

6.4

Industrial enzymes

Immobilisation of enzymes and the use thereof in large-scale biotechnological production

Enzyme action

You will recall from earlier units that enzymes are biological catalysts for metabolic reactions, either anabolic or catabolic. They are useful for industrial processes because of their specificity – they can catalyse reactions between particular chemicals even when in a large mixture of substances, and also because of their performances – most enzymes can function relatively well at quite low temperatures (the typical enzyme optimum temperature is around 40°C).

It is often more efficient to use **isolated enzymes** to carry out the reactions involved in industrial biotechnological processes, rather than growing the whole organism or using an inorganic catalyst. Isolated enzymes can be produced in large quantities in commercial biotechnological processes, where the extraction of a particular enzyme from the fermentation mixture is known as **downstream processing**.

Immobilising enzymes

In order for the product of an enzyme-controlled reaction to be generated, enzyme and substrate must be able to collide and form the **enzyme-substrate complex**. This is most easily achieved by mixing quantities of substrate and isolated enzyme together under suitable conditions for the enzyme to function. The product generated then needs to be extracted from the mixture. This is all a lengthy and costly process.

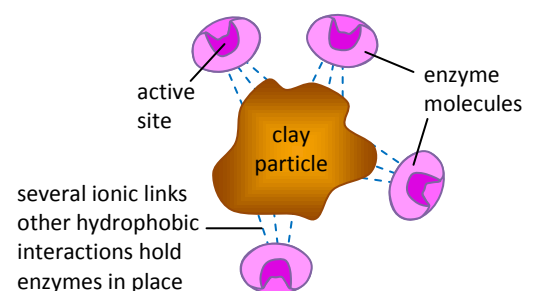
It is possible to **immobilise** enzymes so that they can continue to catalyse the enzyme-controlled reaction but do not mix freely with the substrate as they normally would in a cell. The table below demonstrates the benefits and drawbacks:

Advantages of immobilising enzymes	Disadvantages of immobilising enzymes
Enzymes are not present with products so purification and downstream processing costs are kept to a minimum	Immobilisation requires additional time, equipment and materials and so is more expensive to set up
Enzymes are immediately available for reuse, particularly useful for continuous cultures	Immobilised enzymes can be less active because they do not mix freely with the substrates
Immobilised enzymes are more stable because the immobilising matrix protects the enzyme molecules	Any contamination is costly to deal with because the whole system would need to be stopped

There are four methods of immobilisation of enzymes available to use.

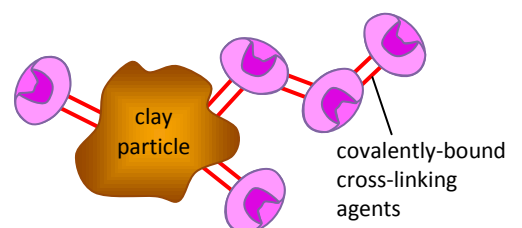
Adsorption

The target enzymes mix with the immobilising support material and bind to it due to a combination of **hydrophobic** interactions and **ionic** links, in a process called **adsorption**. Adsorbing agents used include clay, resin and glass beads. Because the bonding forces are not particularly strong, enzymes can become detached (known as **leakage**). However, provided the enzyme molecules are held so that their active site is not changed and is displayed, adsorption still gives very high reaction rates.



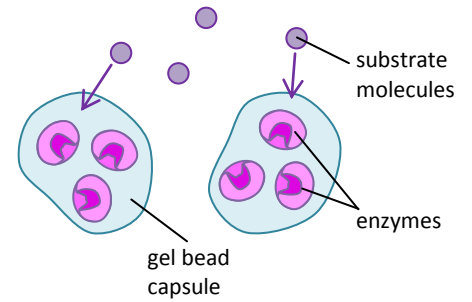
Covalent bonding

Enzyme molecules can also bind to the immobilising support particle by **covalent bonding**. Often, enzymes can form covalent bonds with the support particle as well as each other using a **cross-linking agent**. This method does not immobilise a huge amount of enzymes, but binding is very strong, as covalent bonds are very hard to break, and so the amount of leakage of the enzymes is kept to a minimum.



Entrapment

Enzymes may be trapped, for example, in a gel bead or a network of cellulose fibres. The enzymes are trapped in their natural state (i.e. are not physically bound to another molecule and so their active sites remain intact) however, reaction rates can be reduced because whatever the trapping barrier is must be penetrated by the substrate. This means that the active site of the enzymes is less easily available than it is with adsorbed or covalently-bonded enzymes.



Membrane separation

Finally, enzymes may be physically separated from the substrate mixture by a **partially-permeable membrane**. Most simply, the enzyme solution is held at one side of a membrane, whilst the substrate solution is passed along the other side. Substrate molecules are small enough to pass through the membrane and the products formed can also pass back across the membrane, but the enzymes are too big to fit through the membrane, and so remain enclosed.