Production and Applications of Enzymes

Introduction

- Enzymes are macromolecular biological catalysts.
- Enzymes accelerate or catalyze chemical reactions.
- The molecules at the beginning of the process are called substrates and enzymes convert these into different molecules which are called as products.
- Microbial enzymes are the biological catalysts for the biochemical reactions leading to microbial growth and respiration, as well as to the formation of fermentation products.

Types of Enzymes

Adaptive

- Produced only when the need arises

  Eg. When a cell is deficient of a particular nutrient.

Constitutive

- Produced always irrespective the amount of the substrate.

History

- The first enzyme produced industrially was the fungal amylase.
- By 1969, 80% of all laundry detergents contained enzymes, chiefly Proteases.
- Due to the occurrence of allergies among the production workers and consumers, the sale of such enzyme utilizing detergents decreased drastically.

- 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.
- Microbial rennin is also one of the most significant enzymes. It has been used instead of Calf's rennin in cheeseproduction.

Improved Prospects of Enzyme Application

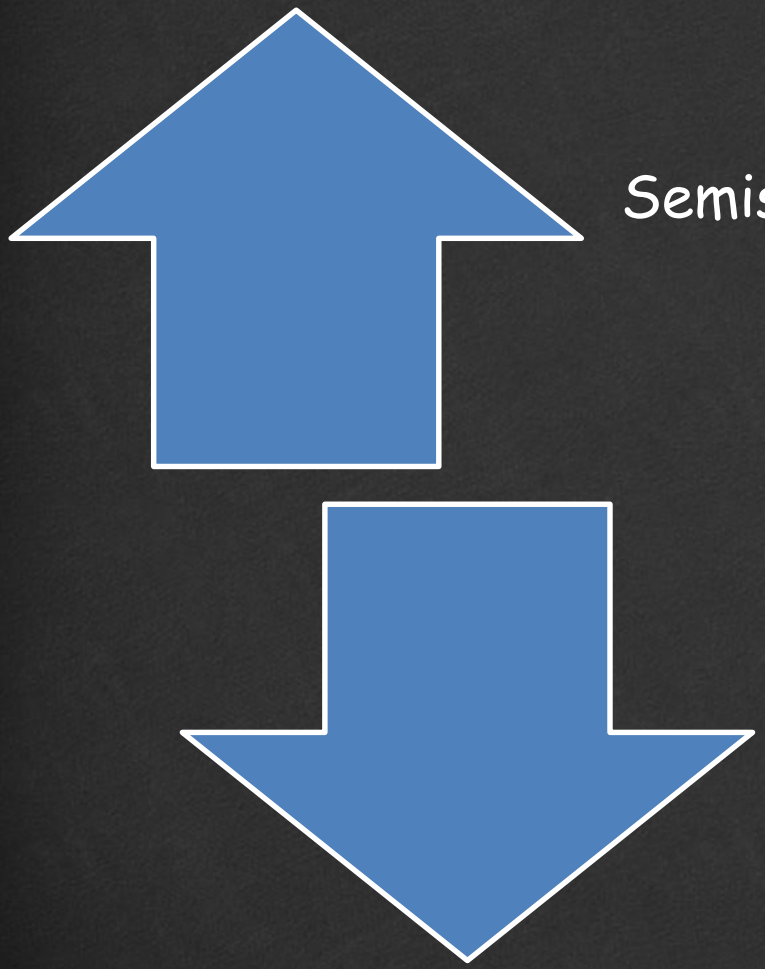
- Microbial Genetics – High yields can be obtained by Genetic manipulation.

  Example – Hansenula polymorpha has been genetically modified so that 35% of it's total protein consists of the enzyme alcohol oxidase.

- Optimization of fermentation conditions (Use of low cost nutrients, optimal utilization of components in nutrient solution, temperature and pH)

- New cell breaking methods like Homogenizer, Sonication etc.
- Modern purification processes like Counter current distribution, Ion-exchange chromatography, Molecular-sieve chromatography, Affinity chromatography and precipitation by using alcohol, acetone, etc.
- Immobilization of enzymes.
- Continuous enzyme production in special reactors.

Methods of Enzyme Production



Semisolid Culture

Submerged Culture

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 – Bran is mixed with solution containing nutrient salts.

pH is maintained at a neutral level. Medium is steam sterilized in an autoclave with stirring.

The sterilized medium is spread on metal trays upto a depth of 1-10 centimeters.

Culture is inoculated in trays.

High enzyme concentration in a crude fermented material.

Enzymes produced by Semi-solid culture

Enzyme	Micro-organisms
α- Amylase	Aspergillus oryzae
Glucoamylase	Rhizopus spp.
Lactase	A. oryzae
Pectinase	A. niger
Protease	A. Niger & A. oryzae
Rennet	Mucor pusillus

Advantages of Semi-solid culture

- It involves comparatively low investment
- Allows the use of substrate with high dry matter content which yields a high enzyme concentration.
- To cultivate those moulds which cannot grow in the fermenters.
- Allows the moulds to develop into their natural state.

Disadvantages of Semi-solid culture

- Requires more space and more labour
- Involves greater risk of infection
- Difficult to introduce automation in such systems

Submerged Culture

- Fermentation equipment used is the same as in the manufacture of antibiotics.
- It is a cylindrical tank of stainless steel and it is equipped with an agitator, an aerating device, a cooling system and various ancillary equipments (Foam control, pH monitoring device, temperature, oxygen tension, etc.)

- Presence of inhibitors or inducers should also be checked in the medium.

  Example –

  - ✓ Presence of Lactose induces the production of β-galactosidase.
  - ✓ Glucose represses the formation of some enzymes (α-amylases).
- Certain surfactants in the production medium increase the yield of few enzymes.
  - ✓ Non- ionic detergents (eg. Tween 80, Triton) are frequently used.

Advantages of Submerged culture

- Requires less labor and space
- Low risk of infection
- Automation is easier

After fermentation

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

AMYLASE

Introduction

- Amylase is an enzyme that catalyzes the hydrolysis of starch into sugars.
- Present in the saliva of humans.
- 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.

Types of Amylases

- α- Amylase
- ß- Amylase
- γ- Amylase

α- Amylase

- Also called as 1,4-α-D-glucan glucanohydrolase.
- Calcium metalloenzymes which cannot function in absence of calcium ions.
- Breaks down long carbohydrate chains of Amylose and Amylopectin.
- Amylose is broken down to yield maltotriose and Maltose molecules.
- Amylopectin is broken down to yield Limit dextrin and glucose molecules.

- Found in saliva and pancreas.
- Found in plants, fungi (ascomycetes and basidiomycetes) and bacteria (Bacillus)
- Because it can act anywhere on the substrate, α-amylase tends to be faster-acting than β- amylase.
- In animals, it is a major digestive enzyme, and its optimum pH is 6.7–7.0

ß- Amylase

- Also called as 1,4-α-D-glucan maltohydrolase.
- Synthesized by bacteria, fungi, and plants.
- During the ripening of fruit, β-amylase breaks starch into maltose, resulting in the sweet flavor of ripe fruit.
- The optimum pH for β-amylase is 4.0–5.0

γ- Amylase

- Also termed as Glucan 1,4-a-glucosidase.
- The γ-amylase has most acidic optimum pH of all amylases because it is most active around pH 3.

Producing strains

- Bacteria – B. cereus, B.subtilis, B. amyloliquefaciens, B. polymyxa, B. licheniformis etc
- Fungi- Aspergillus oryzae, Aspergillus niger, Penicillum, Cephalosporin, Mucor, Candida etc.

Production

- The amylase production to be carried out in 250 mL conical flasks containing 50 mL medium with the following composition:

5 g/L	soluble starch
5 g/L	yeast extract
2.5 g/L	$(NH_4)_2SO_4$
0.2 g/L	$MgSO_4 \cdot 7H_2O$
3 g/L	KH_2PO_4
0.25g/L	$CaCl_2 \cdot 2H_2O$

- It is incubated at 50°C under shaking conditions (200 rpm) and inoculated with 2.5% of 24 h old culture.
- After the specified cultivation time for each set of experimental trials, the culture broths to be centrifuged for 10 min and the cell free supernatant should be used for enzyme determination.

Applications

- Production of sweeteners for the food industry.
- Liquefaction of starch pastes which are formed during the heating steps in the manufacture of corn and chocolate syrups.
- Production of bread.
- Removal of food spots in the dry cleaning industry where amylase works in conjunction with protease enzymes.

LIPASES

Introduction

- Lipases are also called as Glycerol ester hydrolases.
- They are a subclass of esterase.
- It splits fats into mono or di- glycerides and fatty acids.
- They are extracellular enzymes.
- Mainly produced by Fungi.

  Eg: Aspergillus, Mucor, Rhizopus, Penicilium, etc.

- Bacteria producing lipases include species of Pseudomonas, Achromobacter and Staphylococcus.
- Yeasts like Torulopsis and Candida are also commercially used.

- Enzyme production must be induced by adding oils and fats. But in some cases the fats have repressing effect on the lipase production.
- Lipases are generally bound to the cells and hence inhibit an overproduction, but addition of cations such as magnesium ion liberates the lipase and leads to a higher enzyme titer in the production medium.

Production

- Olive oil is being used as the substrate for the lipase enzyme.
- Stable emulsion of olive oil is prepared by vigorous mixing of the oil with a solution of an emulsion stabilizer Triton X-100 in 50 mM potassium phosphate buffer.
- The fermentation should be carried out at 29°C for 96 h supplied with 1% carbon source.
- The type of oil used is very important for both lipase production and fungal growth.

Applications

- Primarily marketed for therapeutic purposes as digestive enzymes to supplement pancreatic lipases.
- In the soap industry, lipases from *Candida cylindraceae* is used to hydrolyze oils.

Proteases

Introduction

- Protease (Mixture of Peptidases and Proteinases) are enzymes that perform the hydrolysis of Peptide bonds.
- Peptide bonds links the amino acids to give the final structure of a protein.
- Second most important enzyme produced on a large scale after Amylase.

Classification based upon the pH in which the Proteases are active

- Alkaline serine Proteases
- Acid Proteases
- Neutral Proteases

Fermentation Process

Cultures are stored in the lyophilized state or under Liquid nitrogen.

Initial cultures are prepared in shake flasks and small fermenters (40-100 m^3) at 30-37°C

The concentration of ammonium ions and amino acids are kept down as they may repress protease production

High oxygen and partial pressure is generally necessary for optimal protease titers

Time span for fermentation is 48-72 hours depending upon the organism

Proteases must be converted in a particulate form before they are added to detergents.

- To prepare a suitable encapsulated product, a wet paste of enzyme is melted at 50-70° C with a hydrophobic substance such as polyethylene glycol and then converted into tiny particles.

Applications

- In Textile industry- to remove proteinaceous sizing.
- In Silk industry- to liberate silk fibers from naturally occurring proteinaceous material.
- Tenderizing Meat
- Used in detergent and food industries.

Peroxidase

Introduction

- Peroxidases are isolated from various sources like plants, animals and microbes.
- More commonly, *Bacillus subtilis* is employed for the production of peroxidases.
- *B. subtilis* is isolated from soil using the serial dilution method.

- **Production:**
- Media for peroxidase:
  - ✓ 50 ml of production media is prepared in conical flask and it is autoclaved at 121°C at 15 lbs pressure for 15 minutes.
  - ✓ Then a loopful of *Bacillus subtilis* culture was inoculated into the production media.
  - ✓ Finally, the bacterial medium is incubated at 37°C for 48 hours in shaking incubator.

Applications

- Peroxidase can be used for treatment of industrial waste waters. For example, phenols, which are important pollutants, can be removed by enzyme-catalyzed polymerization using peroxidase.
- There are many investigations about the use of peroxidase in manufacturing processes like adhesives, computer chips, car parts, and linings of drums and cans.

Thank You