Industrial Microbiology

4th year Biology

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Industrial Enzymes Production:

***Historical aspects*:**

As early as the late 17th and early 18th centuries, the digestion of meat by stomach secretions and the conversion of starch to sugars by plant extracts and saliva were known. However, the mechanism by which this occurred had not been identified.

In 1833, French chemist Anselme Payen discovered the first enzyme, diastase. A few decades later, when studying the fermentation of sugar to alcohol by yeast, Louis Pasteur came to the conclusion that this fermentation was catalyzed by a vital force contained within the yeast cells called "ferments", which were thought to function only within living organisms. He wrote that "alcoholic fermentation is an act correlated with the life and organization of the yeast cells, not with the death or putrefaction of the cells.

In 1877, German physiologist Wilhelm Kühne (1837–1900) first used the term *enzyme*, which comes from Greek /ˈɛnzaɪmz/, "leavened", to describe this process. The word *enzyme* was used later to refer to nonliving substances such as pepsin, and the word *ferment* was used to refer to chemical activity produced by living organisms.

In 1897, Eduard Buchner submitted his first paper on the ability of yeast extracts that lacked any living yeast cells to ferment sugar. He named the enzyme that brought about the fermentation of sucrose "zymase". Following Buchner's example, enzymes are usually named according to the reaction they carry out. Typically, to generate the name of an enzyme, the suffix *-ase* is added to the name of its substrate (e.g., lactase is the enzyme that cleaves lactose) or the type of reaction (e.g., DNA polymerase forms DNA polymers). Having shown that enzymes could function outside a living cell, the next step was to determine their biochemical nature. Many early workers noted that enzymatic activity was associated with proteins, but several scientists argued that proteins were merely carriers for the true enzymes .However, in 1926, James B. Sumner

showed that the enzyme urease was a pure protein and crystallized it; Sumner did likewise for the enzyme catalase in 1937. The conclusion that pure proteins can be enzymes was definitively proved by Northrop and Stanley, who worked on the digestive enzymes pepsin (1930), trypsin and chymotrypsin.

Enzyme structure:

Enzymes are biological catalysts within living cells, catalysts are substances that increase the rate of chemical reactions without being used up. Enzymes are highly specific in their action on substrates.

Enzymes have many natural sources including:

- Microbial (fungi, yeast or bacteria)
- Plant (papaya, pineapple)
- Animal (pancreatic, for example)

Function and structure of enzymes

Enzymes are proteins, and their function is determined by their complex structure. The reaction takes place in a small part of the enzyme called the **active site** (Is particularly important. The shape and the chemical environment inside the active site permit a chemical reaction to proceed more easily) while the rest of the protein acts as "scaffolding"

The amino acids around the active site attach to the **substrate** (reactants that are activated by the enzyme) molecule and hold it in position while the reaction takes place. This makes the enzyme **specific** for one reaction only, as other molecules won't fit into the active site – their shape is wrong.

Many enzymes need **cofactors** (or coenzymes) to work properly. These can be metal ions (such as Fe^{+2}, Mg^{+2}, Cu^{+2}) or organic molecules (such as haem, biotin, FAD, NAD or coenzyme A). The complete active enzyme with its cofactor is called a holoenzyme , while just the protein part without its cofactor is called the apoenzyme.

Classification:

Depending on the **type of reaction catalyzed**, enzymes are divided into six main classes:

1. Oxidoreductase.
Transfer of electrons from one substrate molecule to another (e.g., dehydrogenase, reductase, oxidase).

2. **Transferase**.
Transfer of functional group from one substrate molecule to another (e.g., glycosyltransferase, acetyltransferase, and aminotransferase).

3. **Hydrolase**.

Transfer of functional group from substrate to water (e.g., glycoside hydrolase, peptidase, esterase).

4. **Lyase**.
an enzyme which catalyzes the joining of specified molecules or groups by a double bond.
(e.g., pectate lyase break glycosidic linkages by b-elimination).

5. **Isomerase**.
Transfer of groups from one position to another in the same molecule (e.g., glucose isomerase).

6. **Ligass**.
Addition of function group to substrate usually coupled with ATP hydrolysis (e.g., glycine–tRNA ligase).

Factors affecting enzyme activity:

Several factors affect the rate at which enzymatic reactions proceed: enzyme concentration, substrate concentration, effect of Temperature, effect of pH and the presence of any inhibitors or activators.

1. Enzyme Concentration:

As the concentration of the enzyme is increased, the velocity of the reaction proportionately increases. In fact, this property of enzyme is made use in determining the activities of serum enzymes for diagnosis of diseases.

2. Concentration of Substrate:

Increase in the substrate concentration gradually increases the velocity of enzyme reaction within the limited range of substrate levels. A rectangular hyperbola is obtained when velocity is plotted against the substrate concentration. Three distinct phases of the reaction are observed in the graph.

3. Effect of Temperature:

Velocity of an enzyme reaction increases with increase in temperature up to a maximum and then declines. A bell-shaped curve is usually observed .The optimum temperature for most of the enzymes is between 40°C-45°C. However, a few enzymes (e.g. venom phosphokinases, muscle adenylate kinase) are active even at 100°C. In general, when the enzymes are exposed to a temperature above 50°C, denaturation leading to derangement in the native (tertiary) structure of the protein and active site are seen. Majority of the enzymes become inactive at higher temperature (above 70°C).

4. Effect of pH:

Increase in the hydrogen ion concentration (pH) considerably influences the enzyme activity and a bell-shaped curve is normally obtained. Each enzyme has an optimum pH at which the velocity is maximum. Most of the enzymes of higher organisms show optimum activity around neutral pH (6-8). There are, however, many exceptions like pepsin (1-2), acid phosphatase (4-5) and alkaline phosphatase (10-11) for optimum pH.

5. Effect of Product Concentration:

The accumulation of reaction products generally decreases the enzyme velocity. For certain' enzymes, the products combine with the active site of enzyme and form a loose complex and, thus, inhibit the enzyme activity. In the living system, this type of inhibition is generally prevented by a quick removal of products formed.

6. Effect of Activators:

Some of the enzymes require certain inorganic metallic cations like Mg^{2+}, Mn^{2+}, Zn^{2+}, Ca^{2+}, Co^{2+}, Cu^{2+}, Na^{+}, K^{+} etc. for their optimum activity. Rarely, anions are also needed for enzyme activity e.g. chloride ion (CI^{-}) for amylase.