Chapter 9 - Enzymes

Learning objectives

- To define the term 'metabolism'
- To describe the difference between solar and cellular energy
- To define the term 'enzymes' and describe the structure and function of enzymes in plant and animal metabolism
- To describe the use of immobilised enzymes in bioprocessing
- To prepare an enzyme immobilisation and examine how it is applied
- To explain and investigate the effect of pH and temperature on the rate of enzyme activity.

Chapter 9 - Enzymes

Metabolism - is the sum of all chemical reactions in the body.

Your metabolism is controlled by enzymes.
Enzymes are proteins made in the ribosome from amino acids.

A catalyst is a chemical that speeds up a reaction without being used up.

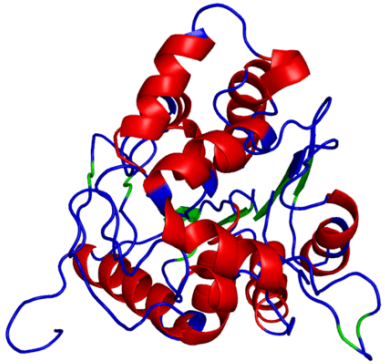
Enzymes are Biological catalysts.

The **substrate** is the substance with which an enzymes reacts. e.g. starch
The **product** is the substance the enzyme forms. e.g. Maltose.
Salivary Amylase is an enzyme that works in the mouth at a pH of 7/8.

Enzyme Shape

Enzymes are 3-D globular in shape.

Amino acids are stuck together and then the long chain is folded.
Enzymes only work if they are folded in the correct way.
The model below is what an enzyme looks like.



They have to have the**correct shape** to fit the substrate.
An enzyme can also add molecules together instead of pulling them apar

Starch + Amylase ⟶ Maltose (+ Amylase)

Substrate + Enzyme ⟶ Product (+ Enzyme)

Metabolic Roles

Enzymes are named by adding **'ase'** to the end.
The sugar sucrose is broken down by the enzyme sucrase.
e.g.Glucose is broken down by the enzyme glucase.

Catabolic enzymes - these CUT large molecules into smaller molecules.
E.g. **Amylase** breaks down food into smaller pieces.
Respiration - breaks down food into smaller pieces.

Anabolic enzymes - these ADD small molecules to make larger ones.
E.g. **DNA polymerase** is an enzyme that forms and repairs DNA.
Photosynthesis - adds small molecules to make large molecules.

Immobilised Enzymes

Bioprocessing is the use of enzyme controlled reactions to produce a product.

Yeast and bacteria are used to make various products like cheeses, yoghurts, breads, beers and wines.

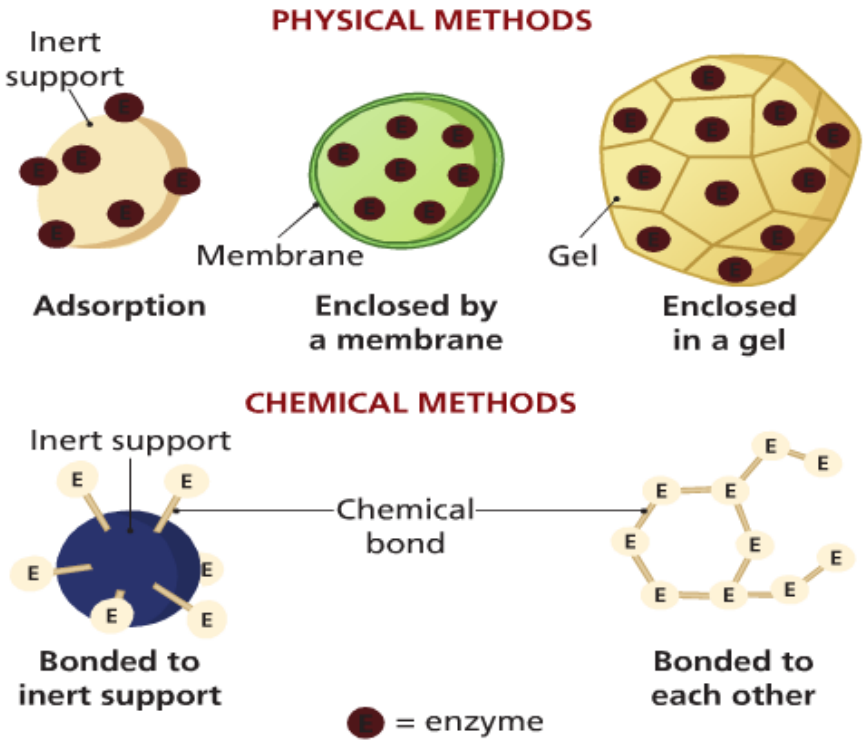
Adding yeast to sugar to make alcohol is often messy, makes the beer cloudy and costs more.

Yeast can be trapped or immobilised in a gel to stop the beer from getting cloudy.

Immobilised enzymes - are enzymes that are attached, or fixed, to each other or to an inert (no reaction) material

Ways of Immobilising Enzymes

The diagram shows various ways of immobilising enzymes.
We will immobilise yeast in a gel called **Sodium Alginate**.



Immobilised Enzyme

Add 0.4 g of **sodium alginate** to 10cm^3 of distilled water in a small beaker. Stir the mixture with a glass rod till smooth, leave for 5 minutes.

In a different small beaker, add 2g of yeast to 10cm^3 of distilled water.
The yeast contains the enzyme **Sucrase**.
Stir the yeast solution and leave for 5 minutes.

In a larger beaker, dissolve 1.5 g of **calcium chloride** into 100 cm^3 of distilled water.

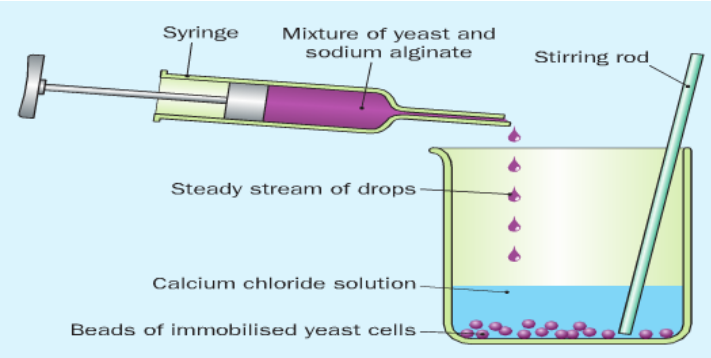
When smooth, add the **yeast** to the **alginate** and stir.

Draw 10ml of this mixture into a syringe and set up on retort stand over the beaker of Calcium Chloride.

The mixture will drip into the beaker forming **beads**.
The beads are **Calcium Alginate,** and have the yeast trapped (immobilised) inside of them.

Leave the beads to harden for 15 minutes.

Wash the beads with distilled water and store in fridge for use.



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Advantages

Immobilised enzymes can be **reused**. They work out cheaper over a long time.

Immobilised enzymes can be **recovered** and **cleaned** at the end of the process.

An immobilised enzyme is protected in gel and so is more **stable** and works longer.

Alcohol made with immobilised enzymes is cleaner and cheaper to make.

Uses

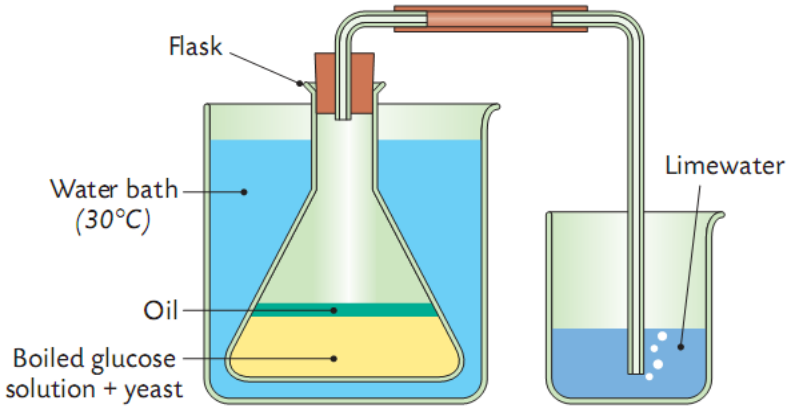
Fizzy drinks are sweetened with a sugar called **Fructose**.
Fructose is made by changing glucose to fructose.
An immobilised enzyme called glucose isomerase is used.

Penicillin acylase is an expensive enzyme that is used to make different forms of **antibiotics** to fight different bacteria.

Lactase is used to turn Lactose into glucose and galactose in **toffee** and caramel.

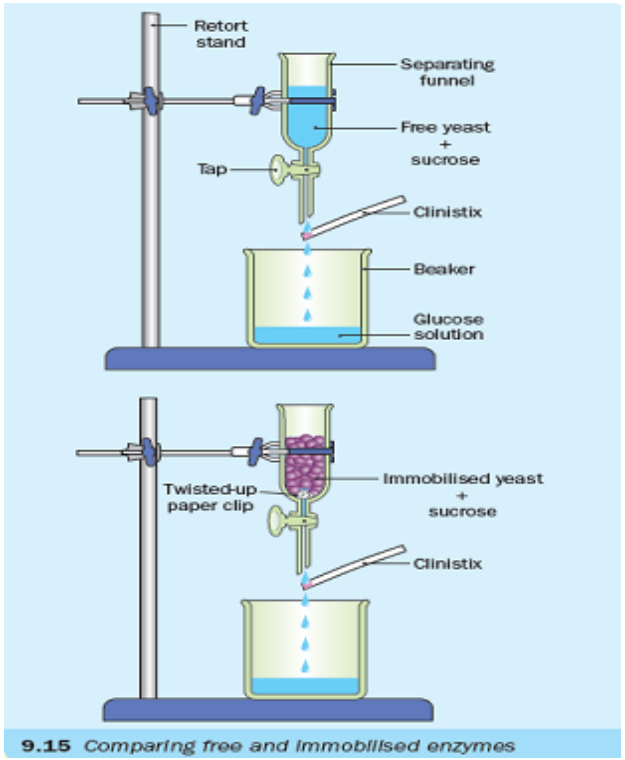
Application of immobilised enzymes

To make alcohol -
We boil water (to remove oxygen).
We add powdered glucose (for food).
The beads are added when the water cools (so enzyme isn't denatured)
After the beads are added to the glucose we add a layer of oil (to block oxygen)
Finally an air-lock is added to stop Oxygen getting in.
The flasks are put in a water bath at 30ºC for 3 or 4 days.
Test for alcohol - We add **potassium dichromate** and sulfuric acid and heat.
The solution will change from **orange** to **green** if there is alcohol present.



Another Application of the beads is to make Glucose.

To make glucose we add the beads to sucrose.
The sucrose solution filters down through the beads and drops of glucose are made.
The drops of glucose are tested using **clinistix** (test for glucose).



9.15 *Comparing free and immobilised enzymes*

Factors affecting enzyme activity

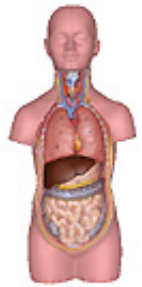
Enzymes work best under ideal conditions. This is called the optimum rate.
There are only 2 factors that we have to deal with, these are,

pH and Temperature

Any change in pH or temperature will change the shape of the enzyme.
This means the enzyme won't work.

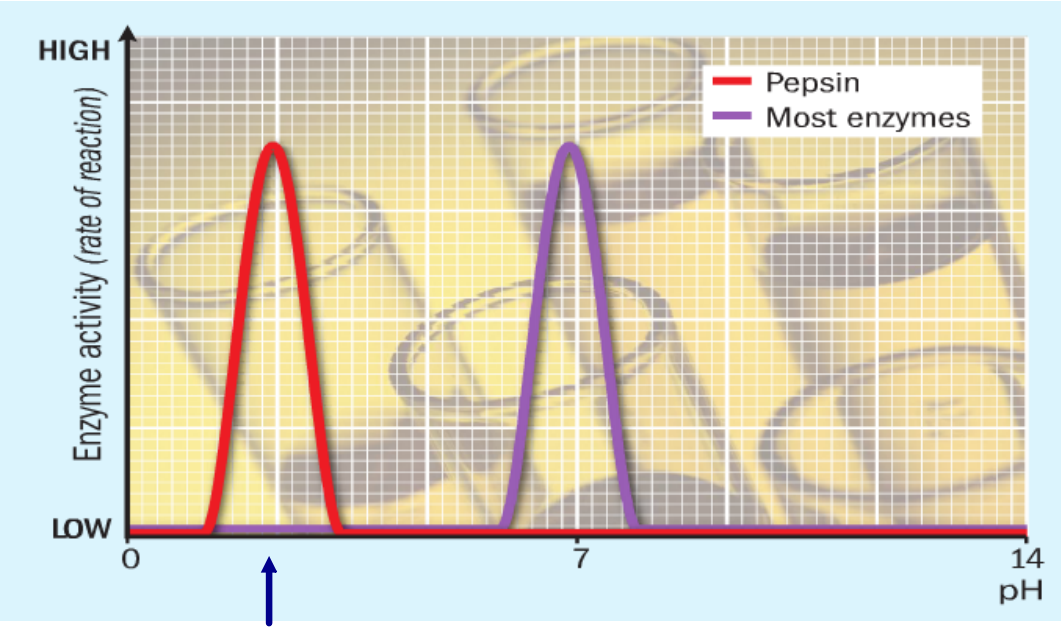
For plants enzymes the optimum (best) pH is **9** and the optimum temperature is **25°C**.

In humans the optimum temperature is body temperature is **37°C**.
The pH varies in different parts of the body.
e.g. in the mouth and pancreas the optimum pH is **7/8**,
(slightly alkaline for **Amylase**)
in the stomach the optimum pH is **2**, (very acidic for **pepsin**).



Graph of pH - Pepsin

Pepsin is found in the stomach. It breaks down proteins into amino acids.
The optimum pH for pepsin is pH 2. Most other enzymes in the body work best at pH 8.



Effect of pH on a Plant Enzymes Activity

Substrate + Enzyme = Product (and enzyme)

$2H_2O_2$ + Catalase = O_2 + $2H_20$ (and Catalase)

(Hydrogen Peroxide) **(from Celery)** **(foam)**

We will add the enzyme to the H_2O_2 in a graduated cylinder.
We will use a few different pH buffers to change the pH.
We need to keep the **temperature the same for all**.
This ensures a fair test and we are only testing one variable/factor, which is pH.

pH

1. Add 3 drops of washing-up liquid.
2. Add 10cm^3 of specific pH buffer.
4. Add 10 cm^3 of H_2O_2 and
5. Add 10cm^3 of Catalase (Celery) into a graduated cylinder.
6. Note how much foam is formed.

pH	Start level	Foam
pH 4	cm^3	cm^3
pH 6	cm^3	cm^3
pH 9	cm^3	cm^3

pH 9 is the optimum pH

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Effect of Temperature on a Plant Enzymes Activity

Substrate + Enzyme = Product (and enzyme)

$2H_2O_2$ + Catalase = O_2 + $2H_20$ (and Catalase)

(Hydrogen Peroxide) **(from Celery)** **(foam)**

We will add the enzyme to the H_2O_2 in a graduated cylinder.
We will use a few different water baths to change the temp.
We need to keep the **pH the same for all**.
This ensures a fair test and we are only testing one variable/factor, which is temperature.

Temperature

1. Add 3 drops of washing-up liquid.
2. Add 10cm^3 of pH9 buffer.
3. Add 10 cm^3 of H_2O_2
4. Place in water bath at 0, 25 or 60 degrees.
5. Place 10 cm^3 Catalase into each water bath also.
4. Add 10cm^3 of Catalase (Celery) into a graduated cylinder when at correct temperature.
5. Note how much foam is formed.

Temp	Start level	Foam
0^{o}C	cm^3	cm^3
25^{o}C	cm^3	cm^3
60^{o}C	cm^3	cm^3
90^{o}C	cm^3	cm^3

25^{o}C is the Optimum Temperature.

Summary of Results

pH

At pH4 - little foam
At pH6 - good bit of foam
At pH9 - lots of foam (optimum pH)
Temperature kept the same at 25°C by Water bath.

Temperature

At 0°C - little foam
At 25°C - lots of foam (optimum temperature)
At 60°C - good bit of foam

At 90°C - little foam (denatured enzyme)
pH kept the same at pH9 by pH buffer.

Enzyme Activity Graph