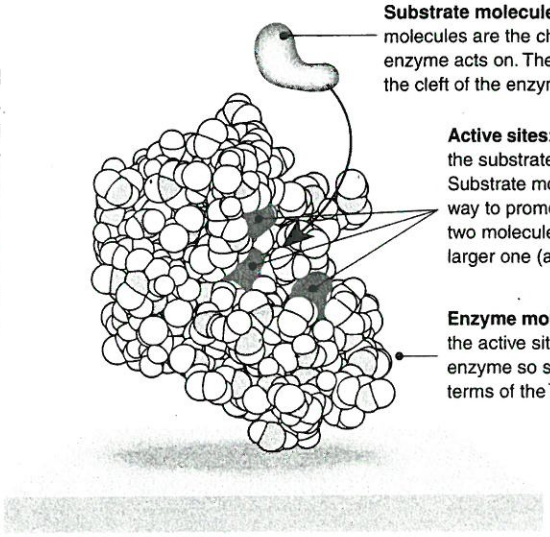
Enzymes

Most enzymes are proteins. They are capable of catalyzing (speeding up) biochemical reactions and are therefore called biological **catalysts**. Enzymes act on one or more compounds (called the **substrate**). They may break a single substrate molecule down into simpler substances, or join two or more substrate molecules chemically together. The enzyme itself is unchanged in the reaction; its presence merely allows the reaction to take place more rapidly. When the substrate attains the required **activation energy** to enable it to change into the product, there is a 50% chance that it will proceed forward to form the product, otherwise it reverts back to a stable form of the reactant again. The part of the enzyme's surface into which the substrate is bound and undergoes reaction is known as the **active site**. This is made of different parts of polypeptide chain folded in a specific shape so they are closer together. For some enzymes, the complexity of the binding sites can be very precise, allowing only a single kind of substrate to bind to it. Some other enzymes have lower **specificity** and will accept a wide range of substrates of the same general type (e.g. lipases break up any fatty acid chain length of lipid). This is because the enzyme is specific for the type of chemical bond involved and not an exact substrate.

Enzyme Structure

The model on the right is of an enzyme called *Ribonuclease S*, which breaks up RNA molecules. It is a typical enzyme, being a globular protein and composed of up to several hundred atoms. The darkly shaded areas are called **active sites** and make up the **cleft**; the region into which the substrate molecule(s) are drawn. The correct positioning of these sites is critical for the catalytic reaction to occur. The substrate (RNA in this case) is drawn into the cleft by the active sites. By doing so, it puts the substrate molecule under stress, causing the reaction to proceed more readily.



Substrate molecule: Substrate molecules are the chemicals that an enzyme acts on. They are drawn into the cleft of the enzyme.

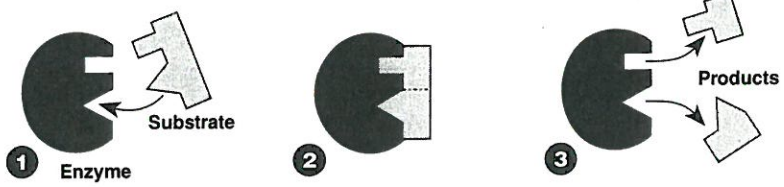
Active sites: These attraction points draw the substrate to the enzyme's surface. Substrate molecule(s) are positioned in a way to promote a reaction: either joining two molecules together or splitting up a larger one (as in this case).

Enzyme molecule: The complexity of the active site is what makes each enzyme so specific (i.e. precise in terms of the substrate it acts on).

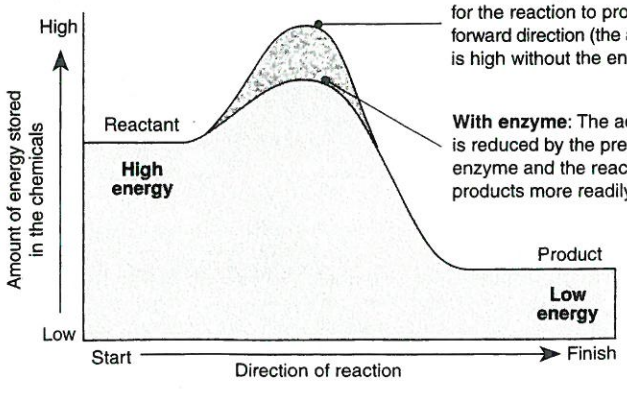
Source: *Biochemistry*, (1981) by Lubert Stryer

How Enzymes Work

The **lock and key** model proposed earlier this century suggested that the substrate was simply drawn into a closely matching cleft on the enzyme molecule. More recent studies have revealed that the process more likely involves an **induced fit** (see diagram on the right), where the enzyme or the reactants change their shape slightly. The reactants become bound to enzymes by weak chemical bonds. This binding can weaken bonds within the reactants themselves, allowing the reaction to proceed more readily.



The presence of an enzyme simply makes it easier for a reaction to take place. All **catalysts** speed up reactions by influencing the stability of bonds in the reactants. They may also provide an alternative reaction pathway, thus lowering the activation energy needed for a reaction to take place (see the graph below).

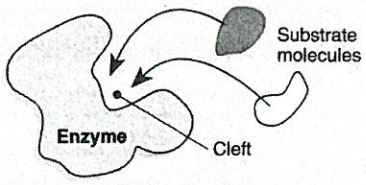


Without enzyme: The energy required for the reaction to proceed in the forward direction (the activation energy) is high without the enzyme present.

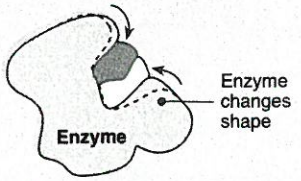
With enzyme: The activation energy is reduced by the presence of the enzyme and the reactants turn into products more readily.

Induced Fit Model

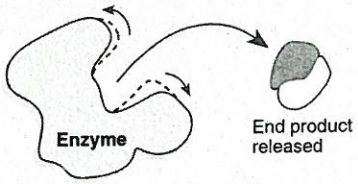
An enzyme fits to its substrate somewhat like a lock and key. The shape of the enzyme changes when the substrate fits into the cleft (called the **induced fit**):



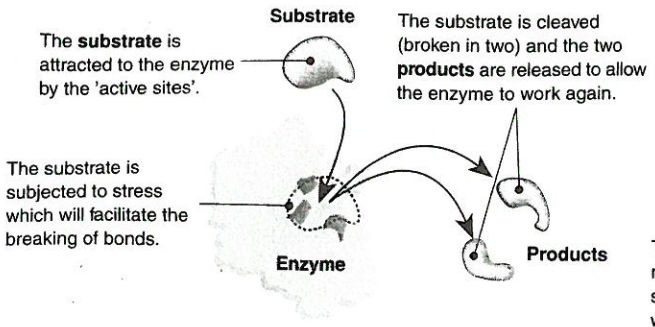
1 Two substrate molecules are drawn into the cleft of the enzyme.

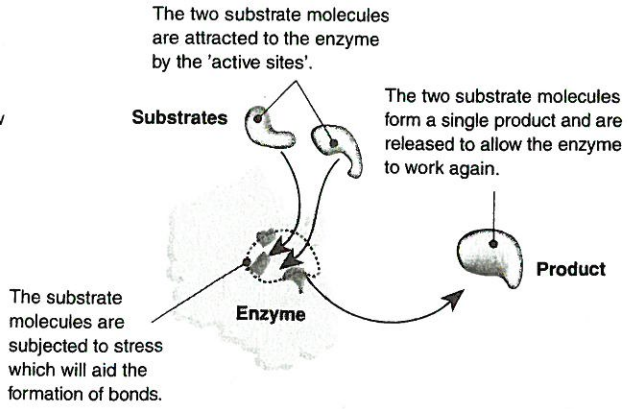


2 The enzyme changes shape, forcing the substrate molecules to combine.



3 The resulting end product is released by the enzyme which returns to its normal shape, ready to receive more.





Catabolic reactions

Some enzymes can cause a single substrate molecule to be drawn into the active site. Chemical bonds are broken, causing the substrate molecule to break apart to become two separate molecules. Catabolic reactions break down complex molecules into simpler ones and involve a net release of energy, so they are called exergonic. **Examples**: *hydrolysis, cellular respiration.*

Anabolic reactions

Some enzymes can cause two substrate molecules to be drawn into the active site. Chemical bonds are formed, causing the two substrate molecules to form bonds and become a single molecule. Anabolic reactions involve the net use of energy (they are endergonic) and build more complex molecules and structures from simpler ones. **Examples**: *protein synthesis, photosynthesis.*

1. Give a brief account of enzymes as **biological catalysts**, including reference to the role of the **active site**:

2. Using examples, distinguish between **catabolism** and **anabolism**, and state whether the product has a higher or lower potential energy than the reactants:

3. Outline the key features of the '**lock and key**' model of enzyme action:

4. Outline the '**induced fit**' model of enzyme action, explaining how it differs from the lock and key model:

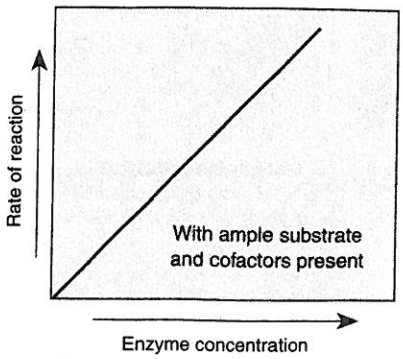
5. Identify two factors that could cause enzyme denaturation, explaining how they exert their effects (see the next activity):

   (a)

   (b)

6. Explain what might happen to the functioning of an enzyme if the gene that codes for it was altered by a mutation:

range of conditions under which they operate properly. For most of the enzymes associated with plant and animal metabolism, there is little activity at low temperatures. As the temperature increases, so too does the enzyme activity, until the point is reached where the temperature is high enough to damage the enzyme's structure. At this point, the enzyme ceases to function; a phenomenon called enzyme or protein **denaturation**. Extremes in acidity (pH) can also cause the protein structure of enzymes to denature. Poisons often work by denaturing enzymes or occupying the enzyme's active site so that it does not function. In some cases, enzymes will not function without cofactors, such as vitamins or trace elements. In the four graphs below, the rate of reaction or degree of enzyme activity is plotted against each of four factors that affect enzyme performance. Answer the questions relating to each graph:



1. **Enzyme concentration**

   (a) Describe the change in the rate of reaction when the enzyme concentration is increased (assuming that substrate and cofactors are not limiting):

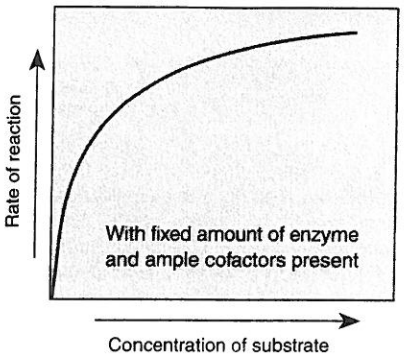
   _____

   _____

   (b) Suggest how a cell may vary the amount of enzyme present in a cell:

   _____

   _____



2. **Substrate concentration**

   (a) Describe the change in the rate of reaction when the substrate concentration is **increased** (assuming a fixed amount of enzyme and ample cofactors):

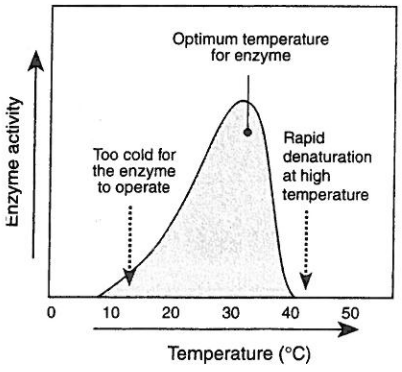
   _____

   _____

   (b) Explain why the rate changes the way it does: _____

   _____

   _____



3. **Temperature**

   Higher temperatures speed up all reactions, but few enzymes can tolerate temperatures higher than 50–60°C. The rate at which enzymes are **denatured** (change their shape and become inactive) increases with higher temperatures.

   (a) Describe what is meant by an optimum temperature for enzyme activity:

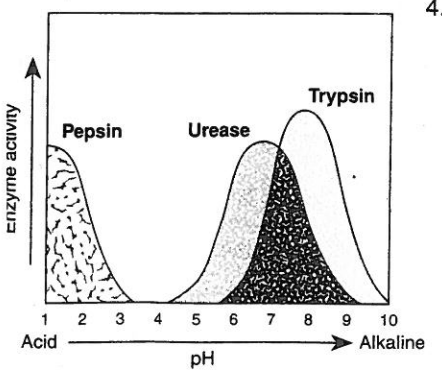
   _____

   _____

   (b) Explain why most enzymes perform poorly at low temperatures:

   _____

   _____



4. **pH (acidity/alkalinity)**

   Like all proteins, enzymes are **denatured** by extremes of **pH** (very acid or alkaline). Within these extremes, most enzymes are still influenced by pH. Each enzyme has a preferred pH range for optimum activity.

   (a) State the optimum pH for each of the enzymes:

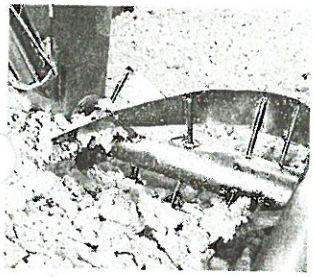
   Pepsin: _____ Trypsin: _____ Urease: _____

   (b) Pepsin acts on proteins in the stomach. Explain how its optimum pH is suited to its working environment:

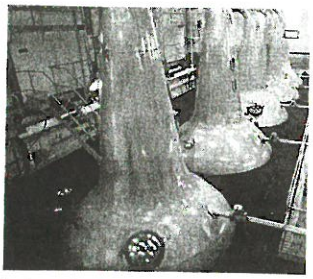
   _____

   _____

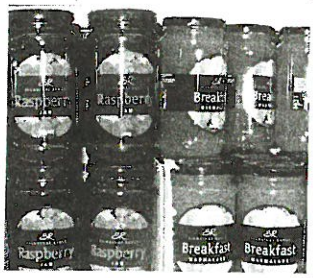
enzymes because of their high productivity, ease of culture in industrial fermenters, and the ease with which they can be genetically modified to produce particular products. In addition, the variety of enzymes available for exploitation is very large. Some of the microorganisms involved in industrial fermentations, and their enzymes and their applications are described below.



Enzymes are used in various stages of **cheese production**, e.g. chymosin from GE microbes now replaces the rennin previously obtained from calves.



In **beer brewing**, **proteases** (from bacteria) are added to prevent cloudiness. Amyloglucosidases are used to produce low calorie beers.

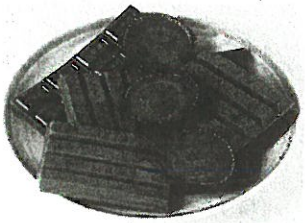


Citric acid is used in **jam production** and is synthesized by a mutant strain of the fungus ***Aspergillus niger***, which produces the enzyme citrate synthase.

Biological detergents use **proteases**, **lipases**, and **amylases** extracted from fungi and thermophilic bacteria to break down organic material in stains.

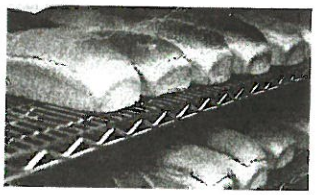
Fungal ligninases are used in **pulp and paper industries** to remove lignin from wood pulp and treat wood waste.

Some of the many applications of microbial enzymes in medicine, industry, and food manufacture.

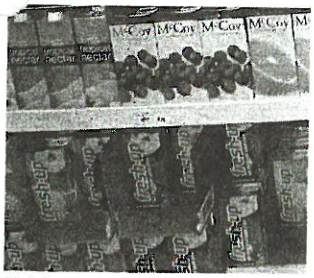


In **soft centered chocolates**, **invertase** from yeast breaks down the solid filling to produce the soft center.

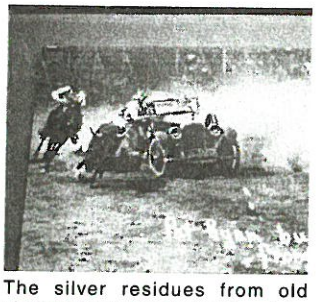
Medical treatment of blood clots employs protease enzymes such as streptokinase from *Streptomyces* spp.



Bacterial proteases are used to break down the wheat protein (gluten) in flour, to produce low gluten breads.

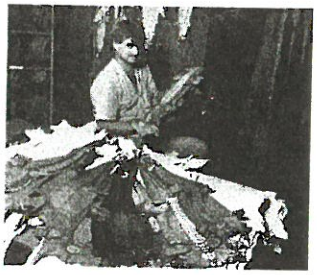


Cellulases and pectinases are used in the manufacture of packaged (as opposed to fresh) fruit juices to speed juice extraction and prevent cloudiness.

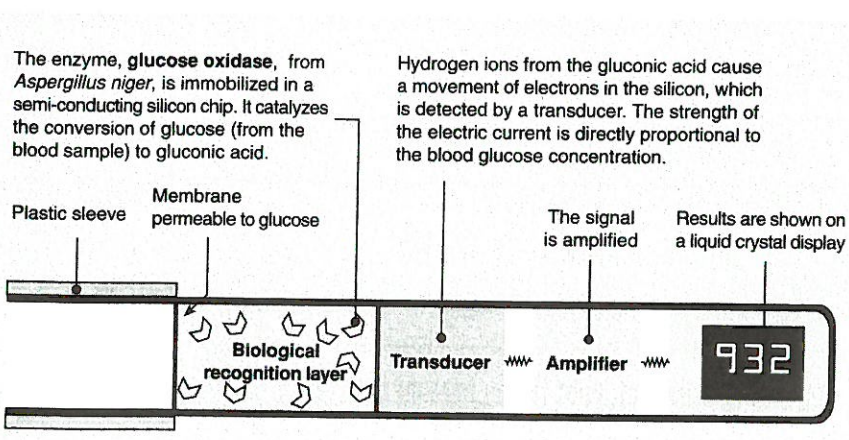


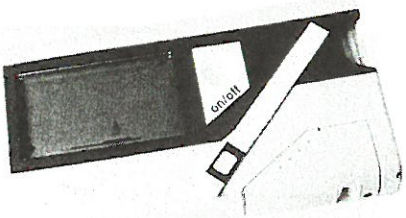
The silver residues from old photographs can be reclaimed for reuse when proteases are employed to digest the gelatin of old films.

The lactase from bacteria is used to convert lactose to glucose and galactose in the production of low-lactose and lactose free milk products.



Tanning industries now use proteases from *Bacillus subtilis* instead of toxic chemicals, such as sulfide pastes, to remove hairs and soften hides.





Biosensors are electronic monitoring devices that use biological material to detect the presence or concentration of a particular substance. Enzymes are ideally suited for use in biosensors because of their specificity and sensitivity. This example illustrates how **glucose oxidase** from the fungus *Aspergillus niger* is used in a biosensor to measure blood glucose level in diabetics.

Periodicals:
Enzyme technology

Web links: *Science in the Box: Enzymes*