Bio Factsheet

Number 47

The Economic Importance of Enzymes

Enzymes are important in commercial processes because they accelerate specific chemical reactions to produce a useful product or effect. Enzymes are sometimes used when still retained within cells; other processes require the enzyme to be extracted from the parent cells and purified. In both cases the efficiency of the process may be increased by trapping the cells or purified enzyme within an insoluble agent. This is called enzyme immobilisation. This Factsheet outlines the processes of enzyme extraction, purification and immobilisation, and summarises the functions of commercial enzymes.

Naming of enzymes

It is important to understand how enzymes are named if their commercial use is to be understood. The names of many enzymes end in the suffix –ase. The first part of the word often indicates the type of molecule on which the enzyme acts (the substrate – see Table 1).

Table 1.

Enzyme	Substrate
proteases	proteins
amylases	carbohydrates
lipases	lipids
cellulases	cellulose
lactases	lactose
pectinases	pectin

Exam Hint: *Make sure you know what categories of enzymes are in your syllabus.*

Enzyme technology

The process of incorporating the enzyme into the commercial process is enzyme technology. The function of this technology is to make the manufacturing process more productive by, for example, reducing waste, removing contaminants and maximising the quantity of product produced in terms of the cost of raw materials.

Enzyme production

Sources of commercial enzymes

A wide variety of microorganisms – bacteria, fungi and yeast – are used as source material. The bacteria Bacillus and fungi Aspergillus are particularly significant. Microorganisms can be genetically engineered to produce higher yields or different types of enzymes.

Purification

The parent cells are first disrupted by physical or chemical means, e.g. grinding or by the addition of alkalis. The cell debris is then removed by filtration or centrifugation. Different enzymes are then precipitated from solution using ammonium sulphate, which is inexpensive and of low toxicity. Further purification can involve a variety of separation techniques such as chromatography, filtration and electrophoresis.

Chromatography and electrophoresis

Chromatography separates the different components of a mixture using a solvent moving through a stationary medium such as chromatography paper. The various components move along the medium at different rates and can then be individually isolated. Electrophoresis achieves a similar effect when an electric current is passed through an appropriate medium and produces positive and negative ends. The different components move through the medium at different rates depending on their size and charge.

Immobilisation

In immobilisation the enzyme is held in a column of insoluble agent through which the substrate flows. Whole cells or extracted enzymes can be immobilised. The binding agent can be organic (e.g. agar gel, cellulose) or inorganic (e.g. porous alumina, porous glass). The mechanism of binding can be physical, as in entrapment within a lattice, or chemical, in which the enzyme forms chemical bonds with the immobilising agent.

An alternative to immobilisation is to operate a so-called batch system in which enzyme and substrate react together in a closed vessel from which the product is periodically extracted.

Examples of commercial enzyme functions

Detection and measurement of glucose

Enzymes can be used to detect molecular markers of clinical importance. One widely quoted example is the detection and measurement of glucose. The amount of glucose in blood or urine is a crucial indicator in the diagnosis and treatment of diabetes mellitus.

Diabetes mellitus

This is caused by a deficiency of the hormone insulin, which is secreted by the pancreas. Well-known symptoms are a high concentration of glucose in the blood and the presence of glucose in the urine.

Glucose can be detected using the enzyme glucose oxidase in a biosensor. A biosensor is an instrument used, as the name indicates, to detect – hence sensor – molecules outside the instrument. The instrument uses some kind of biological system (bio-), for example, an enzyme, to detect this molecule. The reaction produced in the biological system is then converted into electrical activity by a transducer.

Transducer

This word is now frequently used in biology textbooks. The standard dictionary definition can be quite wide, but in biology it is often used to indicate the conversion of some kind of stimulus energy into electrical energy. A rough analogy of biosensors in the human body might be the sensory receptors in the nose, which generate electrical signals in nerve cells as a result of the detection of air-borne chemicals.

A glucose biosensor uses glucose oxidase as its biological system. This enzyme catalyses the reaction between glucose and oxygen to form gluconic acid and hydrogen peroxide. The glucose can then be detected and measured by the quantity of oxygen consumed or gluconic acid produced. These cause changes in the electrical signal generated by the transducer.

An alternative use of glucose oxidase is to use it on a cellulose fibre pad, e.g. 'Clinistix'. The hydrogen peroxide generated as a result of the activity of the glucose oxidase reacts with a colourless compound to form a coloured compound, which indicates the presence of glucose. A second enzyme, peroxidase, also impregnated on the pad, is necessary for the colour reaction.

Genetic Engineering
Genetic engineering depends on three major groups of enzymes which help to copy, cut and join DNA molecules. The enzymes are called reverse transcriptase, restriction endonucleases and DNA ligases respectively.

DNA molecules
DNA means deoxyribonucleic acid. Each chromatid of a chromosome is a single DNA molecule.

Reverse transcriptase is an enzyme which helps construct complementary sections of DNA from messenger RNA. This is an invaluable method of building that section of the DNA which contains the gene required for cloning.

Restriction endonucleases cut DNA molecules at specific base sequences leaving 'sticky ends' - the base sequence which is exposed is then joined to the corresponding base sequence on the other piece of DNA.

DNA ligases glue the DNA fragments together at the sticky ends to make **recombinent DNA** sections into plasmids, which are circular loops of DNA extracted from bacterial cells. The modified plasmids are then inserted into other microorganisms which are then cultured to produce multiple copies of the plasmid.

Penicillin production
Overcoming antibiotic resistance is vital in healthcare. One approach is to develop chemically modified variants of the natural antibiotic. The enzyme penicillin acylase is used to convert natural penicillin G into an intermediate compound, 6-amino penicillanic acid (6-APA), which can be used to produce a range of so-called semisynthetic penicillins.

Lactose-free milk
The presence of lactose in milk produces serious effects in people who are lactose-intolerant. The enzyme lactase is used to remove lactose from milk by converting it to galactose and glucose.

Washing powders
Egg, blood and grease are all targets for enzymes in washing powders. Such powder contains proteases, amylase, lipase and cellulase. The first three help to remove protein, carbohydrate and lipid-based stains; cellulase acts as a conditioner and cleanser for cotton fabrics, removing loose microfibrils (very small fibres) and dirt.

Biological washing powders now occupy a substantial part of the market in Western Europe, having both environmental and energy benefits. They reduce the need for powders with a strong solvent and high phosphate content; their disposal creates fewer environmental problems. Biological washing powders can also be used at relatively low temperatures, resulting in energy saving.

Exam Hint: *Produce a balanced and relevant response to a topic. Develop the proper range of information; do not make an essay on commercial enzymes read like a response to a question on genetic engineering, just because you know a lot about restriction enzymes and DNA.*

High fructose syrups
High fructose syrups are used to replace sucrose as a sweetener in soft drinks and confectionery. The commercial advantage is that fructose is much sweeter than sucrose. Three enzymes are involved – α amylase, amyloglucosidase and glucose isomerase. The raw material is corn starch. Alpha-amylase helps to break the large starch molecules to smaller units called maltodextrins. These are then converted to glucose by amyloglucosidase, which removes glucose units from the ends of the dextrin chains. Glucose isomerase converts glucose to fructose.

Starch $\xrightarrow[\alpha \text{ amylase}]{}$ Maltodextrins $\xrightarrow[\text{amyloglucosidase}]{}$ Glucose

Apple Juice Production
Cold-stored apples develop relatively high levels of soluble pectin because of enzyme changes within the apples during storage. The pectin has a high water-binding capacity and reduces the yield of the pressed juice. The pectin is reduced if the enzyme pectinase is added to the fruit during the crushing stage.

Practice Questions

1. State three advantages of using enzymes commercially compared with other types of catalyst. (3 marks)

2. Describe the commercial use of restriction endonucleases (4 marks)

3. Describe the commercial uses of:
   (a) lipases (3 marks)
   (b) lactases (4 marks)
   (c) pectinases (3 marks)

Answers
(Semicolon indicates marking points)

1. specificity;
   energy saving;
   less pollution;

2. genetic engineering of insulin;
   somatotropin/interferon;
   improving flavour of tomato puree;
   inserting pesticide resistance into plants;
   (credit any valid example)

3. (a) washing powder;
   breaks down lipids;
   to fatty acids and glycerol;

   (b) milk treatment;
   breaks down lactose;
   to galactose and glucose;
   lactose-intolerance;

   (c) apple juice treatment;
   reduces pectin content;
   reduces water-binding capacity of pectin;
   clarifies the juice;

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