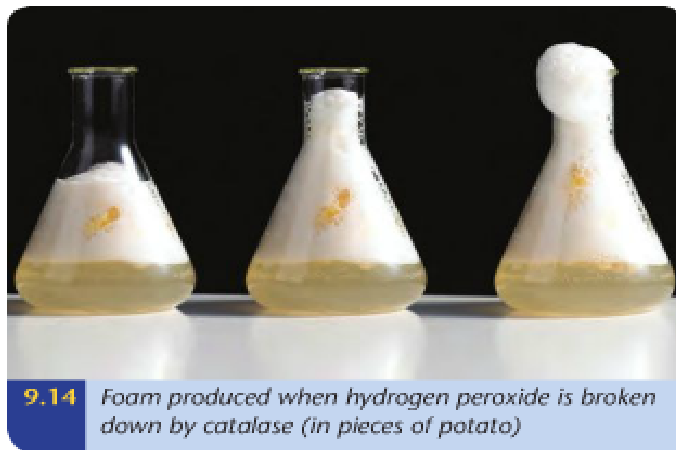
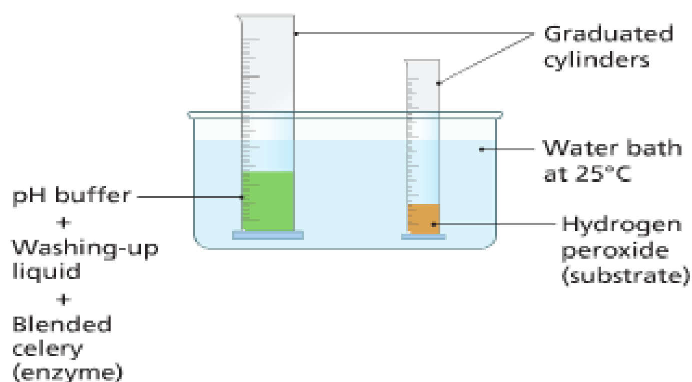
Chapter 9 - Enzymes



9.14 *Foam produced when hydrogen peroxide is broken down by catalase (in pieces of potato)*



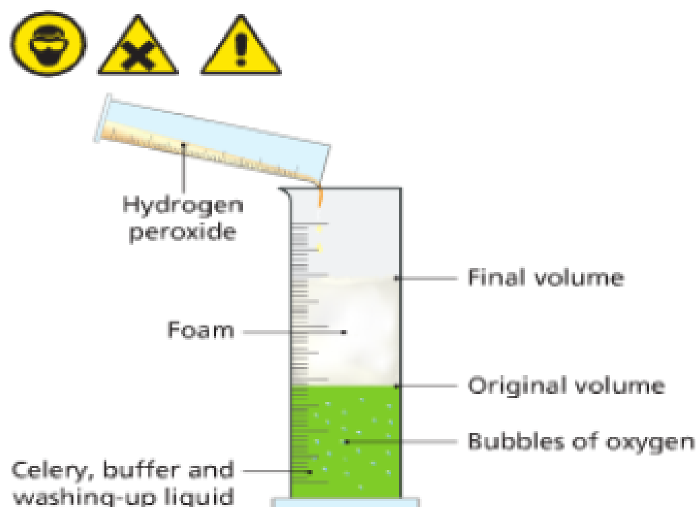
Introduction

Catalase is an enzyme that is found in a wide range of living things, e.g. liver, radishes, celery and potatoes. It converts the toxic substance hydrogen peroxide (H_2O_2) into water and oxygen.

When using catalase the oxygen forms foam (in association with washing-up liquid). The volume of the foam indicates the activity of the enzyme.

1. Place some pH buffer solution 4 in a graduated cylinder (pH buffer 4 ensures that the pH will remain at 4).
2. Using a dropper add one drop of washing-up liquid to the graduated cylinder (the washing-up liquid traps the oxygen that is released, forming foam).
3. Blend some stalks of celery in water in a blender. Filter this solution into a large beaker using coffee filter paper (filtration removes the blended cells and contents; coffee filter paper allows for fast filtration).
4. Add some of the filtrate to the graduated cylinder (the celery contains the enzyme catalase).
5. Add some hydrogen peroxide to a smaller graduated cylinder (hydrogen peroxide is the substrate).

9.15 *Investigating the effect of pH on enzyme activity*



9.16 *Measuring the rate of reaction*

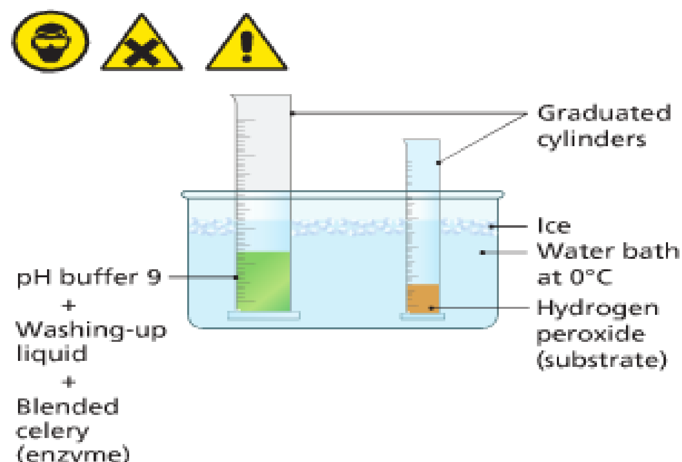
6. Stand both graduated cylinders in a water bath (or a beaker of water) at 25°C for a few minutes (this ensures a constant temperature).
7. Remove the graduated cylinders from the water bath and pour the hydrogen peroxide into the graduated cylinder containing the blended celery.
8. Note and record the volume at the top of the foam after 2 minutes.
9. Calculate the volume of foam produced. This is done by subtracting the original volume of liquid in the graduated cylinder from the volume at the top of the foam after 2 minutes (the volume of foam indicates the rate of the reaction).
10. Repeat steps 1–9 using pH buffers 7, 10 and 13.
11. Record the results as shown overleaf; the first set of figures is filled in as an example.

Activity 8 results				
pH buffer	**4**	**7**	**10**	**13**
Original volume (cm^3)	25			
Volume after 2 minutes (cm^3)	25			
Volume of foam (cm^3)	0			

12. Draw a graph of the results. Put pH on the horizontal axis and the volume of foam produced on the vertical axis. The graph should have a similar shape to that in diagram 9.7 (page 104).
13. Note that catalase is different to most enzymes as it has its maximum activity at pH 9 or 10.
14. As controls, repeat each procedure but do not add blended celery (i.e. no catalase is present) **or** add blended celery that has been boiled (to denature the catalase). In each case no foam is formed.

Activity 9 To investigate the effect of temperature on the rate of enzyme activity

1. Place some pH buffer 9 solution in a graduated cylinder (catalase works best at pH 9; the buffer ensures the pH remains constant at 9).
2. Using a dropper add one drop of washing-up liquid to the graduated cylinder (the washing-up liquid traps the oxygen that is released, forming foam).
3. Blend some stalks of celery in water in a blender. Filter this solution into a large beaker using coffee filter paper (filtration removes the blended cells and contents; coffee filter paper allows for fast filtration).
4. Add some of this solution to the graduated cylinder (the celery contains the enzyme catalase).



9.17 *Investigating the effect of temperature on enzyme activity*

5. Add some hydrogen peroxide to a smaller graduated cylinder (hydrogen peroxide is the substrate).
6. Stand the graduated cylinders in a large beaker of ice-cold water until they are at 0°C.
7. Remove the graduated cylinders from the water bath and pour the hydrogen peroxide into the graduated cylinder containing the blended celery.
8. Note and record the volume at the top of the foam after 2 minutes.
9. Calculate the volume of foam produced, as shown in diagram 9.16 in Activity 8.
10. Repeat steps 1–9 at 10°C, 20°C, 30°C, 40°C, 50°C and 60°C.
11. Record the results as shown; the first set of figures is given as an example.

Activity 9 results

Temperature (°C)	0	10	20	30	40	50	60
Original volume (cm^3)	25						
Volume after 2 minutes (cm^3)	25						
Volume of foam (cm^3)	0						

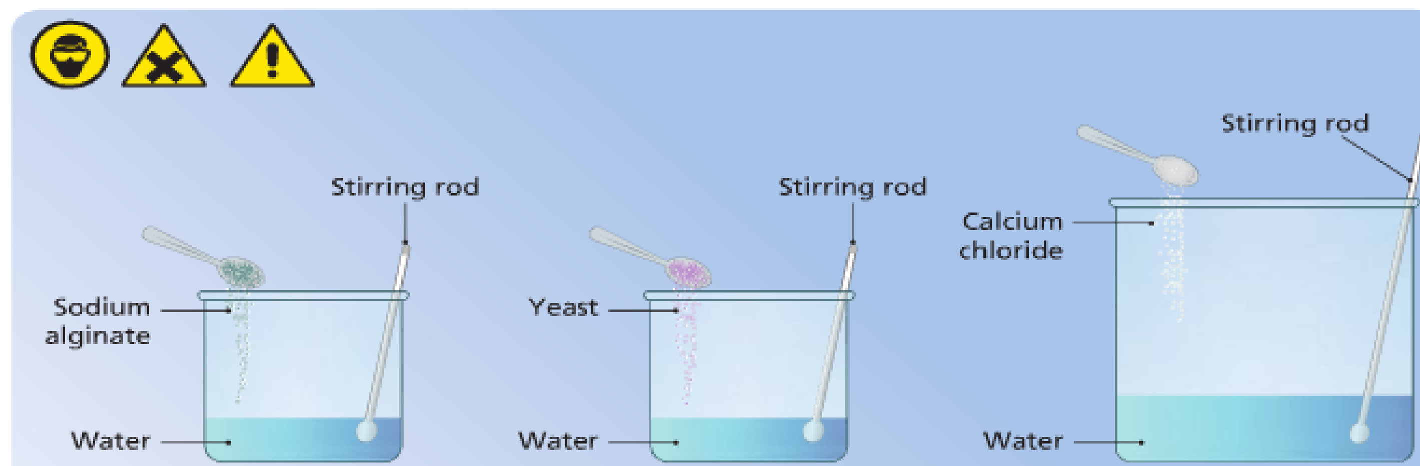
Activity 10 To prepare an enzyme immobilisation and examine its application

Preparing the immobilised enzyme

The formation of alginate beads is a delicate process. All equipment must be clean before use. If possible all the water used in this activity should be distilled water.

In this activity yeast is immobilised. Yeast contains the enzyme sucrase. This means that the enzyme that is immobilised is **sucrase**.

1. Add some sodium alginate to water in a beaker.
2. Stir the mixture with a glass rod until it forms a smooth paste and leave it to soak for 5 minutes (sodium alginate is used to immobilise the yeast (and the enzyme)).
3. Add some yeast to water in a second beaker (the yeast contains the enzyme (sucrase) that is to be immobilised).
4. Stir the yeast solution and leave it for 5 minutes.
5. Dissolve some calcium chloride in water in a larger beaker.



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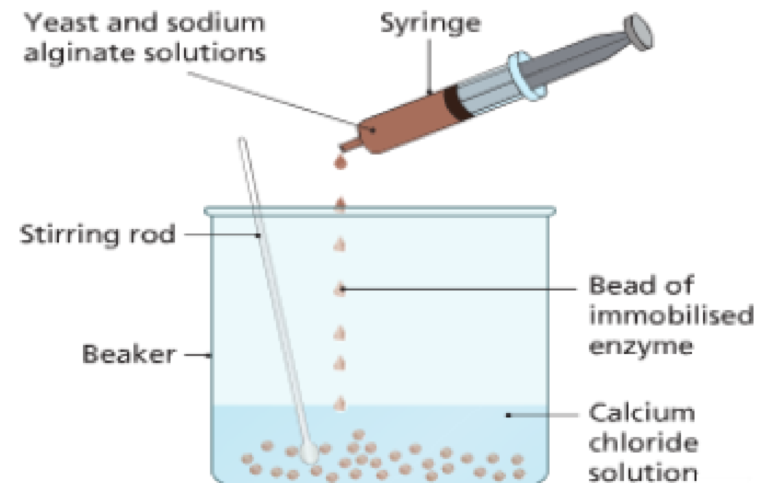
6. Pour the yeast solution into the alginate paste and stir to mix thoroughly.
7. Draw some of the resulting mixture into a syringe (with no needle attached).
8. Slowly and steadily add a series of alginate and yeast drops from the syringe to the calcium chloride solution. Hold the syringe fairly high above the chloride solution and gently stir the solution as you add the drops (this prevents them from clumping). Beads of calcium alginate gel form, enclosing and immobilising some of the yeast cells.



9.19 *Immobilising an enzyme*

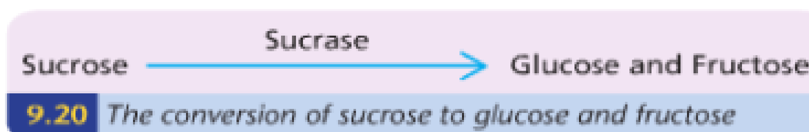
9. Leave the beads in the calcium chloride solution for 15 minutes (this allows the beads to harden).
10. Place the beads in a sieve and rinse them under a tap of running water (this removes any yeast cells from outside the hardened beads). If necessary, the beads can be stored in water or dried on filter paper and stored in a refrigerator.

Application of Immobilised Enzyme

Examining the application of the immobilised enzyme

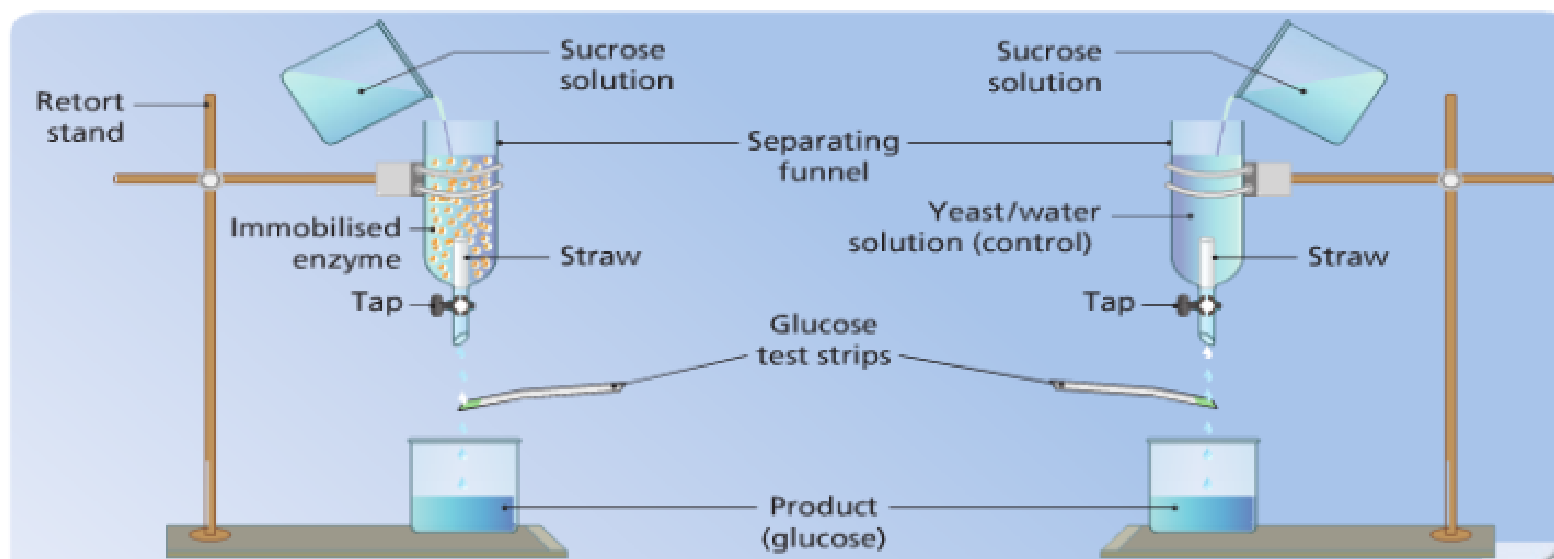
Yeast contains the enzyme sucrase. This enzyme converts sucrose into glucose and fructose. In this activity the ability of immobilised yeast and free yeast to convert sucrose into glucose is compared.

1. Pour the beads of immobilised enzyme into a separating funnel, as shown in diagram 9.21. A short piece of a drinking straw or a twisted-up paper clip should be used (to prevent the beads from blocking the outlet of the funnel).



9.20 *The conversion of sucrose to glucose and fructose*

2. Add some yeast to water in a beaker and stir.
3. Pour this solution into a second separating funnel.
4. Dissolve some sucrose in warm water.
5. Pour half of the sucrose solution into each separating funnel.
6. Test the products by letting them drip onto glucose test strips such as Clinistix or Diastrix.
7. Continue to test until glucose is found coming from each separating funnel.
8. Note and record the time taken for glucose to first form. Note that in most cases glucose is formed more quickly in the separating funnel containing the non-immobilised yeast (the control). Immobilised yeast is slower to **start** forming glucose. This is because it takes longer for the sucrose to penetrate the alginate beads and for the glucose to emerge from the alginate beads. However, once they start producing glucose the immobilised enzymes (or yeast) can be reused very easily.
9. Observe the products in each beaker. Compare the cloudiness of each solution. (The non-immobilised yeast solution contains many yeast cells and is very cloudy or turbid. The product of the immobilised yeast is much clearer because there are no yeast cells present.)
10. Present the results as shown below.



Activity 10 results		
	Immobilised yeast (or enzyme)	Non-immobilised yeast (or enzyme)
Time taken (minutes) for glucose to form		
Cloudiness of product (cloudy or clear)		