Use of microbes in industry

Suman Kumar Mekap
Asst. Professor (Pharmacology)
CUTM, Bhubaneswar

INTRODUCTION

- Bacteria, Fungi, Yeast etc. are used commonly in the production of various fermented products like wine, yogurt etc.

- Strains of *Corynebacterium glutamicum* have been used in the production of the amino acid, L-glutamate.

- Production of food and dairy products. Cheese, yogurt, alcoholic beverages, coffee, tea, vitamins etc. are some of the examples.

- Production of vaccines is another important application of industrial microbiology.

- Antibiotics are another important products produced by using microorganisms.

Enzymes

- Enzymes are macromolecular biological catalysts.
- Enzymes accelerate, or catalyze chemical reactions.
- The molecules at the beginning of the process are called substrates and the enzyme converts these into different molecules, called 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.

STRUCTURE

Protein structure

Scaffold to support and position active site

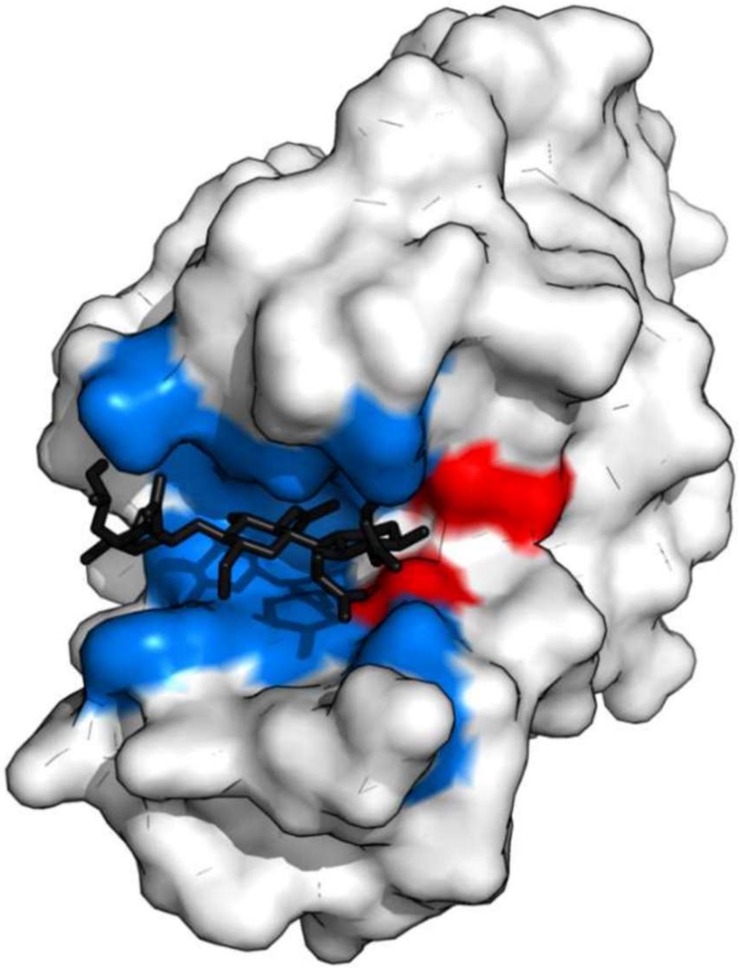
Active site

Binding sites

Bind and orient substrate(s)

Catalytic site

Reduce chemical activation energy



Types of Enzymes

1> ADAPTIVE

- Produced only when the need arises

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

2>CONSTITUTIVE

- Produced always irrespective the amount of substrate.

History

- The first enzyme produced industrially was the fungal amylase Takadiastase which was employed as a pharmaceutical agent for digestive disorders.

- 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 cheese production.

Location of Enzymes

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

- Enzymes which are liberated in the fermentation medium which can attack large polymeric substances are termed as **Exocellular enzymes**.

- Eg: Amylases & Proteases

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, Bead mill, Sonication etc.

- **Modern purification processes** like Counter current distribution, Ion-exchange chromatography, Molecular-sieve chromatography, Affinity chromatography and precipitation by using alcohol, acetone.

- 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 while stirring.
- The sterilized medium is spread on metal trays up to a depth of 1-10 centimeters.
- Culture is inoculated either in the autoclave after cooling or in trays.
- High enzyme concentration in a crude fermented material is obtained.

Enzymes produced by Semi-solid culture

Example

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. Hence it yields a high enzyme concentration in the crude fermented material.
- To cultivate those moulds which cannot grow in the fermenters due to wall growth.
- 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

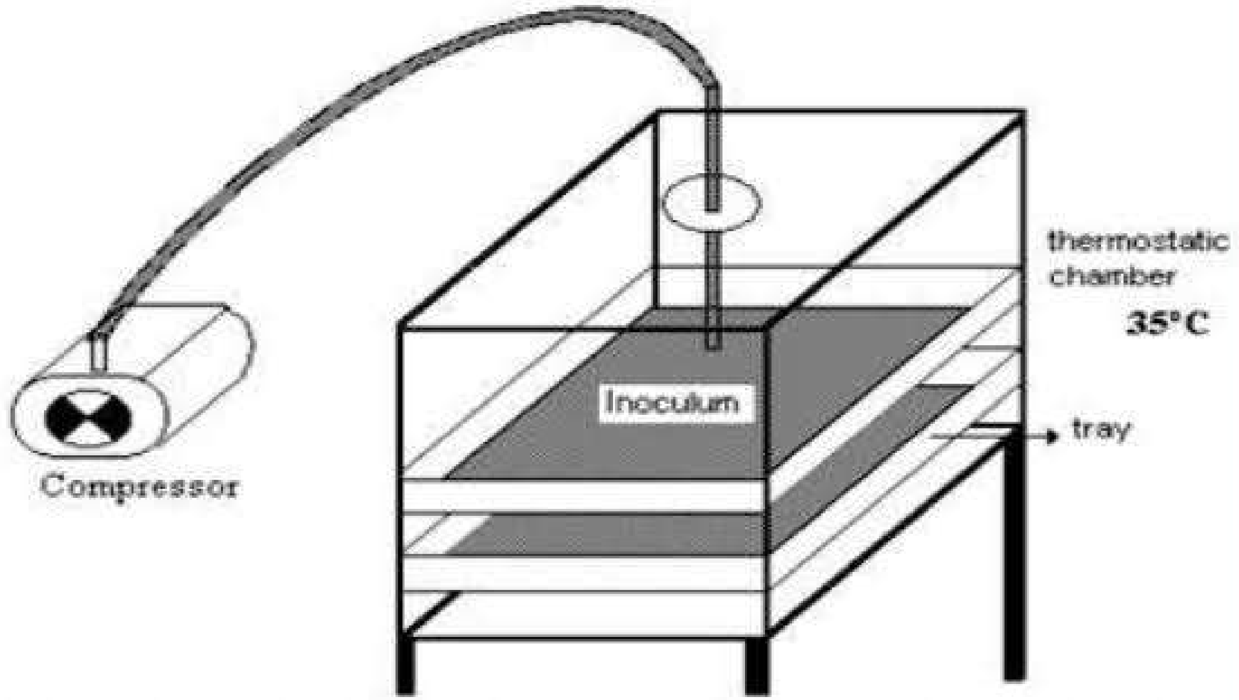


***Figure 1.** Solid substrate fermentation of cactus in trays inside of thermostatic chamber at 35°C. The initial pH was adjusted to 3.0 and 60% of initial moisture. A nitrogen source was made of 11% ammonium and 2% urea (on a nitrogen basis), 5% potassium phosphate, and 3.2 g spore/100 g substrate.*

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 devise, temperature, oxygen tension etc.)
- Good growth is not enough to obtain a higher enzyme yield.
- Certain surfactants in the production medium increases the yield of certain enzymes.
- Non- ionic detergents (eg. Tween 80, Triton) are frequently used.

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

  Example – Presence of Lactose induces the production of β-galactosidase.

- As the inducers are expensive, constitutive mutants are used which do not require an inducer.

- Glucose represses the formation of some enzymes (α-amylases). Thus the glucose concentration is kept low.

- Either the glucose can be supplied in an incremental manner or a slow metabolizable sugar (Lactose or metabolized starch)

Advantages of Submerged culture

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

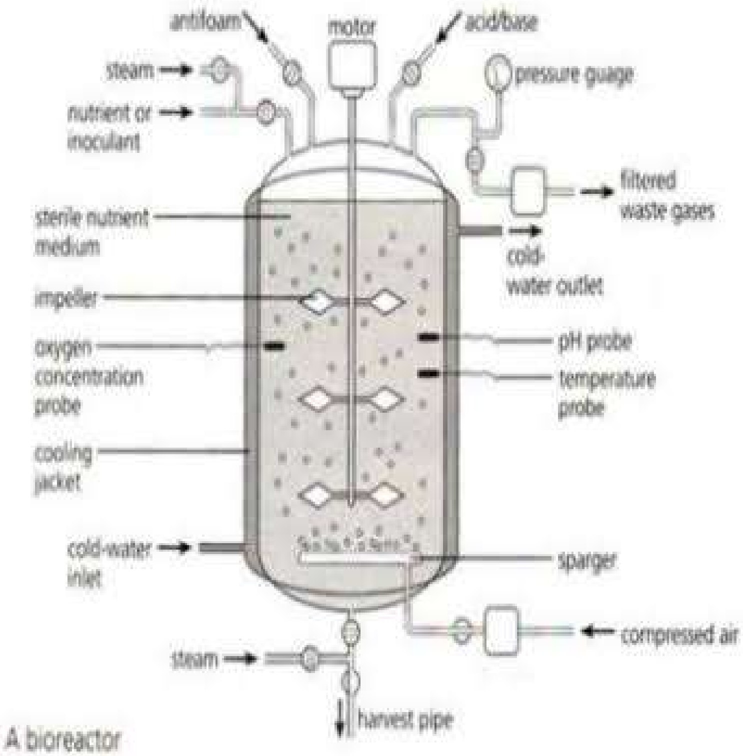
Disadvantage of Submerged Culture

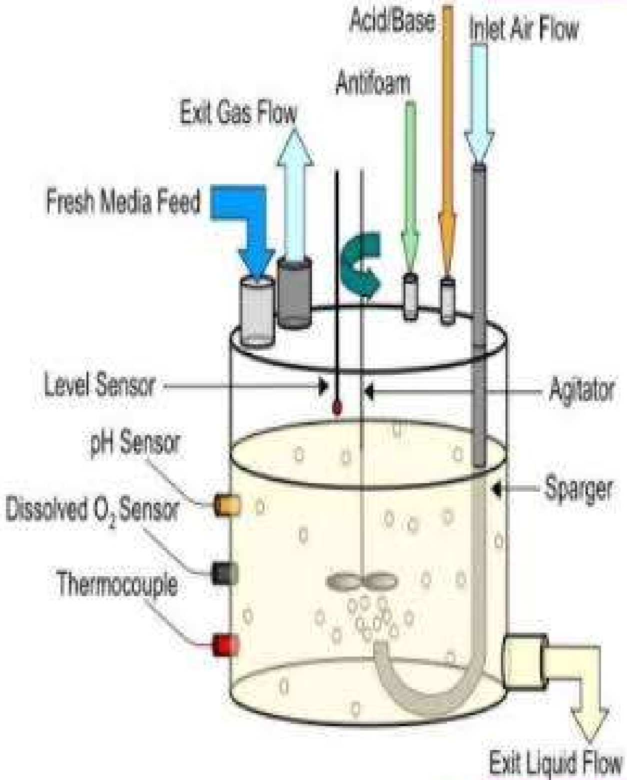
- Initial investment cost is very high.

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 of the refrigerated broth with adjusted pH.
- To obtain a higher purity of the enzyme, it is precipitated with acetone, alcohols or inorganic salts (ammonium or sodium sulphate).
- In case of large scale operations, salts are preferred to solvents because of explosion hazards.

OPEN AND CLOSED FERMENTER





1. AMYLASE

- Amylase is an enzyme that catalyses 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 metallo-enzymes 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.
- Working from the non-reducing end, β-amylase catalyzes the hydrolysis of the second α-1,4 glycosidic bond, cleaving off two glucose units (maltose) at a time.
- 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-α-glucosidase.

- Cleaves α(1–6) glycosidic linkages, as well as the last α(1–4) glycosidic linkages at the non reducing end of amylose and amylopectin, yielding glucose.

- The γ-amylase has most acidic optimum pH of all amylases because it is most active around pH 3.

Effects of α-Amylases

- Break down the starch polymer but does not give free sugar Starch- Liquefying

- Gives free sugars Saccharogenic

Producing strains

- Bacteria – *B. cereus, B.subtilis, B. amyloliquefaciens, B. polymyxa, B. licheniformis* etc.

- Fungi – *Aspergillus oryzae, Aspergillus niger, Penicillum, Cephalosporin, Mucor, Candida* etc.

Applications

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

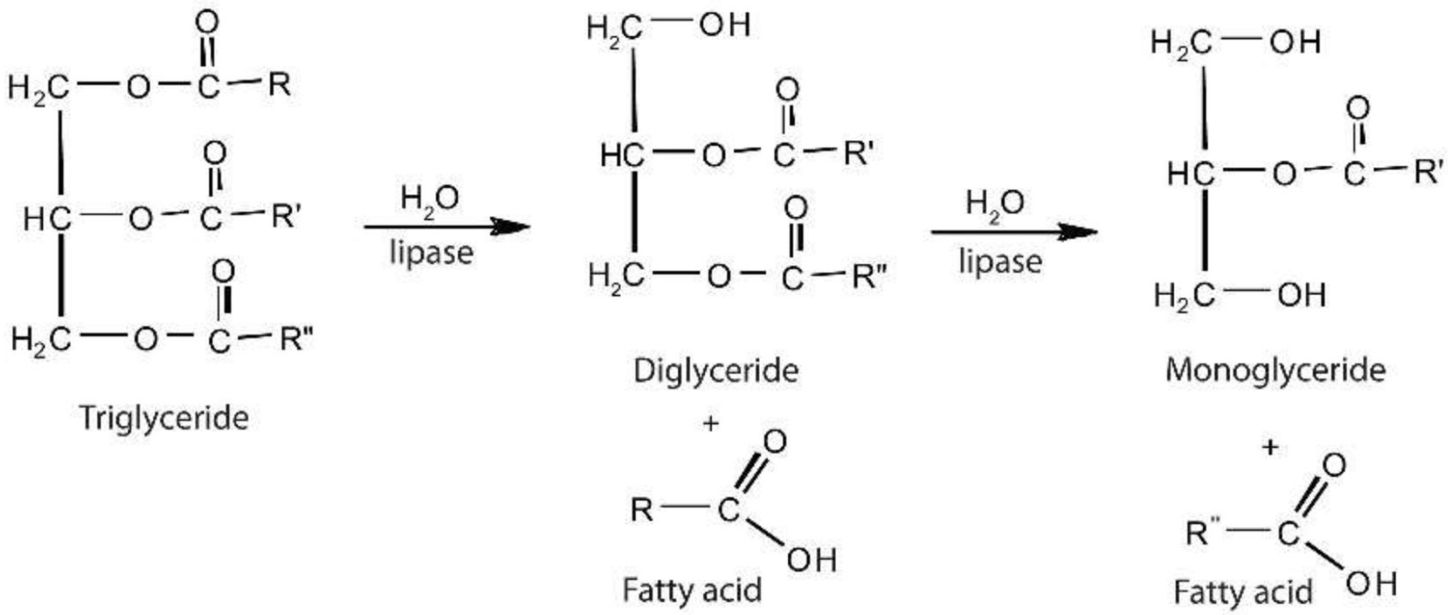
2. LIPASES

Introduction

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

Mode of Action



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

Applications

- Primarily marketed for therapeutic purposes as digestive enzymes to supplement pancreatic lipases.

- Since free fatty acids affect the odour 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.

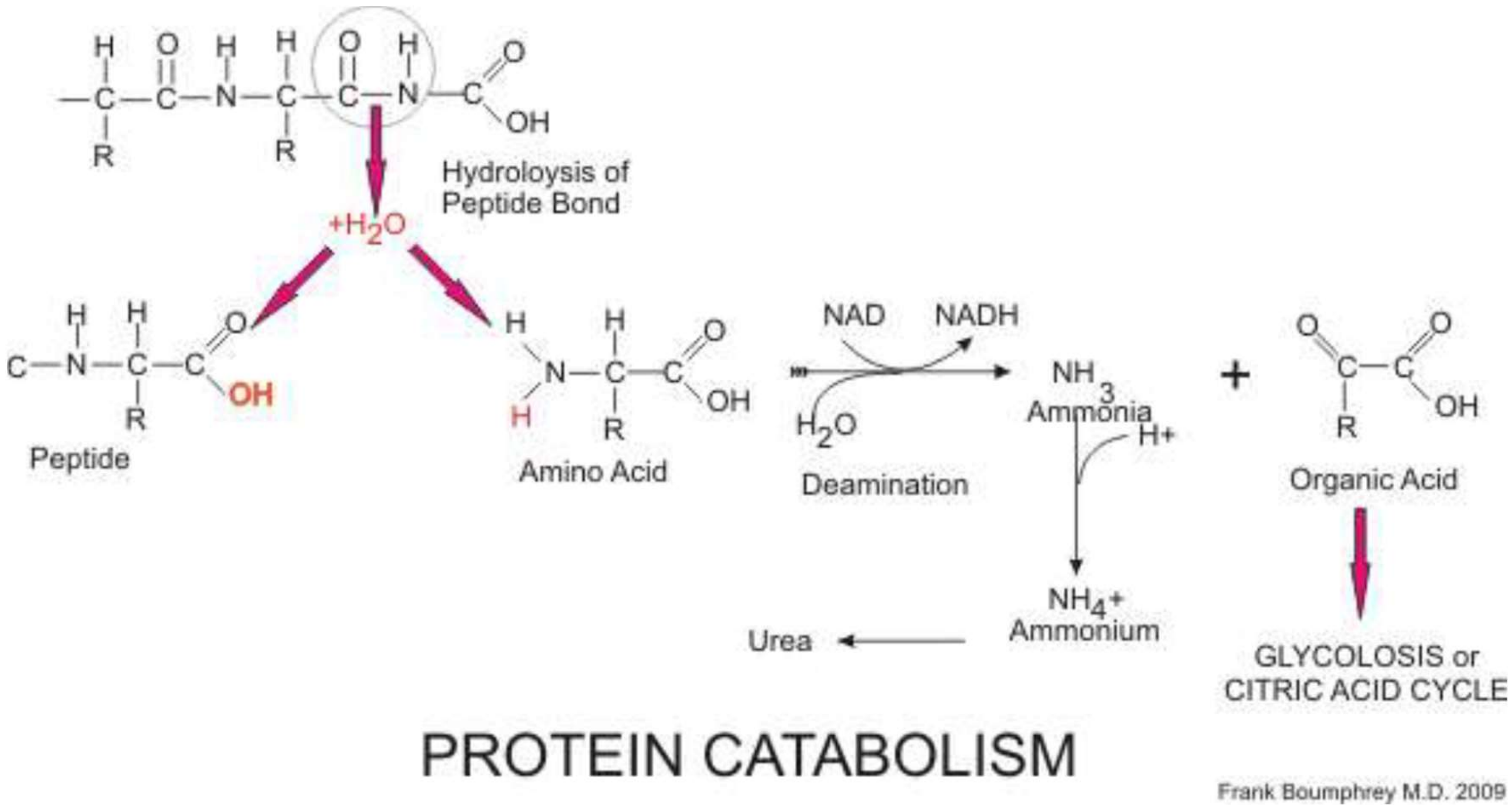
- In the soap industry, lipases from *Candida cylindraceae* is used to hydrolyze oils.

3. 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.
- Proteinases are extracellular and Peptidases are endocellular.
- Second most important enzyme produced on a large scale after Amylase

Mode of Action



Classification

Based upon the residues in the Catalytic site

- Serine Protease
- Threonine Protease
- Aspartate Protease
- Cysteine Protease
- Glutamatic acid Protease
- Metalloproteases eg: Zinc

Classification Based upon the pH in which the Proteases are Active

- Alkaline serine Proteases
- Acid Proteases
- Neutral Proteases

Alkaline Serine Proteases

- pH of the production medium is kept at 7.0 for satisfactory results.

- Have serine at the active site

- Optimum temperature maintained is 30^o to 40^o C.

- Important producers are *B. licheniformis, B. amyloliquefaciens, B. firmus, B. megaterium, Streptomyces griseus, S. fradiae, S. rectus and fungi like A. niger, A. oryzae, A. flavus*.

- Enzymes used in detergents are chiefly proteases from bacillus strains (**Bacillopeptidases**)

- Best known proteases are **Subtilisin Carlsberg** from *B. licheniformis* and **Subtilisin BPN** and **Subtilisin Novo** from *B. amyloliquefaciens.*

- These enzymes are not inhibited by EDTA (Ethylene diamine tetraacetic acid) but are inhibited by DFP (Di isopropyl fluorophosphate)

Proteases for the Use in Detergent industries

- Stability at high temperature
- Stability in alkaline range (pH- 9 to 11)
- Stability in association with chelating agents and perborates
- But shelf life is affected in presence of surface active agents.

Screening

- Because the enzymes should be stable in alkaline conditions, screening for better producers is done by using highly alkaline media.

- It was found that *B. licheniformis* and *B. subtilis* showed growth is the range of pH 6-7 by new strains were found to grow even in pH 10-11.

- Genetic Manipulation can also be carried out.

Fermentation Process

- Cultures are stored in the lyophilized state or under Liquid nitrogen.
- Initial cultures are carried out in shaken flasks and small fermenters (40-100 m3) at 30-37^{o} C
- Fed-Batch culture is generally used to keep down the concentration of ammonium ions and amino acids as they may repress protease Production
- High oxygen 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.

Neutral Proteases

- They are relatively unstable and calcium, sodium and chloride must be added for maximal stability.

- Not stable at higher temperatures

- Producing organisms are *B. subtilis, B. megaterium* etc.

- They are quickly inactivated by alkaline proteases.

Acid Proteases

- Similar to Mammalian pepsin

- It consists of Rennin like proteases from fungi which are chiefly used in cheese production

- They are used in medicine, in the digestion of soy protein for soya sauce production and to break down wheat gluten in the baking industry

Applications

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

4. PENICILLINASE

- **Syn:** β-lactamase
- It is a bacterial enzyme, produced by *bacillus* species & certain strain of *staphylococcus*.

Description:

- It contain β-lactam ring .
- It includes penicillin derivatives.
- It inhibit bacterial cell wall synthesis

Preparation:

- Obtained from *B.subtilis & B.cereus*

It is divided in to 2 class

1) Penicillin amidase or acylase
2) β-lactamase

- Amidase attack on acyl group attached to the nucleous thats why its called acylase also. This enzyme more specific with Pen –V & K (Phenoxymethylpenicillin)

- β-lactamase act on basic nucleus itself,

- This enzyme more specific with Pen –G (Benzylpenicillin) & X (Hydroxybenzylpenicillin) & lesser with Pen-V (Phenoxymethylpenicillin) .

Identification Test:

- Each strip dipped with benzylpenicillin & pH indicator, bromocresol purple.
- Positive produce- penicilloic acid
- These cause fall in pH
- Purple to yellow

Use:

- Inactivation of penicillin
- Antigen-antibody reaction.

THANK YOU

Happy to answer if you have any Queries…?