Bio Factsheet

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Industrial Uses of Enzymes

This Factsheet will outline the major advantages and uses of enzymes in commercial and industrial processes. More than 300 enzymes are now used commercially. Most of these come from bacteria, fungi and yeast.

Advantages of using enzymes
- they are highly specific –the enzyme's active site has a strong affinity for a particular substrate.
- Compared with inorganic catalysts, they are highly efficient – very small quantity of enzyme catalyses the production of a huge quantity of products.
- they work at normal temperatures and pressures, so need much less energy than most chemical catalysts.

Sources of industrial enzymes
Most enzymes are obtained from microorganisms. The advantages of microoganisms are:-
- they have high growth rates and contain a larger proportion of enzymes in relation to their body mass than plants or animals.
- they can be grown in huge quantities in bulk fermenters.
- their growth is not limited by season or climate.
- they occur naturally in a wide range of habitats, and so some contain enzymes that will function under extremes of temperature or pH.
- they can be genetically manipulated, for example a human gene for insulin production has been introduced into the bacterium E. coli.

In many processes the enzymes are used in an **immobilised form**.

Immobilised enzymes
One disadvantage of simply adding an enzyme to the substrate in solution is that it might then be difficult to separate the enzyme from the products. This might mean we could not re-use the enzyme, which would be expensive.

An alternative is to use immobilised enzymes. Immobilised enzymes are bound to a surface so that they are not allowed to dissolve and usually they cannot move. There are several ways of holding and immobilising the enzymes (Fig1).

The advantages of immobilising the enzymes are:
- the enzyme can be recovered and re-used, over and over again.
- since it is protected by the inert matrix, the enzyme may be more stable at extreme pH or temperatures and it may, therefore, last longer.
- the product is not mixed in with and doesn't need separating from the enzyme. This reduces costs
- it allows continuous processing rather than a batch system. This allows for a much smaller set-up; the plant may be prefabricated cheaply off-site so capital costs are a lot less.
- the productivity of an enzyme, when immobilised, is greatly increased as it may be more fully used at higher substrate concentrations for longer periods than the free enzyme

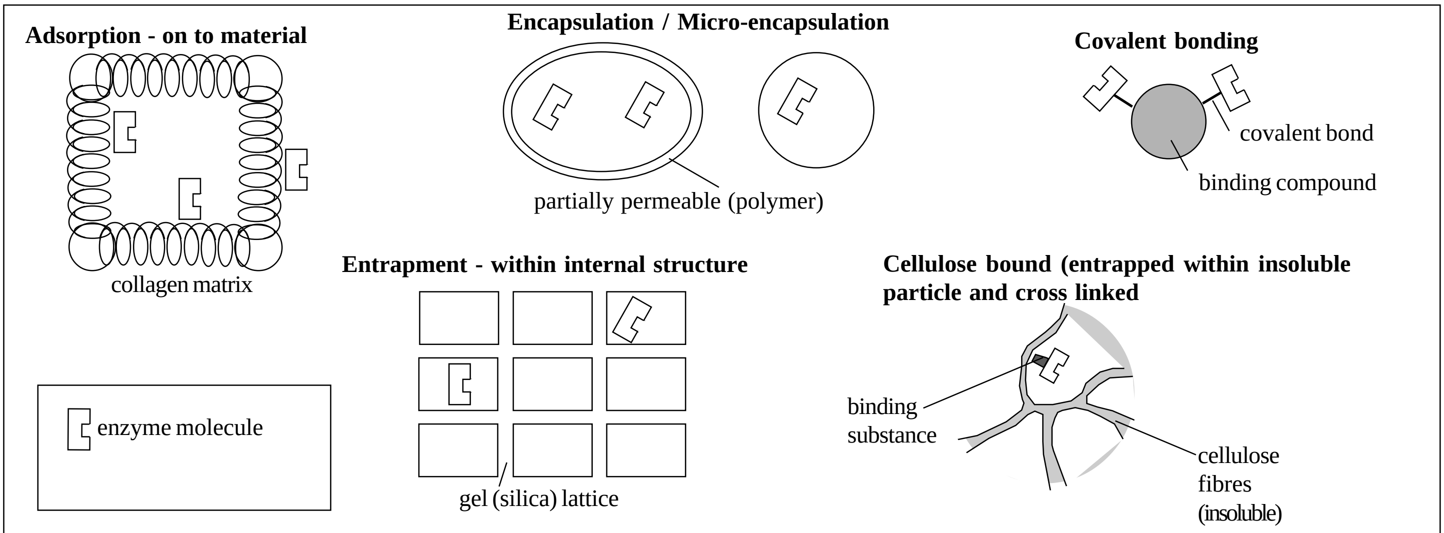
Table 1 summarises some of the more important industrial uses of immobilised enzymes

Table 1. Important industrial uses

Enzyme	Product
Glucose isomerase	High -fructose corn syrup
Glucoamylase	Glucose
Invertase	Invert sugar
Aminoacylase	Amino acids
Penicillin amidases	Penicillins
Aspartate ammonia-lyase	Aspartic acid
Cyanidase	Formic acid (from waste cyanide)
Lactase	Lactose-free milk and whey
Lipase	Cocoa butter substitutes
Nitrile hydratase	Acrylamide
Raffinase	Raffinose-free solutions
Thermolysin	Aspartame

Typical Exam Questions - Everything on this page!

Fig 1. Immobilised enzymes



Proteases

Proteases are used to recover protein from parts of animals (and fish) would otherwise go to waste after butchering. About 5% of the meat can be removed mechanically from bone. To recover this, bones are mashed, incubated at 60°C with neutral or alkaline proteases for up to 4 h. The meat slurry produced is used in canned meats and soups.

Biological detergents

Enzymes for "biological" washing powders originally came from bacteria adapted to live in hot springs (45°C+). Most are now produced through genetic engineering so that they are not denatured by high temperatures, the alkaline pH (many detergents contain phosphate ions) or by the strong oxidising agents contained in most detergents.

The main enzymes are proteinases, lipases, amylases and cellulases, the latter brightening and softening cotton fabrics in particular.

Use in Medicine

Table 2 summarises some important medical uses of enzymes.

Table 2. Therapeutic enzymes

Enzyme	Reaction	Use
Urokinase	Plasminogen → plasmin	Blood clot
Collagenase	Collagen hydrolysis	Skin ulcers
b-Lactamase	Penicillin → penicilloate	Penicillin allergy
Glutaminase	Penicillin…. semi-synthetic penicillin	Increased production of penicillins
Lysozyme	Bacterial cell wall hydrolysis	Antibiotic
Rhodanase	$S_2O_3^{2-} + CN^- \rightarrow SO_3^{2-} + SCN^-$	Cyanide poisoning
Ribonuclease	RNA hydrolysis	Antiviral
Streptokinase	Plasminogen → plasmin	Blood clots
Trypsin	Protein hydrolysis	Inflammation

Unfortunately, enzymes are difficult to use therapeutically because:
- as foreign proteins they may trigger an **immune response**, with life-threatening consequences;
- they are too large to be distributed simply within the body's cells.
- they are destroyed within the body very quickly
- they are required to be extremely pure and can therefore prove expensive

Lactose-free milk

People who are unable to produce lactase in their pancreatic juice cannot digest milk sugar (lactose) in their small intestine. As a result, bacteria in the large intestine feed on the lactose there, producing fatty acids and methane; nausea, diarrhoea and dehydration may result. Thus, ordinary milk has to be avoided in favour of lactose-free milk which is produced by passing milk through a column containing immobilised lactase usually obtained from *Aspergillus spp*. or the yeast Kluyveromyces. The lactase hydrolyses the lactose to glucose and galactose.

Other uses of lactases include the production of ice cream and sweetened flavoured and condensed milks. Hydrolysing some of the lactose gives a sweeter, more viscous product which does not crystallise if condensed or frozen. It also reduces the amount of whey which would otherwise have to be disposed of – tricky because of its high BOD.

Production of syrups containing maltose

Maltose syrups can be produced by treating barley starch with amylase. Maltose syrup can be used in frozen desserts –it is very sweet and doesn't crystallise above 4°C

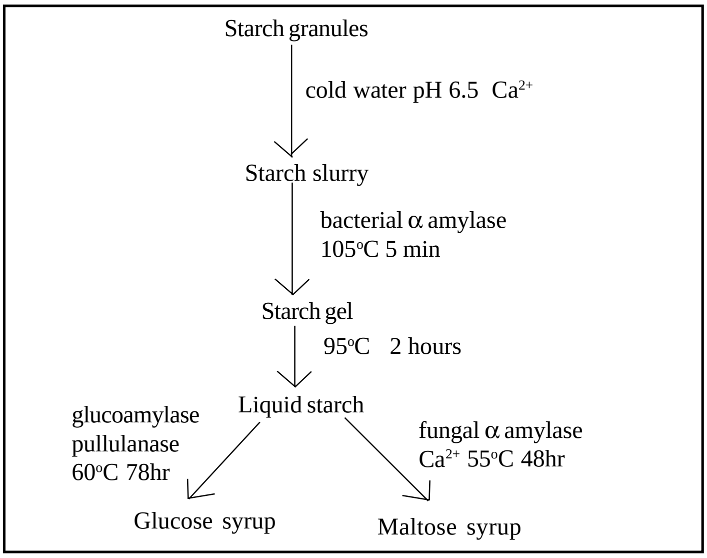
Table 3 The relative sweetness of food ingredients

Food Ingredient	Relative sweetness (by weight, solids)
Aspartame	180
Fructose	1.3
Hydrolysed sucrose	1.1
HFCS (55% fructose)	1.1
Sucrose	1.0
Glucose	0.7
Galactose	0.7
Hydrolysed lactose	0.7
Glucose syrup 97 DE	0.7
High-conversion syrup 65 DE	0.5
Maltose	0.3
Maltose syrup 44 DE	0.3
Lactose	0.2
Raffinose	0.2

Starch hydrolysis

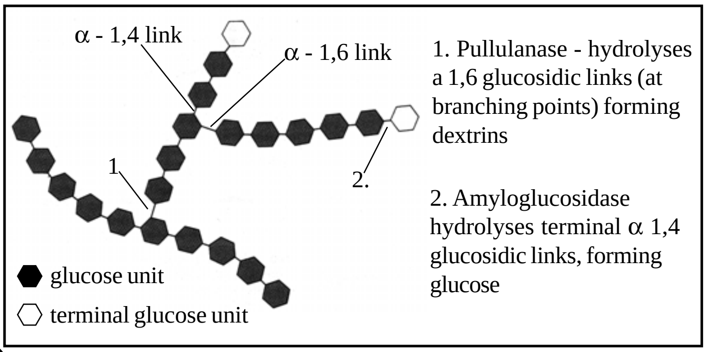
Microbial enzymes are used to produce glucose and maltose syrup from starch. (Fig 2)

Fig 2 Glucose and maltose syrup production



The amylase amyloglucosidase breaks alpha-1,6 links within the polysaccharide chains whilst pullulanase hydrolyses the alpha-1,6 links at branching points in the polysaccharide (Fig 3)

Fig 3. Site of enzyme action



High fructose syrup
A sweetener that is widely used in food and drinks is high-fructose corn syrup (HFCS). It is manufactured from starch in corn fruits (maize). The grains are milled to a starch slurry and the enzyme amylase is added. This produces a glucose syrup, which is then decolourised and concentrated. Glucose syrup can be converted to fructose syrup by passing it down a column of immobilised glucose isomerase enzyme. Fructose has the same energy content as glucose but is much sweeter – so much less is needed.

Fruit juice production
In order to extract fruit juice, plant cell walls have to be broken open. This could be done using high temperatures but this would affect the colour and flavour of the juice.

Instead, the fruit is crushed, and hydrolytic enzymes including cellulases, hemicellulases, xylanase and pectinases are added to break open the cell walls. Addition of pectinase, xylanase and cellulase releases more juice from the pulp. Pectinases and amylases are also used to clarify the juice.

Textiles
Starch has for a long time been used as a protective glue of fibres in weaving of fabrics. This is called sizing. Enzymes are used to remove the starch in a process called desizing. Amylases are used in this process since they do not harm the textile fibres.

The manufacture of denim involves amylases –to digest the starch which is used as a glue during weaving – and cellulases which partially digest the indigo dye to produce a bleached or faded effect.

Laccase, a polyphenol oxidase from fungi which degrades lignin, is also used as a textile bleaching agent.

Animal feed
Beta-glucanase added to chicken feed digests beta – glucan which otherwise causes viscosity in the chicken's gut. The effect of the enzyme is to increase the conversion efficiency so that the chicken gains more weight per unit of feed. A similar improvement in conversion efficiency can be achieved by adding xylanases to wheat-based feeds. The enzymes release nutrients from the wheat fibres and accelerate their uptake by the chickens.

Phytase - a phosphoesterase – releases phosphate from the phytic acid which is a common compound in plant based animal feeds. The net effect is to reduce the amount of phosphorus which has to be added to the feed and reduces the amount in the faeces – reducing eutrophication.

Foodstuffs
During breadmaking, xylanases decrease water absorption by the dough – and lipases and proteinases improve dough-handling properties. Glucose oxidase is added to replace chemical oxidants.

The glucose and oxygen content of foodstuffs can be reduced by the use of glucose oxidase enzyme as follows:

$$\text{glucose} + O_2 + H_2O \xrightarrow[\text{Aspergillus niger or Penicillium}]{\text{glucose oxidase from}} \text{gluconic acid} + H_2O_2$$

This improves the storage time of the foodstuffs. If the aim is to avoid production of any hydrogen peroxide the glucose oxidase is added with catalase (also obtained from A. niger) and this enzyme immediately breaks down any hydrogen peroxide into water and oxygen.

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

Several commercial instruments are available which apply this principle for measurement of molecules like glucose, lactate, lactose, sucrose, ethanol, methanol, cholesterol and some amino acids.

These two enzymes are also used to remove glucose from egg-white before drying for use in the baking industry, to remove oxygen from the head-space above bottled and canned drinks and to reduce browning in wines and mayonnaises.

Pulp and Paper
Xylanases hydrolyse xylan and this effectively produces lignin fragments in the pulp which reduces the need for chlorine- based bleaches. Starch is used to alter important properties of the paper such as strength, stiffness and erasability. In order to get just the right combination of these properties, amylases are used. Lipases help to digest pitch – a sticky substance found in some conifers – cellulase enzymes are used to help in the removal of ink from recycled paper

Leather
Alkaline proteases are used to remove hair from hides and are also added in the soaking phase of leather manufacture. The proteases improve water uptake by the dry skins and digests proteins, softening the leather.

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Summary

Enzyme	Example of source	Industrial application
Cyanidase	*Bacillus pumilis*	Producing formic acid from waste cyanide
Glucoamylase	*A. niger*	Producing glucose
Protease	*Bacillus subtilis*	Removing hair from hides. Softening leather.Tenderising meat
Glucose isomerase	Many fungi	High-fructose corn syrup
Glucose oxidase	*A. niger*	Replaces chemical oxidants in baking
Lactase	*Kluyveromyces fragilis*	Lactose-free milk and whey. Longer-lasting, sweeter yoghurt.Improved scoopability and sweetness of ice creams
Penicillin amidases	*E. coli*	Producing penicillins
β-glucanase	*Trichoderma* fungus	Breaks down glucan in chicken feed, increasing its digestibility
Thermolysin	*Bacillus spp*	Producing aspartame
α-amylase	*Bacillus licheniformis*	Reduces starch in cane sugar. Liquefaction of mashes in brewing. Digestion of starch glue in weaving
α-amylase glucomylase	*Aspergillus niger*	Production of glucose syrup. Production of low-carbohydrate beer
Cellulase	*Trichoderma reesei*	Juice clarification. Breakdown of cellulose microfibrils to reduce viscosity of wort in brewing. Bleaching of denim. Removal of inks in paper recycling
Pectinases	*Aspergillus niger*	Increase fruit juice production and clarification of juice. Setting jam
Xylanases	*Trichoderma* fungus	Reduces lignin in paper pulp, reducing need for bleaching
Lipase	*Aspergillus spp*	Used in detergents to break down fats