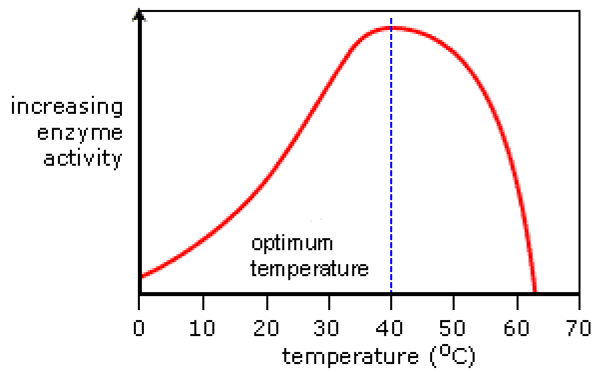
03 Enzymes

#21 Enzymes and reactions

Many chemical reactions can be speeded up by substances called **catalysts.** Within living organisms, these reactions (metabolic reactions) are controlled by catalysts called **enzymes.** Enzyme molecules are **proteins**.



Key definitions

Catalyst	substance	that **speeds up** a chemical reaction is **not changed** by the reaction
Enzyme	protein	that functions as **biological catalyst**
Substrate	chemical compound	the enzyme work on
End product	result	of the reaction

Enzymes and reactions

Most enzyme names end in –ase, e.g. lipase, protease.

Enzymes

Speed up	or	Slow down	reaction
Build up (synthesise)	or	Breake down	molecules

Enzymes usually speed up reactions, but some slow them down.

Some enzymes help to build up molecules (synthesise them), e.g.

Maltose $\xrightarrow{\textbf{starch phosphorylase}}$ Starch

Others are involved in breaking them down, e.g.

Protein $\xrightarrow{\textbf{protease}}$ Amino Acids

Starch $\xrightarrow{\textbf{amylase}}$ Maltose

<u>**Temperature, pH and enzymes**</u>

The activity of enzymes is affected by temperature and pH.

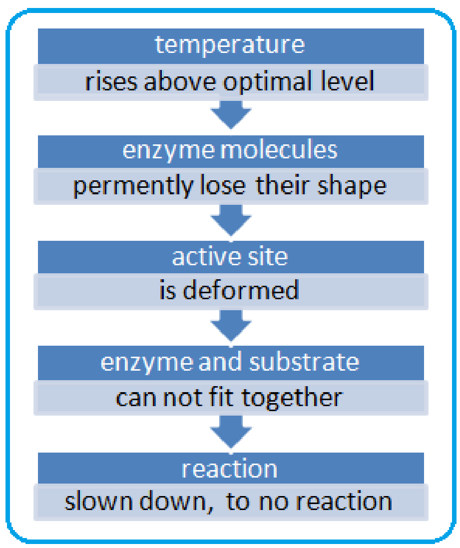
Effect of temperature on enzymes

The **optimum** (best) temperature for enzyme-controlled reactions is 37^0C (body temperature).

As the temperature increases, the rate of reaction increases. But very **high** temperatures **denature** enzymes.

The graph shows the typical change in an enzyme's activity with increasing temperature.

The enzyme activity gradually increases with temperature up to around 37ºC, or body temperature. Then, as the **temperature** continues to **rise**, the rate of reaction falls rapidly as heat energy **denatures** the enzyme. Most enzymes are denatured above 50^0C.



Common misconceptions

- Enzymes are not denatured by low temperatures – they are just slowed down, and will work again when the temperature is suitable.
- Once an enzyme is denatured, the damage is permanent.

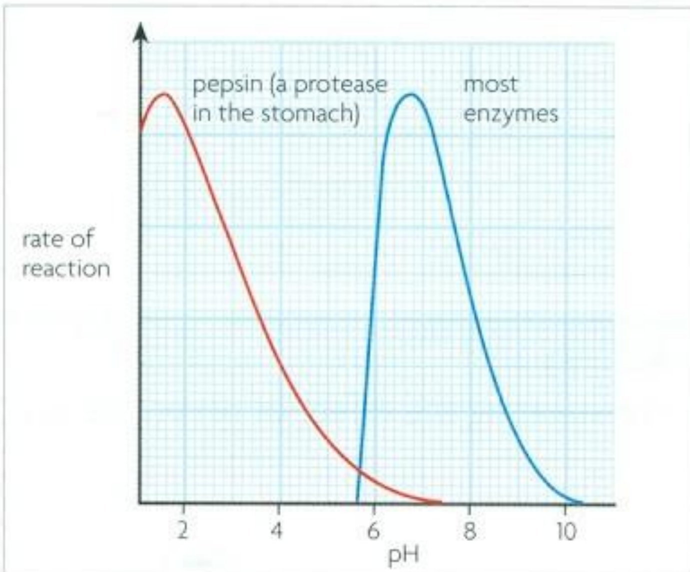
Effect of pH on enzymes

- The pH of a solution is how acidic or alkaline it is.
- Different enzymes work best at different pH values.
- The optimum pH for an enzyme depends on where it normally works.
- It is around neutral (**pH= 7**) for most enzymes but there are some exceptions.

Enzymes	Substrate	End products	Location	pH
Salivary amylase	starch	Maltose Glucose	mouth	6.8
Pancreatic lipase	fat	Fatty acids, Glycerol	duodenum	9.0
Protease	protein	Amino acids	stomach	2.0
			duodenum	9.0

Changes in pH also alter an enzyme's shape and slow down its activity, but this can usually be **reversed** if the optimum pH is restored.

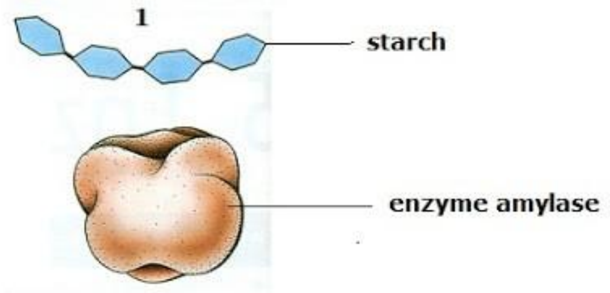
An extreme pH can **denature** enzymes – the active site is deformed permanently.



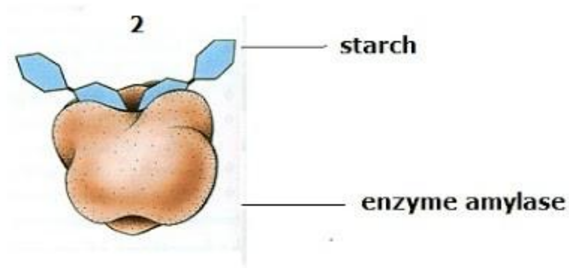
How pH affects enzyme activity.

#22 Enzymes - 'Lock and key' model

Enzymes are very **specific,** each kind of enzyme catalyse one kind of reaction only. To catalyse a reaction, **enzyme** molecule and **substrate** molecule need to meet and **joint** together by a temporary bond.

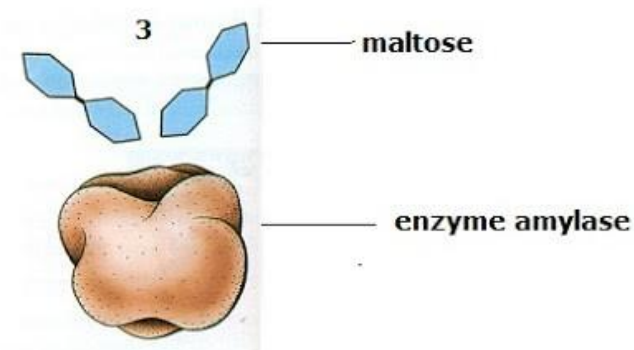


Each molecule has a special shape and an **active site** into which its **substrate** molecule fits exactly.



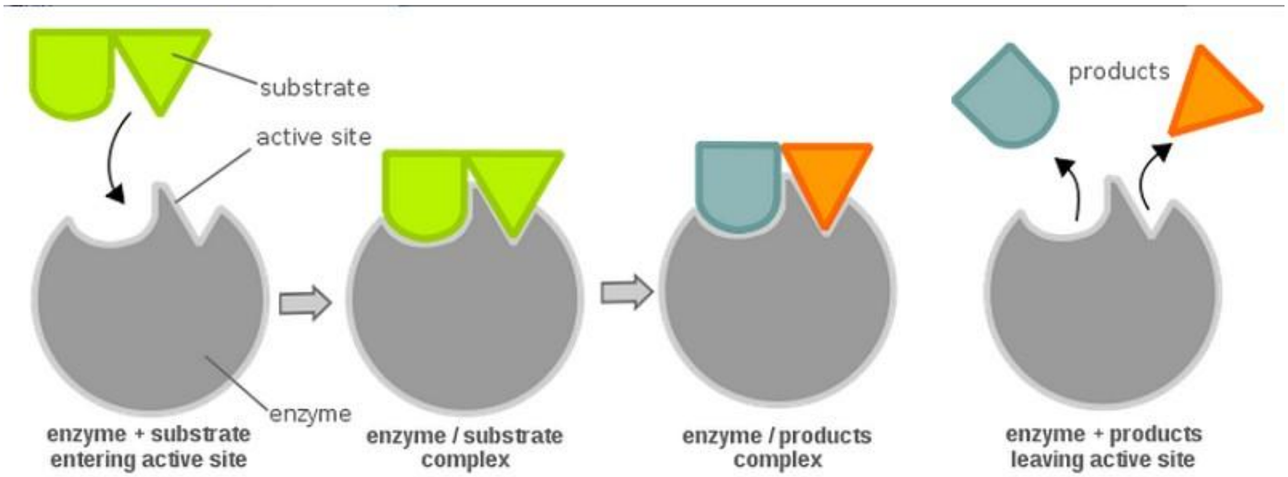
This enzyme is **amylase**, and its **active site** is just the right size and shape for a **substrate** molecule (**starch** in this case).

The starch **slots** into the active site.



The starch is **split** into **maltose** molecules.

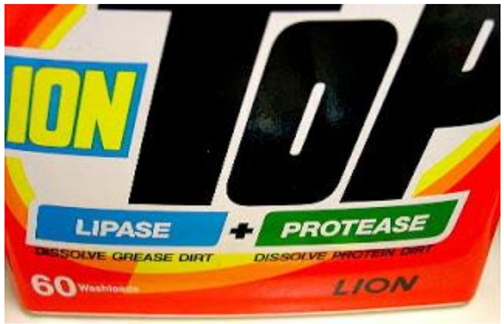
The enzyme is unaltered, and ready to accept another part of the starch molecule



23 Role of enzymes in germinating seeds

- Seeds contain **stored food** in the cotyledons to provide energy and materials for growth. This is usually in the form of **starch** – a large, *insoluble* molecule (long chain of glucose), that keeps the food immobile. The starch needs to be changed into a *soluble* molecule (**sugar**) with help of **enzymes** for the seeds to make use of.
- In the presence of **H_2O**, **Gibberellin** or **gibberellic acid** (GA) stimulates the production of **amylase**.
- **Amylase** breaks down **starch** to **maltose**, allowing for the formation of ATP (via glucose).
- The **energy** produced in the embryo is used to facilitate germination.
- The **glucose** produced may also be used to synthesis cellulose - for cell wall formation.
- **Warmth** helps speed up the process.

#24 Use of enzyme in biological washing powders



Biological washing powders contain **protease** and **lipase** to remove **protein** stains and **fat**/grease from clothes. The enzymes break down proteins or fats on the fabric, forming water-soluble substances that can be washed away.

Example: Blood contain the red protein Haemoglobin (Hb). The Proteases in biological washing powder break Hb molecules into smaller molecules, which are not coloured and which dissolve in water and can be washed away.

This makes the washing powder more effective than detergent alone, especially at lower temperatures. This save energy (no need to boil water), but if the temperature is too high, the enzyme will be denatured.

The differences between biological and regular washing powders

	Biological washing powder	Regular washing powder
Detergents Mix greasy dirt with H_2O so it can be washed away	(+)	(+)
Enzymes **Braking** down stains and dirt in fabrics	(+) **Proteases** → proteins (blood, egg, gravy*…) **Amylases** → starches **Lipases** → fats and grease **Cellulase** → micro fibrils on cotton, brightening color of washed clothes They work efficiently at 40°C.	(-)
Remove **difficult stains** (blood, gravy, egg yolk, sweat, fats and grease)	**Easily** by decomposing the stains.	**Difficultly.** **Heat** alone makes stains coagulate and attach more firmly to the clothing.

*Gravy: juices that drip from cooking meat.

Do it your self

Boil two standard eggs together, and push two teaspoons into the yolks so that there is some yolk left on the spoons.

Dissolve equal amounts of **ordinary** and **'biological'** detergents in two separate glasses of water, and leave a yolk-stained spoon in each glass. After some time you will see that the spoon in the ordinary detergent still has **yolk** on, but the yolk on the other spoon has been digested by the 'biological' detergent. This will happen if the 'biological' detergent really contains
enzymes that break down the proteins in egg yolk.

Try this

Figure above shows a box of biological washing powder.

a) Explain why:
i) The presence of protease and lipase would make the washing powder more effective than ordinary detergent [3 marks]

ii) The powder should not be used in boiling water [2 marks]

b) Silk is a material made from protein. Explain why the biological washing powder should not be used to wash silk clothes [2 marks]

Answer:

a) i) Protease and lipase are enzyme
They break down stains better than ordinary detergent
Protease breaks down protein; lipase breaks down fat
ii) Enzymes are denatured at high temperature
b) There is protease in the biological washing powder
This would digest the protein in the silk so the clothes would get spoiled.

Benefit from using enzymes in cleaning products, toothpaste and other products in our home.

Additional resources:

- http://www.saasta.ac.za/biosciences/enzymes.html
- http://isbibbio.wikispaces.com/biological+washing+powders+and+enzymes

#25 Use of enzymes in the food industry

Enzymes are an integral component of modern **fruit juice** manufacturing and are highly suitable for optimising processes. Fruit juices are **extracted** using an enzyme called **pectinase**.

Pectin is a substance which helps to stick plant cells together. Fruits like apple or orange contain a lot of pectin. The braking down of **pectin** makes it much easier to squeeze juice from the fruit.

Pectinase is widely used in order to:

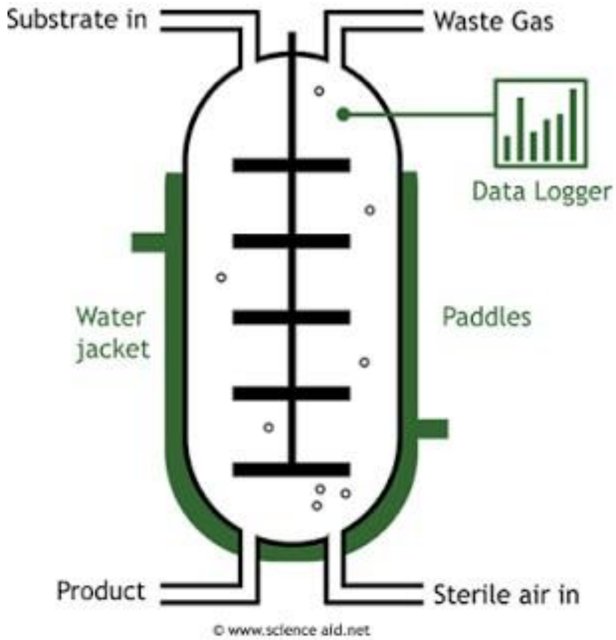
- increase **extraction** of juice from raw material
- increase **processing efficiency** (pressing, solid settling or removal)
- generate a final product that is **clear** and visually attractive

Enzymes are sometimes used when making baby foods. **Proteases** are used to treat some high-protein foods, they break down **proteins** to **polypeptides** and **amino acids** for young baby to **absorb** the food **easier**.

Use of enzymes in the food industry

Process	Use
Baking	Enzymes in yeast convert **sugar** → ethanol and **CO_2** **CO2** makes the bread dough **rise**
Brewing	Enzymes in yeast convert **sugar** → **ethanol** and **CO_2** **Ethanol** makes the drink **alcoholic** CO_2 makes the drink **fizzy**
Chees making	Enzyme **rennin,** extracted from cow's stomachs, is used to **clot** milk. Rennin can now be made using genetically engineered bacteria.
Making baby foods	**Trypsin** (a protease) is used to **predigest** baby foods.

#26 Use of microorganisms and fermenter to manufacture enzymes



We obatain many **enzymes** from **microorganisms**. The enzymes that are used in industry are usually obtained from microorganisms. These include **bacteria** and microscopic **fungi**, such as **yeast**. The microorganisms are grown inside large vessels called **fermenters.**

- Inside the **fermenter**, the microorganisms are provided with everything they need to grow and reproduce, e.g.:**O_2**, supply of **nutrients**, a suitable **pH** and **temperature**.
- The microorganisms make the enzymes and release them into the **liquid** in which they are growing.
- The liquid can then be collected from the fermenter, and the enzymes **purified** before use.

Closer look at fermenter

The fermenter is a large, sterile container with a stirrer, a pipe to add feedstock* (molasses* or corn-steep liquor), and air pipes to blow air into the mixture.
The microorganisms are added and the liquid is maintained around 26^0C and a pH of 5-6.

Use of enzymes in the food industry

Process	Use
Baking	Enzymes in yeast convert **sugar** → ethanol and CO_2 $CO2$ makes the bread dough **rise**
Brewing	Enzymes in yeast convert **sugar** → ethanol and CO_2 Ethanol makes the drink alcoholic CO_2 makes the drink fizzy
Chees making	Enzyme **rennin,** extracted from cow's stomachs, is used to **clot** milk. Rennin can now be made using genetically engineered bacteria.
Making baby foods	Trypsin (a protease) is used to predigest baby foods.

The enzymes produced by the microorganisms may be extracellular or intracellular:

1. **Extracellular** enzymes are extracted from the feedstock by filtering.
2. To extract **intracellular** enzymes the microorganisms are filtered from the feedstock, then crushed and washed with water. The enzymes are now in solution.

Explain words:

* ***Feedstock***: Raw material (input) fed into a process for conversion into something different (output).

* ***Molasses (syrup):*** Thick, dark brown, uncrystallized juice obtained from raw sugar during the refining process.

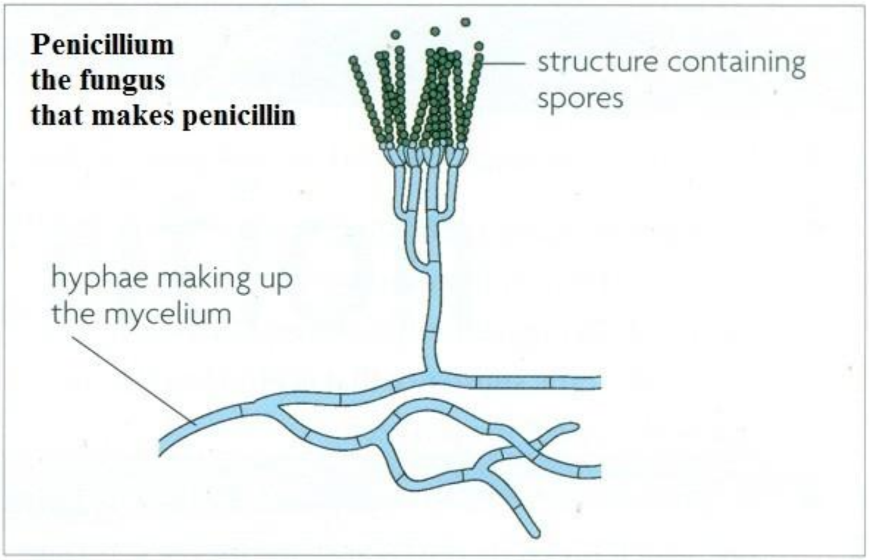
****Corn-steep liquor***: a concentrated fluid obtained by soaking corn grains in water (containing 0,2% SO_2) for 36 — 40 hours at 46 — 50°C.

#27 Use of microorganisms to manufacture antibiotic penicillin

Three colonies of a Penicillium mold growing on an agar medium.

Antibiotics are substances which kill **bacteria** without harming human cells. They help to cure bacterial infections. **Penicillin** is made by growing the fungus ***Penicillium*** in a large **fermenter**.

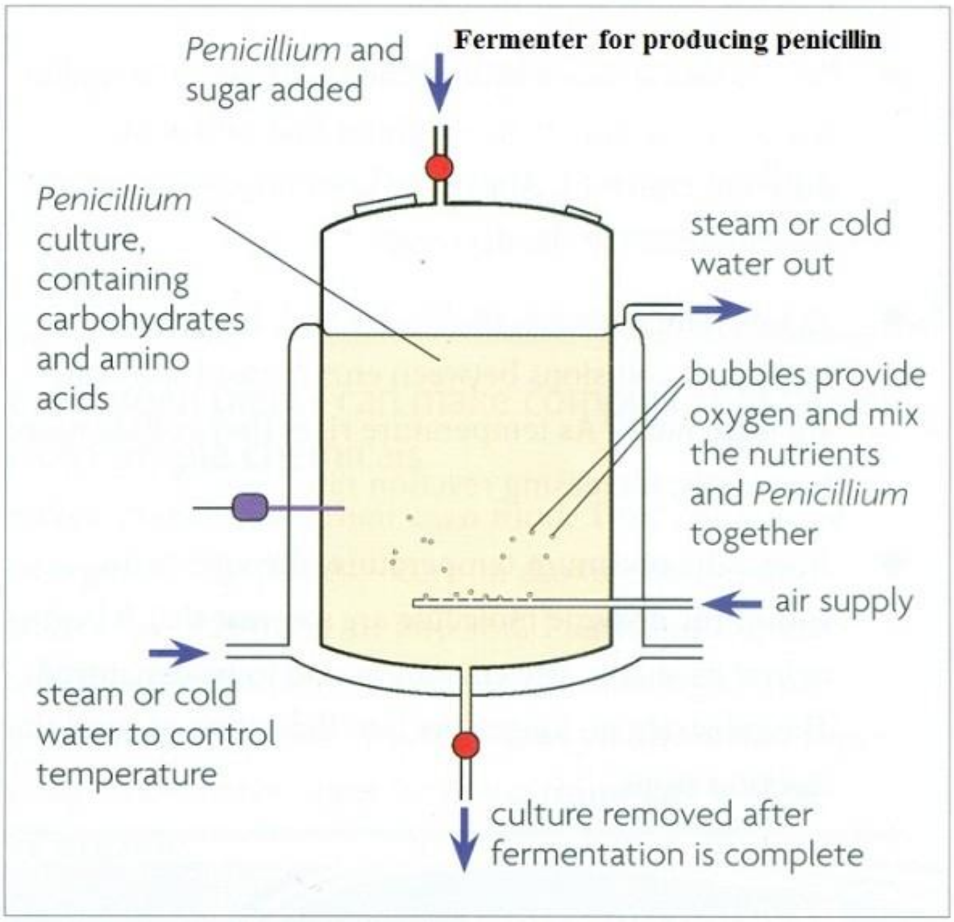
Often, it is easier to use the **whole microorganisms** rather than extract its enzymes from it. The microorganism is grown in a fermenter, its enzymes **convert** a **substrate** to a desired **product**. The production of antibiotic **penicillin** is one example.



Fermenter

The fungus is grown in a **culture** medium containing **carbohydrates** and **amino acid**. This looks like watery porridge and is stirred continuously to:

- Keep the fungus in contact with fresh supplies if nutrients.
- Mix O2 into the culture
- Roll the fungus up into little **pellets** (this facilitates the ***separating*** of the liquid part containing penicillin from the fungus lately).



For first **15-24 h**, the fungus just grows. After that it begins to secret **penicillin**. Rate of production depends on how much sugar is available:

- A lot of sugar à not much penicillin
- No sugar à no penicillin

So **small amount** of sugar have to be fed all the time that the fungus is producing penicillin.

The culture is kept going until the **rate** of production is so **slow** that is not worth waiting more (often after a week). Then it is **filtered**, and the liquid is treated to **concentrate** the penicillin in it.

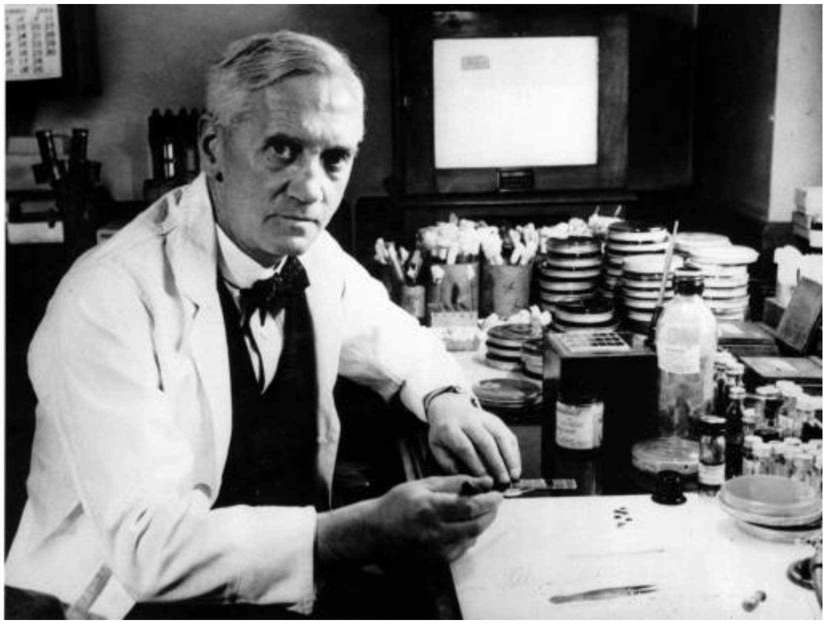
History (not included in the syllabus)

Thousands of glass fermentation vessels like this one were used in Glaxo (now GlaxoSmithKline) laboratories to produce penicillin.

Credits: Science Museum

The penicillium mould was grown on the surface of a liquid filled with all the nutrients it needed. This approach was replaced by the method of growing the mould within large industrial fermenters. The antibiotic was first used in the early 1940s and saved the lives of many soldiers during the Second World War.

Discovering of Penicillin



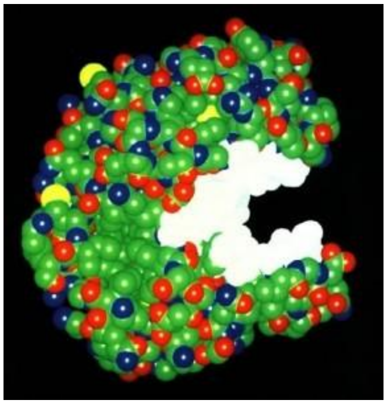
Sir Alexander Fleming, 1952 (photo AP)

Penicillin was discovered **by chance.** **Alexander Fleming -** the Scottish bacteriologist - accidentally left a dish of staphylococcus bacteria uncovered for a few days. He returned to find the dish dotted with bacterial growth, apart from one area where a patch of **mould** (Penicillin notatum) was growing. The mould produced a substance, named **penicillin** by Fleming, which inhibited bacterial growth and was later found to be effective against a wide range of harmful bacteria.

However, it was not until **World War II** that penicillin, the **first antibiotic,** was finally isolated by Howard Florey and Ernst Chain. Fleming, Florey and Chain received a Nobel prize in 1945, for their discovery which revolutionised medicine and led to the development of lifesaving antibiotics.

Additional sources: **Royal Society of Chemistry**
sciencemuseum

#28 Summary of Enzymes



- **Enzymes** are specialized **protein** molecules facilitating most of the body's **metabolic processes** – such as, supplying energy, digesting foods, purifying your blood, ridding the body of waste products. Enzymes are vital to our health and **change** the **rate** at which **chemical reactions** happen, but without any external **energy** source added or by being changed themselves.
- Enzymes are **proteins** that work as biological **catalysts**.
- Enzymes are named according to the **substrate** on which they act. **Proteases** act on proteins, **carbohydrases** on carbohydrates and **lipases** on fats (lipids). The substance that is produced by the reaction is called the **product**.
- An enzyme molecule has a depression called its **active site**, which is exactly the right shape for the substrate to fit into. The enzyme can be thought of as a **lock**, and the substrate as the **key**.
- **Reactions** catalysed by enzymes work **faster** at **higher temperatures**, up to an optimum that differs for different enzymes. Above the **optimum** temperature, reaction rate rapidly decreases.
- At low **temperatures**, molecules have low kinetic energy, so collisions between enzyme and substrate molecules are infrequent. As temperature rises they collide more frequently, increasing reaction rate.
- **Above** the **optimum temperature**, the vibrations within the enzyme molecule are so great that it begins to lose its shape. The **enzyme** is said to be **denatured**. The substrate no longer fits into the active site and the **reaction stops**.

- Reactions catalysed by enzymes work fastest at a particular pH. The **optimum pH** for most enzymes is around **pH7** (neutral), but some have an optimum pH much higher or lower than this.
- **Extremes of pH** cause enzyme molecules to **lose** their **shape**, so they no longer bind with their substrate.
- **Amylase** is found in **seeds**. When the seed begins to **germinate**, the amylase is activated and catalyses the breakdown of **insoluble starch** to **soluble maltose** in the seed. The maltose is used by the growing embryo as an **energy** source and to make **cellulose** for new cell walls.
- **Biological** washing **powders** contain enzymes, often obtained from **microorganisms** such as **bacteria** or **fungi**. The enzymes break down proteins or fats on the fabric, forming watersoluble substances that can be washed away.
- **Pectinase** is used to break down cell walls in **fruits**, making it easier to extract **juice** from them.
- The antibiotic **penicillin** is made by cultivating the fungus **Penicillium** in a fermenter. The fermenter is kept at the correct pH and temperature for the enzymes of the fungus to work well.