# Topic 2 – Practical 3

## *Immobilised enzymes*

### Safety

• Care must be taken when handling enzymes. Wear eye protection and protective gloves.

• Enzymes are irritants. If enzyme comes in contact with skin or eyes, rinse with plenty of water.

### Apparatus and materials

• 2 cm3 lactase • small sieve

• 30 cm3 5% lactose (found in full fat milk) • two 10 cm3 syringes

• 8 cm3 2% sodium alginate made in distilled water • three 100 cm3 beakers

• 100 cm3 2% calcium chloride solution • glass rod or spatula

• 10 cm3 syringe without plunger • distilled water bottle

• small piece of muslin or nylon gauze • glucose test sticks

• screw clip • retort stand

### Introduction

Immobilised enzymes used in industry are attached to inert insoluble materials such as agar gels, cellulose and polyacrylamides. A substrate flows over the immobilised enzyme and reacts as it does so; the product then flows out of the reaction container. Immobilised enzymes can be re-used many times and the product that emerges does not have to be separated from other reagents, saving production costs for manufacturers. Immobilised enzymes are used in the production of lactose-free milk used by people who are unable to produce the enzyme lactase and digest milk naturally.

In this practical you can investigate on a small scale how immobilised enzymes are prepared and used. You will prepare the immobilised enzyme in alginate beads, which harden as they are dropped in calcium chloride solution.

### Procedure

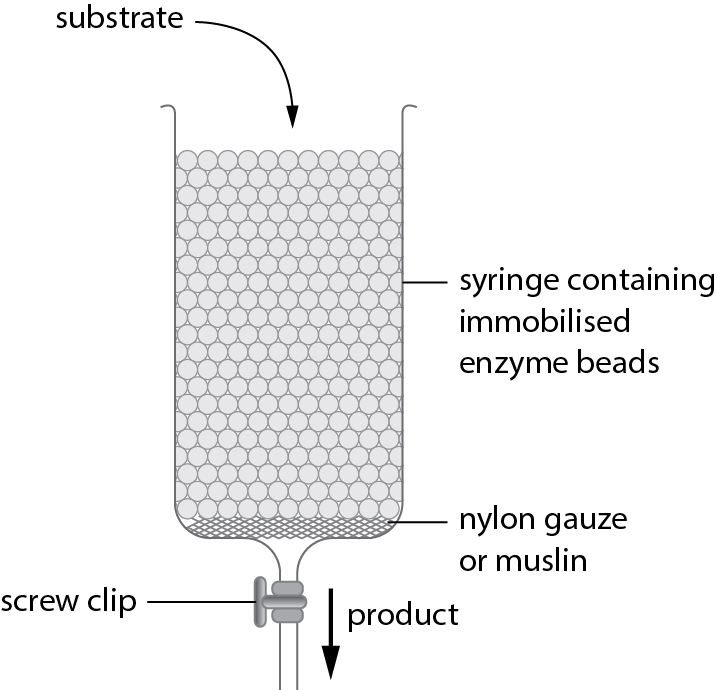
**1** Thoroughly mix 8 cm3 sodium alginate solution and 2 cm3 lactase enzyme in a beaker, using a glass rod or spatula. Draw the mixture up into a 10 cm3 syringe.

**2** Place approximately 100 cm3 calcium chloride solution in a beaker. Drop the enzyme alginate mixture carefully into this solution from the syringe. Alginate beads will form. Leave for about 15 minutes to become firm.

**3** Strain the beads through the small sieve and rinse with distilled water.

**4** Place a small piece of muslin or nylon gauze into the end of the 10 cm3 syringe without a plunger, and add the beads.

**5** Fix this syringe in an upright position as shown below, attach the screw clip, and place a beaker underneath.



**6** Pour distilled water into the top of the column and adjust the screw clip to obtain a suitable flow rate. Note this in a suitable way, for example, cm3/min or drops/min.

**7** Once a suitable flow rate has been established, repeat step **6** but replace the water with 10 cm3 lactose solution. At the correct flow rate, collect a sample and test it with the glucose test sticks. Use the scale on the sticks to estimate the concentration of product produced.

**8** The experiment can now be repeated using different flow rates.

### Questions and further work

**1** Design a similar investigation to determine the effect of substrate concentration on the concentration of product.

**2** List any factors that may affect the accuracy of your experiment and suggest ways to overcome them.