Enzymes

Enzymes are biological catalysts made of protein. They alter the rate of chemical reactions without themselves being chemically changed at the end of the reactions.

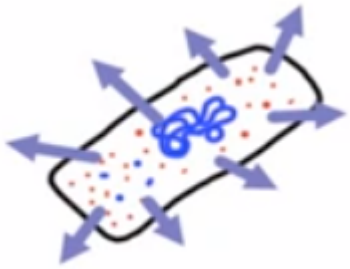
- Enzymes are present in all living things.
- They are nature's hardest workers.
- Enzymes are known as **biocatalysts.**
- Enzymes are catalytic molecules and are not directly used up by the process in which they are used.

Advantages	Disadvantages
They are **specific in their action** and are therefore **less likely to produce unwanted by-products**	They are **highly sensitive to changes in physical and chemical conditions** surrounding them.
They are **biodegradable** and therefore cause less environmental pollution	They are **easily denatured** by even a small increase in temperature and are **highly susceptible to poisons and changes in pH**. Therefore the **conditions in which they work must be tightly controlled**.
They work in **mild conditions**, i.e. low temperatures, neutral pH and normal atmospheric pressure, and therefore are **energy saving**	The enzyme substrate mixture **must be uncontaminated with other substances** that might affect the reaction.

Microbes are the most common source of industrial enzymes.

- Microorganisms produce enzymes inside their cells (**intracellular** enzymes)
- Microorganisms also secrete enzymes for action outside the cell (**extracellular** enzymes)

- The microorganisms selected are usually cultured in large fermentation chambers (**fermenters)** under controlled conditions to maximize enzyme production.
- The microorganisms may have specific genes introduced into their DNA through ***genetic engineering***, so that they produce enzymes naturally made by other organisms if necessary.

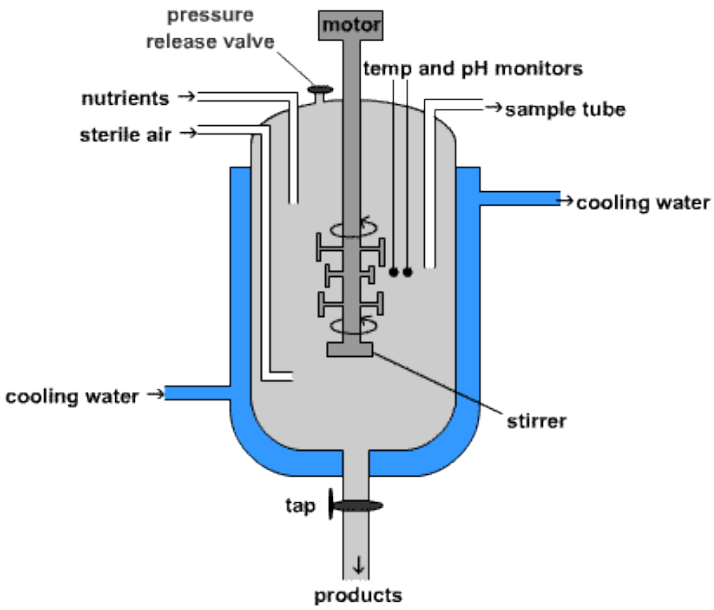


Producing and Extracting Enzymes

Producing substances such as penicillin from microbes on an industrial scale requires massive number of organisms to be grown for commercial use. They are grown in large vessels called **fermenters**.

The large stainless steel cavity is filled with a sterile nutrient solution, which is then **inoculated** with a pure culture of the carefully selected microbes.

Paddles rotate the mixture so that the suspension is mixed well. As the nutrients are used up, more can be added.



Advantages of a fermenter

- Allows production of enzyme-producing microbes on a large scale
- Helps maintain pH, oxygen concentration and temperature for optimum growing of microbes and creation of enzymes
- Helps remove air, carbon dioxide and other gases produced by cell metabolism leave the fermenter by an exhaust pipe.

Requirements for the production of microbes in fermenters:

- **Oxygen** is needed for aerobic respiration of (some) micro-organisms – others are strict anaerobes and oxygen must be excluded
- A source of c**arbohydrate** is needed as an energy source for respiration to release energy needed for growth.

- A source of **nitrogen** is needed need nitrogen for **protein synthesis** – Ammonia and urea are both widely used as sources of useable nitrogen

Extracting extracellular enzymes

Extracellular enzymes are present in the culture outside the microbial cells, since they have been secreted.

- They are often soluble in water, so they can readily be extracted from the culture medium and purified.
- Extracellular enzymes are cheaper to produce as they are easy to extract.

Comparing intra- and extra- cellular enzymes

Intracellular enzymes	Extracellular enzymes
More difficult to isolate	Easier to isolate
Cells have to be broken apart to release them	No need to break cells – secreted in large amounts into medium surrounding cells
Have to be separated out from cell debris and a mixture of many enzymes and other chemicals	Often secreted on their own or with a few other enzymes
Often stable only in environment inside intact cell	More stable
Purification/down streaming processing is difficult/expensive	Purification/down streaming processing is easier/cheaper

Reason for necessity of cell-free enzymes

- When whole cells are used, some of the substrate is used in the cell metabolism
- The optimum condition fo*r individual enzymes is often different to that of a whole cell.
- No wasteful side reactions.

Uses of Enzymes in the Industry

- **Detergents**
    - Largest application of industrial enzymes for the last forty years.
    - Uses **protease, amylase and lipase**
    - Removes stains like blood (proteins) starch (carbohydrates) and grease (fats)
    - By lower the optimal water temperature for getting clothes clean by up to 30 degrees, enzymes have helped to reduce the amount of energy it takes to heat the water for the laundry.
    - Helps reduce CO_2 emissions.
    - Also reduces length of time taken to clean clothes.
    - Now using **thermostable** enzyme from *Staphylothermus marinus* (thermophilic bacteria)

- **Paper bleaching and pulping**
    - Biotechnology has modified microbes to produces an enzyme that can replace one of the chemical mixtures formerly used in the paper pulping processes.
    - Uses **ligninases and amylase**
    - Removal of lignin from pulverized wood, prior to use of wood cellulose in manufacturing processes
    - Partial breakdown of starch to produce smooth 'quality' paper
    - Enzymes are more environmentally friendly than chlorine-bleaching methods.
- **Manufacture of vitamins and pharmaceuticals**
    - Enzyme-based processes require less energy than traditional methods, therefore cheaper

    - Previously, the only way to produce the important B2 vitamin was a lengthy chemical-intensive process.
    - By utilizing biotechnology, researchers have engineered microbes that produce Vitamin B2 as a natural by-product of a vegetable oil fermentation process.

- **Medical**
    - Removal of blood clots and in wound cleaning, treatment of *thrombosis* (**trypsin**)
    - Used in biosensors
    - Used to test for glucose in urine (**glucose oxidase and peroxidase**)

- **Textile industry**
    - Remove starch from fibres (which help to protect from mechanical damage during weaving)
    - Uses **amylase**

- **Food Industry**
    - Produce fructose syrup from glucose (**glucose isomerase**)
    - Fruit juice production – increase volume of extracted juice and to remove cloudiness due to presence of pectins (**cellulase, hemicellulase, pectinase**)
    - Pre-digestion of some baby foods (**trypsin**)
    - Meat tenderization – **proteases** added to meat to break down tough and inelastic connective tissue and collagen (**papain, ficin**)
- **Lactose-free milk**
    - helps to avoid lactose-intolerance in people
    - their body does not produce enough of the lactase enzyme needed to break down lactose, resulting in gastrointestinal difficulties.
    - lactose is broken down into glucose and galactose using **lactase**

    - resultant milk is sweeter as the 2 separate sugars are sweeter together than lactose
    - Same method also used for other food products such as cottage cheese and ice cream.

- **Fermentation**
    - Conversion of carbohydrates in organic matter by microorganisms or enzymes into acids or alcohols. (**rennin**)
    - E.g. Milk spoils easily without refrigeration. When it is fermented into cheese, it can last for months. This is an efficient form of food storage for places which may not have efficient refrigeration systems.
    - Fermented food makes up one-third of humans' diet.
    - Enzymes can help make fermentation more efficient, to increase the quality, safety and product consistency of preserved foods.

- **Biodegradable plastics**
- **Bioethanol**

Immobilization of Enzymes

Enzymes are catalytic molecules and are not directly used up by the process in which they are used.

However due to denaturation, they do loose activity with time. Therefore they should be stabilized against denaturation.

The enzyme can be imprisoned allowing it to be reused but also preventing contamination of the product – this is known as immobilisation.

Advantages of immobilisation	Disadvantages of immobilisation
Easier to separate enzyme and products	Immobilisation may alter shape of enzyme
Allows catalysis in unfavourable media	May alter catalytic ability
Increases stability and can be manipulated easily	Enzyme may become detached
Allows continuous production/enzyme used for longer	Expensive
Enzyme can be recovered and reused	
Enzyme does not contaminate product/no purification required	
Extends the life of proteolytic enzymes by preventing them digesting each other.	

There are four main methods available for immobilising enzymes:

- Adsorption in glass or alginate beads – enzyme is attached to the outside of an inert material
- Cross-linkage to another chemical e.g. cellulose or glyceraldehydes.
- Entrapment in a silica gel – enzyme is held in a mesh or capsule of an inert material.
- Membrane confinement