Enzyme Technology

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Immobilised Enzymes

The costs associated with the use of enzymes for industrial purposes can also be reduced by immobilising the enzymes.

Enzymes for industrial processes are more valuable when they are able to act in an insolubilised state rather than in solution.

Enzymes are immobilised by binding them to, or trapping them in a solid support.

Various methods for immobilising enzymes are available.

Enzymes that are immobilised have a greater value in industry than those in solution.
Methods of enzyme immobilization

Enzymes are held on to a solid support (matrix) by weak forces such as hydrogen bonding.

Enzymes are trapped within the structure of a solid polymer (usually in the form of beads) – the enzyme is trapped rather than bound.
Enzymes are covalently bonded to a matrix such as cellulose or collagen.

Another more expensive method involves enzymes which are both covalently bonded to, and cross-linked within, a matrix.

Cross-linking and covalent bonding may cause some enzymes to lose their catalytic activity especially if the active site is involved in forming the linkages.
Advantages of Immobilising Enzymes

Compared with free enzymes in solution, immobilised enzymes have a number of advantages for use in industrial processes.

The stability of many enzymes is increased when they are in an immobilised state; they are less susceptible to changes in environmental conditions such as temperature and pH fluctuations.

Immobilised enzymes can be recovered and re-used, reducing overall costs.

The products of the reaction are not contaminated with enzyme eliminating the need to undertake costly separation of the enzyme from the product.

Immobilising enzymes allows for continuous production of a substance with greater automation.
Enzyme Immobilisation and Thermostable Enzymes in The Production of High Fructose Syrup

This industrial process involves the conversion of cheap corn starch into a high fructose syrup for use as a sweetener in confectionary and drinks.

Starch paste is incubated with the thermostable enzyme alpha amylase at 90°C for a couple of hours.

Alpha amylase catalyses the hydrolysis of the starch into short glucose chains called dextrins.

The temperature is raised to 140°C to denature the amylase and then lowered to around 55°C before adding the fungal enzyme amyloglucosidase.

Amyloglucosidase catalyses the hydrolysis of dextrins into glucose molecules.
The final stage involves the conversion of glucose syrup into the much sweeter fructose syrup using the enzyme glucose isomerase.

Glucose isomerase is immobilised in rigid granules and packed into a column.

Glucose syrup is poured into the top of the column and is hydrolysed as it contacts the immobilised enzyme.

Fructose syrup emerges from the end of the column free from contamination with enzyme.
Enzymes as Analytical Agents

The sensitivity and specificity of enzymes makes them useful tools in medicine for the detection and measurement of chemicals in fluids such as blood and urine.

Because of their specificity, enzymes will bind to only one substrate – they can therefore be used for the identification of a specific substance in a biological sample.

Because of their sensitivity, enzymes are able to detect the presence of specific molecules even when they are present at very low concentrations.

The enzyme glucose oxidase is used in an immobilised form for the detection of glucose in biological fluids.
Glucose Measurement using 'Clinistix'

This method relies upon the specificity of the enzyme glucose oxidase, allowing glucose to be detected in the presence of other sugars. N.B. Benedict's test is not specific for glucose as it gives a positive reaction with all reducing sugars.

This test uses a plastic strip (clinistix) for the detection of glucose in the urine of diabetics.

When the clinistix is dipped into a urine sample (containing glucose), the glucose oxidase catalyses the conversion of glucose to hydrogen peroxide:

\[
\text{Glucose} + \text{O}_2 \rightarrow \text{gluconic acid} + \text{hydrogen peroxide (H}_2\text{O}_2)\
\]

In the presence of the enzyme peroxidase, the chromagen dye is oxidised by the hydrogen peroxide to produce a colour change on the fibre pad:

\[
\text{DH}_2 \text{ (chromagen dye)} + \text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{D}\
\]

The amount of coloured compound (D) produced is a direct measure of the amount of glucose in the sample.

At the tip of the clinistix is a cellulose fibre pad on to which glucose oxidase, peroxidase and a chromagen dye are immobilised.
Glucose Measurement using 'Clinistix'

The colour of the pad on the clinistix is compared with a colour chart to determine the amount of glucose present in the sample.

- No glucose
- Increasing amounts of glucose
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Biosensors are electronic monitoring devices that make use of an enzyme’s specificity and the technique of enzyme immobilisation.

Immobilised enzymes bind with specific molecules even when they are present in very low concentrations.

The enzyme reaction brings about a change that is converted into an electrical signal by a transducer.

The electrical signal is amplified and gives a read-out on a small display screen.
A biosensor has been developed for detecting glucose in the blood of diabetics.

Glucose oxidase oxidises any glucose present in the blood to release electrons – these are detected by the transducer and converted into an electrical current.

The current generated is proportional to the amount of glucose present in the sample and this is displayed as a digital read-out.