# Enzymes production: Amylase & Protease

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# History

- Enzymes such as amylases, carboxymethylcellulases and proteases are widely used in the industry for the manufacture of pharmaceuticals, foods, beverages and confectioneries as well as in textile and leather processing, and waste water treatment.
- 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.

# Methods of Production

- Semisolid Culture & Submerged Culture

**Semisolid culture**

- The culture was grown on the surface of the suitable semisolid substrate. (Moistened wheat or rice bran with nutrients).
- Bran is mixed with solution containing nutrients salts, pH is maintained at neutral level.
- Medium is steam sterilized in an autoclave while stirring.
- The sterilized medium is spread on the metal trays upto a depth of 1-10 cms.
- Culture is inoculated in trays.
- High enzyme concentration in a crude fermented material.

# Semisolid Culture

**Advantages**

- 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.
- Allows the microorganism to develop into their natural state.

**Disadvantages**

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

# Submerged Culture

- Fermentation equipment used is 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.
- Presence of inhibitors or inducers should also be checked in the medium.
- Certain surfactants in the production medium increases the yield of certain enzymes.
- Non- ionic detergents (eg. Tween 80, Triton) are frequently used.

**Advantages**

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

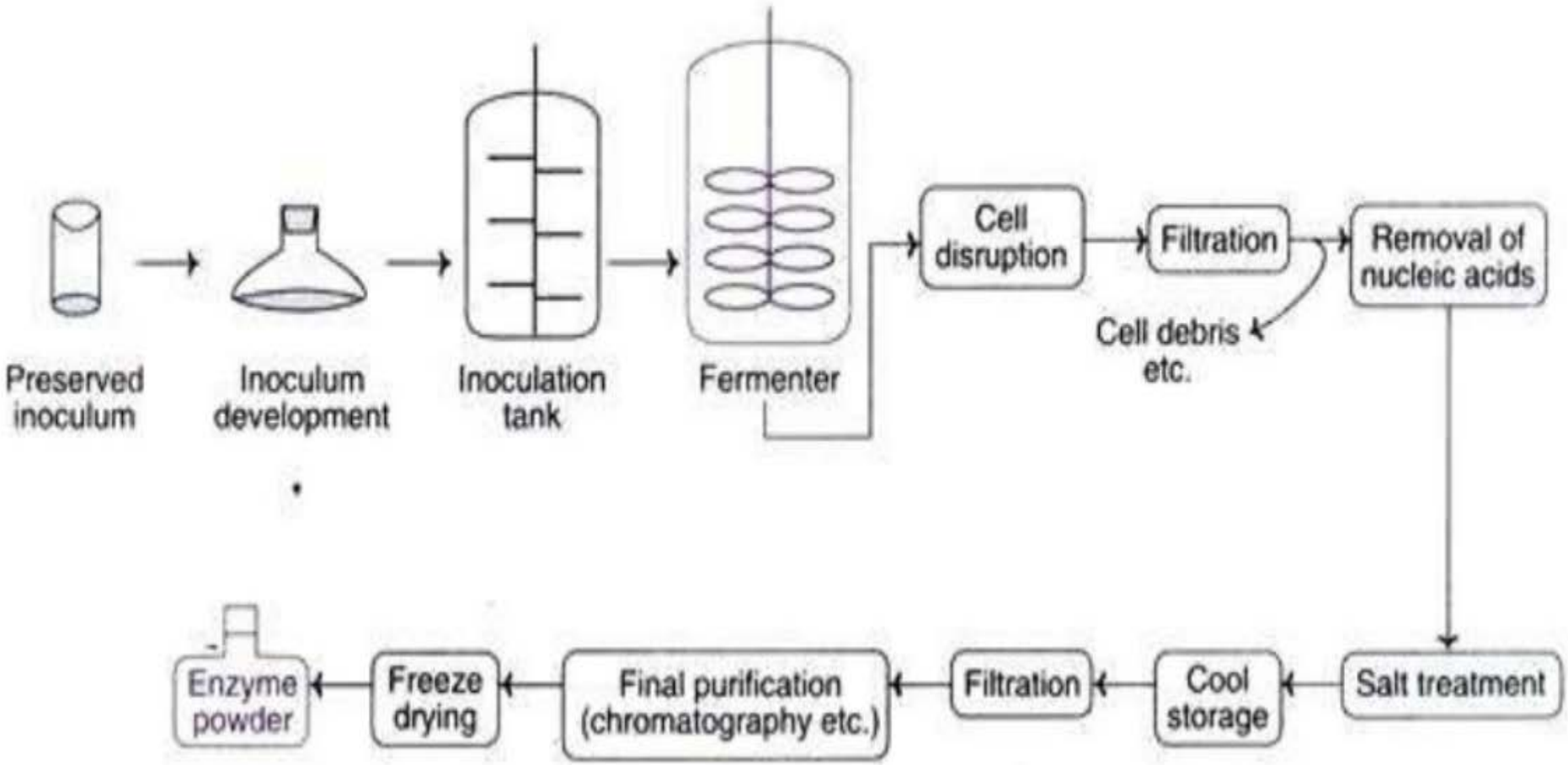
**Disadvantage**

- Initial investment cost is very high.

# Downstream processing

- 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 sulfate).
- In case of large scale operations, salts are preferred to solvents because of explosion hazards.

# PRODUCTION OF ENZYMES BY MICROORGANISMS



# 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.

# TYPES OF AMYLASES

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

# Substrates and Producing organisms

**Substrate materials**

- Wheat bran
- Gram husk
- Rice bran
- Bagasses
- Paper pulp
- other starchy substances
- Carbon sources (Maltose, sucrose and glucose)
- Nitrogen sources (ammonium sulphate, ammonium chloride and ammonium hydrogen phosphate)

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

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

# Fungal α-Amylase

- Fungal α-amylase is produced commercially by employing either *Aspergillus oryzae* or *Aspergillus niger*.
- Stationary culture method is used when *A. oryzae* is employed.
- Wile submerged culture method is used when *Aspergillus niger* is employed.

**The following medium is generally employed for submerged fermentation:**

| Component | Amount (g liter$^{-1}$) |
|---|---|
| Corn starch | 24 |
| Corn steep liquor | 36 |
| Potassium chloride | 0.2 |
| Sodium monohydrogen phosphate | 47 |
| Calcium chloride | 1 |
| $MgCl_2 \cdot 6H_2O$ | 0.2 |

Amylase biosynthesis is inhibited when there is glucose in the medium. The medium is steam sterilized. The sterilized medium is passed into a production fermenter for α-amylase production.

# Fermentation Process

- A cylindrical fermenter made up of stainless steel is generally used in the fermentation process.
- It is equipped with an agitator, an aerating device, a cooling system and other ancillary equipment like a device for foam control, monitoring of pH, temperature and control of oxygen tension etc.
- Medium is taken in the fermenter and is inoculated with spores of the selected species of the fungus.
- The spores are allowed to germinate and produce sufficient mycelium by controlling the fermentation conditions.
- Control of fermentation conditions plays a vital role in the success of the process, includes pH, temperature, aeration, agitation, oxygen supply etc.
- The optimum pH for the fermentation is 7.0.
- Calcium carbonate is used as buffer to maintain pH.
- The fermentation process is generally operated at a temp. of 30 to 40°C.
- Aeration and agitation of the production medium is needed because of high viscosity of the medium due to the presence of mycelial mat.

# Harvest & Recovery

- The following steps are followed during the recovery of the enzyme after the completion of fermentation.
- In order to avoid denaturation of the enzyme, the fermentation broth is subjected to rapid cooling at 5°C temperature immediately and the enzyme is extracted.

  1. Separation of fungal mycelium is accomplished by filtration of the refrigerated broth.

  2. The suspended particles present in the broth are removed with flocculating agents like calcium phosphate.

  3. The enzyme is precipitated, in order to get high degree of purity, by using acetone or alcohol or even inorganic salts like ammonium sulphate or sodium sulphate.

  4. Sometimes fractional precipitation of the enzyme is done to obtain it in purest form.

# Bacterial α -Amylase

- The following medium is generally employed in a submerged culture method.

| Substances | Amount (in %) |
|---|---|
| Lactose | 4.5 |
| Ground soyabean meal | 1.85 |
| $MgSO_4 \cdot 7H_2O$ | 0.04 |
| Hodag KG –1 antifoam | 0.05 |
| Amber BYF (Autolysed brewers yeast fractions) | 1.50 |
| Distillers dried solubles | 0.70 |
| N-Zamine (enzyme casein hydrolyzate) | 0.65 |
| Water | 90.40 |

- **Fermentation Process :** The fermentation is continued upto 4-6 days.
- The pH of the medium is maintained at 7.0.
- Calcium carbonate is used as a buffer for maintaining neutral pH.
- The temperature is maintained at 30-40°C.
- The production of amylase starts when the bacterial density reaches $10^9$-$10^{10}$ cells $ml^{-1}$.
- However, the enzyme production increases just before the growth rate of the microorganism decreases and spore formation begins.
- **Recovery:** Bacterial α-amylase is harvested by the same method that is used for the recovery of fungal α-amylase.
- The most active liquid enzyme preparation contains 2% amylase protein and solid preparation contains 5% amylase proteins.

# 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.

# Protease Production

- 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.

# Industrial Production

- Commercially produced microbial proteases contribute to approximately 2/3 of all enzyme sales.
- Bacteria- *Bacillus, Pseudomonas, Clostridium, Proteus*, and *Serratia*
- Fungi- *Aspergillus niger, Aspergillus oryzae, Aspergillus flavus*, and *Penicillium roquefortii*.
- Bacillus species are mostly used in the commercial production of proteases. □
- The fungal proteases present a wider pH activity range- wider range of uses.
- There are two types of proteases generally produced at industrial level: (a) alkaline serine proteases and (b) acid proteases.
- Alkaline serine proteases- *Bacillus licheniformis* by submerged culture method.
- Acid proteases- fungi by either semisolid culture or submerged culture method.

# Fungal Protease

- Commercial production of fungal protease- *Aspergillus flavus, Aspergillus wentii, Aspergillus oryzae, Mucor delemar, Mucor miehei* and *Amylomyces rouxii*.
- The fungus is usually grown on wheat bran, although other media are sometimes employed, under fermentation conditions similar to those for amylase production.
- At sporulation, the various fungal proteolytic enzymes are present in the medium, and the proteases are recovered by procedures similar to those for mold amylases.
- The optimum temperature of the fermentation is 30°C & requires 3 days for completion.
- *Mucor miehei*- Acid proteases by submerged culture method (The optimum temperature of the fermentation is 30°C but requires 7 days for completion).

# Bacterial Protease

- Bacterial protease production- strains of *Bacillus subtilis*, and the fermentation conditions are similar to those for amylase production by this organism.
- However, the *Bacillus subtilis* strains are specially selected for high protease activity and not for amylase activity.
- A high carbohydrate content medium is utilized to stimulate protease activity and depress amylase production, although the final product does contain some amylase activity.
- The fermentation is incubated 3 to 5 days at 37°C in pans containing a shallow layer of fermentation medium, and the harvest procedure is similar to that for bacterial amylase.
- *Bacillus licheniformis* - alkaline serine proteases by submerged culture method.

# Industrial Applications

- Detergent industry
- Leather industry
- Food industry
- Dairy industry
- Baking and brewing industry
- Soy sauce production
- Meat tenderization
- Synthesis of aspartame
- Pharmaceutical industry
- Therapeutics
- Photography industry
- Management of industrial wastes
- Degumming of silk