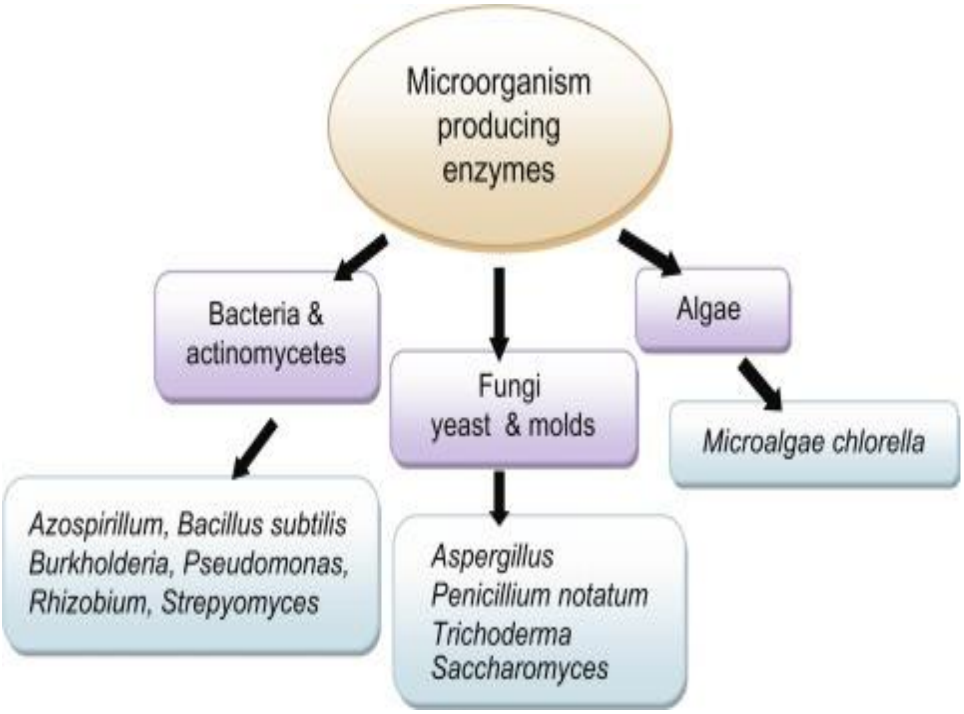
Enzymes are naturally occurring, biological catalysts that are mainly used to control certain biochemical reactions in the living system. Enzymes have a wide range of applications in the production of both medical and non-medical field. Apart from the plants and animals, enzymes are also obtained from certain microbes and are referred to as the microbial enzymes. Microorganisms are majorly used for the production of industrial enzymes through the safe gene transfer methods. The first industrially produced microbial enzymes were obtained from the fungal amylase in the year 1896 and were used to cure indigestion and several other digestive disorders.



History of enzymes

1. The first enzyme produced industrially was the fungal amylase Takadiastase which was employed as a pharmaceutical agent for digestive disorders.
2. By 1969, 80% of all laundry detergents contained enzymes, chiefly Proteases.
3. Due to the occurrence of allergies among the production workers and consumers, the sale of such enzyme utilizing detergents decreased drastically.
4. Special techniques like micro-encapsulation of these enzymes were developed which could provide dustless protease preparation. It was thus made risk free for production workers and consumers.
5. Microbial rennin is also one of the most significant enzymes. It has been used instead of Calf's rennin in cheese production.

Location of Enzymes

I. **Endocellular enzymes** - Enzymes which are produced within the cell or at the cytoplasmic membrane are called as endocellular enzymes.

II. **Exocellular enzymes**- Enzymes which are liberated in the fermentation medium which can attack large polymeric substances are termed as Exocellular enzymes. Eg: amylases & proteases

No.	Sources	Enzyme	Micrsmoorgan
1	**Bacterial**	Protease	*Bacillus subtills*
2		Amylase	*Bacillus subtills*
3		Pencillinase	*Bacillus subtills*
4	**Yeast**	Lactase	Sacchromyces fragllis
5		Invertase	Sacchromyces cerevusae
6	**Fungus**	Protease	*Aspergillus niger*
7		Amylase	*Aspergillus oryzae*
8		Pectinase	*Aspergillus niger*
9		Catalase	*Aspergillus niger*
10		Glucose oxidase	*Aspergillus notanum*
11		Glucosidase	*Aspergillus flaves*

Types of enzymes

A. Amylase- Amylase is an enzyme that catalyses the hydrolysis of starch into sugars.It is present in the saliva of humans. It perform thr hydrolysis of starch with amylase will first result in the formation of a short polymer dextrin and then the disaccharide maltose and finally glucose. Glucose is not as sweet as fructose. Thus the next step would be the conversion of glucose to fructose by the enzyme glucose isomerase. Produces by

a. Bacteria – B. cereus, B.subtilis, B.amyloliquefaciens, B. polymyxa, B.licheniformis etc

b. Fungi – Aspergillus oryzae, Aspergillus niger, Penicillum, Cephalosporin, Mucor, Candidae etc.

Types of Amylases

1. α- Amylase - It is also called as 1,4-α-D-glucan glucanohydrolase

I. It breaks down long carbohydrate chains of amylose and amylopectin.

II. Amylose is broken down to yield maltotriose and altose molecules.

III. Amylopectin is broken down to yield dextrin and glucose molecules.

IV. In animals, it is a major digestive enzyme, and its optimum pH is 6.7–7.0

2. **ß- Amylase- It is a**lso called as 1,4-α-D-glucan maltohydrolase.

1. Synthesized by bacteria, fungi, and plants.
2. Working from the non-reducing end, β-amylase catalyzes the hydrolysis of the second α-1,4
3. glycosidic bond, cleaving off two glucose units (maltose) at a time.

4. During the ripening of fruit, β-amylase breaks starch into maltose, resulting in the sweet flavor of ripe fruit.
5. The optimum pH for β-amylase is 4.0–5.0

γ- Amylase

1. Also termed as Glucan 1,4-α-glucosidase.
2. Cleaves α(1–6) glycosidic linkages, as well as the last α(1–4) glycosidic linkages at the
3. nonreducing end of amylose and amylopectin, yielding glucose.
4. The γ-amylase has most acidic optimum pH of all amylases because it is most active around pH 3.

Uses of amylase

1. **Production** of sweeteners for the food industry.
2. Removal of starch sizing from woven cloth
3. Liquefaction of starch pastes which are formed during the heating steps in the manufacture of corn and chocolate syrups.
4. Production of bread and removal of food spots inthe dry cleaning industry where amylase works in conjunction with protease enzymes

c. Lipase

1. Lipases are also called as glycerol ester hydrolases.
2. They are a subclass of esterases
3. It splits fats into mono or diglycerides and fatty acids.
4. They are extracellular enzymes
5. Mainly produced by fungi Eg: Aspergillus, Mucor, Rhizopus, Peniciilum etc. Bacteria producing lipases include species of Pseudomonas, Achromobacter and Staphylococcus Yeasts like Torulopsis and Candida are also commercially used.

Uses of Lypase

1. Primarily marketed for therapeutic purposes as digestive enzymes to supplement pancreatic lipases.
2. Since free fatty acids affect the odor and taste of cheese, and the cheese ripening process is affected by lipases, microbial affects during the aging process can be due to lipase action.
3. In the soap industry, lipases from Candida cylindraceae is used to hydrolyze oils.

d. Pectinase

1. Pectinase is an enzyme that breaks down pectin, a polysaccharide found in plant cell
2. walls.
3. Pectinase enzymes include Pectolyase, Pectozyme and Polygalacturonase.

4. Pectin is the jelly-like matrix which helps cement plant cells together and in which other cell wall components, such as cellulose fibrils, are embedded.
5. Basic structure of a pectin consists of α-1,4linked Galactouronic acid with upto 95% of it's
6. carboxyl groups esterified with methanol.
7. Pectinase might typically be activated at 45 to 55 °C and work well at a pH of 3.0 to 6.5. Example- Aspergillus niger, A. wentii, Rhizopus etc Fermentation with Aspergillus Niger runs for 60-80 hours in fed batch cultures at pH 3-4 and 37o C using 2% sucrose and 2% pectin.

Uses of Pectinase

1. Pectinase enzymes are commonly used in processes involving the degradation of plant materials, such as speeding up the extraction of fruit juice from fruit, including apples.
2. Pectinases have also been used in wine production since the 1960s
3. Helps to clarify fruit juices and grape must, for the maceration of vegetables and fruits and for the extraction of olive oil.
4. By treatment with pectinase, the yield of fruit juice during pressing is considerably increased.

e. Proteases

1. Protease (Mixture of Peptidases and Proteinases) are enzymes that perform the hydrolysis of Peptide bonds.
2. Peptide bonds links the amino acids to give the final structure of a protein.
3. Proteinases are extracellular and Peptidases are endocellular.
4. Second most important enzyme produced on a large scale after Amylase.
5. Enzymes used in detergents are chiefly proteases from bacillus strains (Bacillopeptidases)
6. Best known proteases are Subtilisin Carlsberg from B. licheniformis and Subtilisin BPN and
7. Subtilisin Novo from B. amyloliquefaciens.
8. These enzymes are not inhibited by EDTA (Ethylene diamine tetraacetic acid) but are
9. inhibited by DFP (Di isopropyl fluorophosphate)

Uses of Protease

1. Textile industry to remove proteinaceous sizing.
2. Silk industry to liberate silk fibers from naturally occurring proteinaceous material in
3. which they are embedded.
4. Tenderizing of Meat
5. Used in detergent and food industries

Methods of enzyme production

1. **Semisolid Culture**- The enzyme producing culture is grown on the surface of a suitable semi-solid substrate (Moistened Wheat or Rice Bran with nutrients) Preparation of Production Medium –

I. Bran is mixed with solution containing nutrient salts. pH is maintained at a neutral level. Medium is steam sterilized in an autoclave while stirring.
II. The sterilized medium is spread on metal trays upto a depth of 1-10 centimeters.
III. Culture is inoculated either in the autoclave after cooling or in trays.
IV. High enzyme concentration in a crude fermented material.

Enzymes produced by Semi-solid culture.

No.	Enzyme	Micro-organisms
1	α- Amylase	Aspergillus oryzae
2	Glucoamylase	Rhizopus spp.
3	Lactase	A. oryzae
4	Pectinase	A. niger
5	Protease	A. niger , A. Oryzae
6	Rennet	Mucor pusillus

Advantages of Semi-solid culture

I. It involves comparatively low investment
II. Allows the use of substrate with high dry matter content. Hence it yields a high enzyme concentration in the crude fermented material.
III. To cultivate those moulds which cannot grow in the fermenters due to wall growth.
IV. Allows the moulds to develop into their natural state.

Disadvantages of Semi-solid culture

I. Requires more space and more labour
II. Involves greater risk of infection
III. Difficult to introduce automation in such systems

Submerged Culture- Fermentation equipment used is the same as in the manufacture of antibiotics. • It's a cylindrical tank of stainless steel and it is equipped with an agitator, an aerating device, a cooling system and various ancillary equipment (Foam control, pH monitoring device, temperature, oxygen tension etc). Good growth is not enough to obtain a higher enzyme yield.

1. Presence of inhibitors or inducers should also be checked in the medium. Example – Presence of Lactose induces the production of β- galactosidase.
2. As the inducers are expensive, constitutive mutants are used which do not require an inducer.
3. Glucose represses the formation of some enzymes (α-amylases). Thus the glucose concentration is kept low.
4. Either the glucose can be supplied in an incremental manner or a slow metabolizable sugar (Lactose or metabolized starch).

5. Certain surfactants in the roduction medium increases the yield of certain enzymes Non- ionic detergents (eg. Tween 80, Triton) are frequently used.

Advantages and disadvantages of submerged culture

I. Requires less labor and space
II. Low risk of infection
III. Automation is easier
IV. Initial investment cost is very high.

After fermentation

1. Once fermentation is finished, the fermented liquor is subjected to rapid cooling to about 5o C in order to reduce deterioration.
2. Separation of micro-organisms is accomplished either by filtration or by centrifugation of the refrigerated broth with adjusted pH.
3. To obtain a higher purity of the enzyme, it is precipitated with acetone, alcohols or inorganic salts (ammonium or sodium sulfate).
4. In case of large scale operations, salts are preferred to solvents because of explosion hazards.