

Enzymes outside the body

Enzymes allow chemical reactions in all living things to proceed quickly, under conditions where they would normally be very slow. Scientists realised that enzymes could be isolated and used to catalyse reactions outside living organisms. This was the start of a major biotechnology industry, which is still developing.

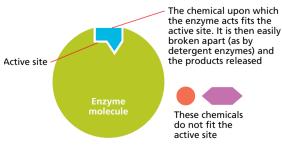
Enzyme
Carbohydrase
Lipase
Protease
Protein

Above: A computer graphic showing the structure of the enzyme lipase. Each amino acid appears in a different colour.

 Work out exactly how many combinations of amino acids there could be for a chain of 50, with any of the 20 occurring at each position. nzymes are catalysts made by living organisms. There are thousands of different chemical reactions taking place in living things and almost every reaction has its own associated enzyme. There are, therefore, vast numbers of different enzymes.

How can there be so many? Enzymes are proteins, polymers of amino acids. Most of the biological molecules you need to know about are made up of pretty standard basic units — for example starch and glycogen are made up of lots of glucose molecules. But when proteins are formed there are 20 *different* amino acids to be drawn upon. Box 1 gives you some idea of how such a vast diversity of proteins can be built from 20 amino acids.

Figure 1 The active site in an enzyme molecule.



Enzyme literally Changing pH or temperature alters means in yeast. Changing pH or temperature alters the shape of the active site

HOW DO ENZYMES WORK?

The shape of each enzyme is determined by the sequence of amino acids it contains and the way in which they link up to form a particular three-dimensional molecule. One part of this molecule is an active site — the part of the enzyme that interacts with the chemical or chemicals involved in the reaction it catalyses (Figure 1). This site recognises the shape of the chemicals and reduces the amount of

BOX 1 ENZYME DIVERSITY

There are 20 common amino acids. Each amino acid molecule includes a standard component (which is involved in the link with other amino acids as the protein forms) but the rest of the molecule varies greatly between amino acids. In some the rest of the molecule mixes with water, in others it doesn't, some carry a small positive charge, others a negative charge.

Imagine an enzyme to be 50 amino acids long. How many possible combinations are there? At each of the 50 positions in the amino acid chain there could be any of 20 amino acids. This provides vast numbers of possible combinations — and some proteins are made up of much longer chains of amino acids.

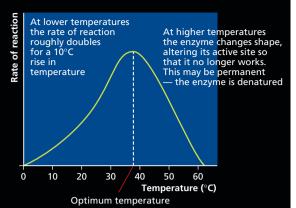


Figure 2 The effect of temperature on a reaction catalysed by an enzyme.

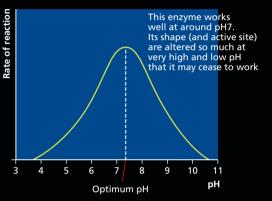
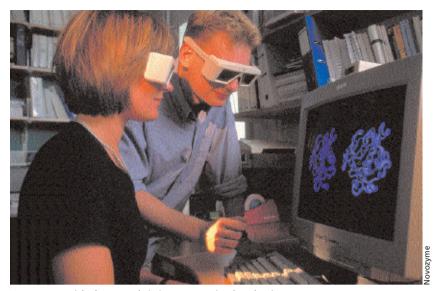


Figure 3 The effect of pH on a reaction catalysed by an enzyme.

energy needed to join them together — or split them apart, as happens in digestive processes.

Enzymes are affected by temperature and pH (Figures 2 and 3). At both high temperatures and extremes of pH, they change shape. The active site changes shape too, and no longer matches up with the chemicals involved in the reaction, which slows down or stops. The effect on protein structure can be permanent, in which case the enzymes are denatured.

Figure 2 shows the effect of temperature upon enzymes in humans or other organisms living in temperate regions. The temperature of their surroundings does not normally go above 30°C and their body temperature does not exceed 37°C, so their enzyme systems are unaffected.



Computer-aided research is important in developing enzymes for industrial use. The goggles provide a three-dimensional image.

Some organisms, especially bacteria, live in extreme conditions, for example around volcanic steam vents. There are evidently some proteins that are much less affected by what we regard as 'extreme' temperatures.

BIOLOGICAL DETERGENTS

The widest general commercial use of enzymes is in detergents. Before the First World War Biotex — a prewash for soaking stained clothes — was developed by a Dutchman who had noticed that extracts from the pancreas could digest protein stains. By 1914 tablets of a product called Burnus containing the protease trypsin were being sold in Germany. It took many years to develop a way of including enzymes in washing powder granules (see Box 2). They needed to be encapsulated and stable in the presence of bleaching agents in the powder. Before automatic washing machines people washed clothes at high temperatures which would denature most common enzymes. Nowadays most domestic washing is done at 40°C, or at most 60°C.

The enzymes incorporated into modern detergents are shown in Table 1. These detergents are effective

One of the first enzymes to have its structure determined was lysozyme, which occurs in tears. When bacteria land on the eye it breaks open their walls and kills them.

- Find out how and why enzymes are 'immobilised'.
- Look at packaging on prepared foods to see if enzymes are mentioned. If they are, can you work out why they were used?

Cotton fibres develop from unicellular hairs that grow out from the seed coats inside the fruit of the cotton plant. They die, leaving nothing but the cellulose cell wall and these fibres are spun to

make thread.

Table 1 The label on detergent packets often says no more than 'contains enzymes'. Which enzymes are used?

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Enzymes	What they do	End product
Amylases (carbohydrases)	Hydrolyse starch	Soluble sugars
Lipases	Break down fats and oils	Soluble glycerol and fatty acids
Proteases	Break down proteins	Soluble peptides or amino acids
Cellulases (carbohydrases)	Work on loose cellulose fibres sticking out from the surface of cotton clothes. This helps maintain the original appearance of the cloth	Soluble sugars

Enzymes in pure form as powders could cause allergic responses if inhaled. Precautions are taken in detergent factories to prevent workers inhaling them.

Allergies have been reported by users of 'biological' detergents, but there is little hard evidence for these.

Right: Gene technology is used in the development of enzymes.

> Find out about the role of enzymes in the production of 'corn syrup'.

BOX 2 CHRONOLOGY OF ENZYME-CONTAINING DETERGENTS

- **1833** Payen and Persoz isolate the enzyme diastase from germinating barley.
- **1835** Bercelius demonstrates that starch can be broken down more efficiently with malt extract from germinating seeds than with sulphuric acid and coins the term **catalysis**.
- **1878** Kühne introduces the term **enzyme** for the substances in yeast responsible for fermentation.
- **1913** Otto Röhm patents the use of pancreatic enzymes in prewash soaking solutions.
- **1926** James Summers identifies urease as a protein after purification and crystallisation.
- 1920s K. Linderstrøm-Lang and M. Ottesen isolate subtilisin, an alkaline protease produced by bacteria. In the 1950s a similar enzyme product was the first important protease used in laundry detergents.
- 1930s The use of enzymes in detergents their largest industrial application begins slowly, based on Röhm's 1913 patent on the use of pancreatic enzymes in prewash solutions.
- **1950** Novo Nordisk launches the first enzyme produced by growing bacteria in a fermenter, a bacterial alpha-amylase.
- **1963** A protease with a low alkaline optimum pH (Alcalase®) is isolated the first breakthrough for detergent enzymes.
- **1980s** A lipase (Lipolase®) is developed for detergents, using a genetically-modified bacterium.





Before automatic washing machines people washed clothes at high temperatures.

at moderate temperature and pH values. The presence of enzymes gives:

- shorter washing times by quickly degrading dirt;
- reduced energy consumption because lower wash temperatures can be used;
- reduced water consumption;
- minimal impact on the environment because they are biodegradable and a renewable resource.

IMPROVING ENZYMES

The enzymes included in detergents might be improved in two ways. First, microorganisms are being screened all the time to see if they have enzymes that might be useful. Enzymes which are stable at higher temperatures might be found in bacteria which live in hot environments. The genes for the enzymes of interest might then be genetically engineered into other bacteria which are grown in bioreactors to produce the enzyme in quantity. Second, genetic engineering techniques might be used to produce modified enzyme structures with improved performance.

Examples of ways in which this could be of benefit include making enzymes which are adapted to work under more alkaline conditions (pH 8–12) in automatic dishwashers. Industrial laundries often operate at higher temperatures — for this purpose enzymes that are stable at 55°C are developed.

In some hot countries washing is traditionally done in cold water. Enzymes which offer efficient operation at temperatures of 20°C and below are needed.

Nigel Collins is an editor of CATALYST.