the reaction in terms of enzymes activity.

Design a further investigation, using this apparatus, to test the rate of flow through the column on the rate of breakdown of sucrose.

Lesson Plan

Producing a model industrial immobilised enzyme column

Context

To build a working model of an industrial immobilised enzymes column. This will improve understanding of the nature and applications of immobilised enzymes in industry, in the context of the 9700 syllabus – "immobilise an enzyme in alginate and compare the ease of recovering the enzyme and ease of purification of the product compared to the same enzyme that has not been immobilised".

Key aims of lesson

This practical is designed to enable students to visualise an immobilised enzymes column and demonstrate the ability of immobilised enzymes to be re-used.

Intended learning outcomes

By the end of this practical and its write-up the student should be able to:

- Set up an effective working model of an immobilised enzyme column.
- Understand the usefulness of immobilised enzymes in biotechnology.
- Identify variables that should be controlled.
- Use relevant methods, including the use of a control.
- Design a method to test the effect of the rate of flow through the column on the hydrolysis of sucrose.

Resources required

White board or flipchart and suitable pens or blackboard and chalks

Practical materials specified on the Technical Information sheet

Some spare copies of the student worksheet

Planned activities (timings can be altered to suit shorter or longer lessons)

Timings/ minutes	**Teacher / Student Activities**
End of previous lesson	**Preparation** – student given theory on immobilised enzymes, students to consider industrial applications of enzymes in biotechnology. Students to be reminded of Benedict's test, as learned in AS syllabus.
0-4	**Introduction** to the aims, intended outcomes and shape of the lesson - teacher led oral presentation
4-8	**Context** - review industrial uses of enzymes, reasons for use of immobilised enzymes and methods of immobilisation. Also, review Benedict's test.
8-12	**Introduction to method** - teacher demonstration of alginate bead production. Teacher may have a column already set up to enable students to visualise it.

12-20	**Student preparation exercises** Students list the variables that should be controlled, using their knowledge of enzymes and describe ways in which each variable may be controlled. Suggest a suitable control experiment to prove the model is working correctly.
20-50	**Carrying out the practical** - students carry out the practical work and tidy away apparatus when they have finished.
50-60	**Drawing together the threads** - teacher-led class discussion on the skills that have been developed and the knowledge the students have gained. Students begin evaluation and design of a follow up investigation. These are completed as homework.

Useful Information - Producing a model industrial immobilised enzyme column

- Possible variables to control include: temperature, bead size, column volume / dimensions, time which sucrose remains in column, concentration of sucrose / sucrase / sodium alginate solutions, pH (you may wish to include a buffer e.g. citrate-phosphate buffer (pH 7) into the alginate / sucrase mixture.
- If a positive result is not achieved when doing the Benedict's test, it may be necessary to leave the sucrose in the column for a longer period of time.
- Students should recognise the need to devise a method of passing sucrose solution through the column at a variable rate. Suggestions could be using a burette or a larger syringe and controlling the clip flow. Adding a known volume of sucrose above the beads and recording the time taken for the entire volume to pass through the column. Students should also suggest a means of quantifying the amount of sucrose hydrolysed. This could be using glucose testing strips or making a series of known glucose concentrations and comparing the colour to a Benedict's trust carried out on the collected filtrate.

Technical Information- Producing a model industrial immobilised enzyme column

The **apparatus and materials** required are listed below. The amount given is **per student or one** group if students are to work in groups.

It is convenient to make up more of the reagents than is required in order to give sufficient quantities for accurate measurements.

- 4 cm^3 of 1% Sucrase solution provide
- 6 cm^3 of 2% Sodium alginate solution
- 1 mol dm^{-3} Calcium chloride solution (the molecular weight of sucrose = 342g) – provide enough for 50 cm^3 per student
- 10 cm^3 graduated pipettes, syringes / measuring cylinders – 2 per student
- Beakers (at least 50cm^3 volume) – 2 per student
- Dropping / teat / Pasteur pipettes – 1 per student
- 15 cm^3 syringe – 1 per student
- 2 cm^2 pieces of muslin or gauze – 1 per student
- Stop clock / watch – 1 per student
- Bunsen burner, tripod and gauze – 1 per student (for Benedict's test, alternatively you may use a water bath set to >60°C)
- Narrow rubber tubing cut to >5cm lengths – 1 per student
- Rubber tubing / Hoffman clips – 1 per student
- Boiling tube – 1 per student
- Clamp stand – 1 per student

- Sodium alginate powder takes a long time to dissolve, so ensure this is prepared well in advance, it has a shelf life of a few months.
- Sucrase and sucrose solutions will keep for up to 1 week in a fridge, calcium chloride solution will keep indefinitely.
- You may wish to prepare the syringes by putting the square of muslin in and attaching the rubber tubing prior to the practical lesson, to save time.

Safety Precautions/Risks.

Safety information on the use of enzymes may be found at http://www.ncbe.reading.ac.uk/NCBE/SAFETY/enzymesafety1.html

Calcium chloride = H (Irritant only) 

Sucrase enzyme = H 

A risk assessment should be carried out as a matter of course.