Module-36: L-Lysine Fermentation

Introduction

- There are 20 naturally occurring amino acids, which are required for the synthesis of variety of proteins besides other biological functions.
- However, all these 20 amino acids need not to be taken in the diet.
- Based on the nutritional requirements amino acids are grouped into two classes: essential and non-essential.
- The amino acids which cannot be synthesized by the body and therefore, need to be supplied through the diet are called essential amino acids.
- Opposite to that the amino acids which can be synthesized by the body and therefore, need not to be supplied through the diet are called non-essential amino acids.
- They are required for proper growth and maintenance of the individual.
- L-Lysine is commercially important amino acid as it is one of essential amino acids.
- Generally L-Lysine is used in the animal feed industry as feedstuff additive.
- It is not used solely but mixed with several common livestock like cereals which do not have enough amount of L-Lysine.
- Thus, protein quality of certain foods (e.g. wheat based foods) is improved resulting in an improved growth and tissue synthesis.
- It is mainly used for mono gastric animals like humans, rats, pigs, dogs, cats, horses and rabbits.
- It improves the feed quality by increasing absorption of other amino acids.
- There are various chemical & biochemical methods available for L-Lysine production.
- One can choose the method; whichever is more economic.

History

- In 1889, first it was isolated from casein.
- After that for half a century L-lysine has been mass-produced by bacterial fermentation of carbohydrates.
- Lysine was commercially introduced as a feed additive around 1960.
- Recent method of lysine production is based on fermentation of carbohydrates like, beet and cane sugar or starch sugars.
- In 1978 first fermented L-lysine was produced by Japanese Company “Kyowa Hakko Kogyo”.
- Three amino acids which are produced at large scale includes,
  - L-lysine
  - L-glutamic acid
  - DL-methionine.
• So, lysine is one amongst them which covering more than 90% of total world amino acid production.

**Fermentation Microorganisms**

- Gram+ve Corenebacterial strains like *Corynebacterium glutamicum*, *Brevibacterium flavum* etc. are used for industrial production.
- Kinoshita *et al* (1958) first reported the fermentative production of L-lysine using homoserine auxotrophs of *Corynebacterium glutamicum*.
- This auxotroph requires L-homoserine or a mixture of L-threonine and L-methionine for growth, and produces greater than 20 g per liter of L-lysine in the fermentation broth.
- In addition, the L-lysine is not destroyed by the organism during the fermentation, because it also lacks the ability to produce l-lysine decarboxylase.
- The biotin requirement of this organism was not lost as a result of the mutation and, therefore, both biotin and L-homoserine must be initially supplied in the medium.
Fermentation Medium

- Apart from physical parameters like pH, Agitation and aeration rate and temperature; media composition is very important factor.
- The *seed culture* to be prepared includes:
  - Glucose: 20g
  - Peptone: 10g
  - Meat extract: 5g
  - Sodium chloride: 2.5g in 1 liter of tap water
- Seed culture obtained is reinoculated for 2nd *seed culture* in media containing:
  - Molasses: 200g
  - Soy protein hydrolysate 18g in 1L tap water
- After obtaining first and second media *production media* is used which includes:
  - Corn steep liquor
  - Ammonium sulphate
  - Glycerol
  - CaCO₃
  - Incubation for 72 hours at 28 degrees

Raw Materials

- Various sources of carbohydrates can be used as raw material but now selection of raw material is based on geographic location.
- For example, Sugar from beet and cane is used as raw material in regions with intensive sugar manufacturing.
- As we know cultivation of Starchy crops such as cane and beet is economic as well as feasible.
- Their use as raw material becomes feasible in various countries including the United States.
- In the United States cane sugar is frequently used for industrial applications.
- Besides sugar, corn has recently become part of a biofuel economy.
- A new trend in this field is the use of cassava, which is the main crop of tropical area.
- Because of this Lysine production is progressively migrating to tropical or subtropical areas where cheap sources of carbohydrates are available.

Effect of Oxygen

- As L-lysine fermentation is an aerobic fermentation process it demands large amounts of oxygen.
- Air necessity is satisfied by the air saturation in bioreactor.
- To maintain aerobic conditions, oxygen containing gaseous mixtures (e.g. atmospheric air or pure oxygen) is aseptically added to the culture.
Effect of pH
- The pH is an essential parameter which strongly influences microbial fermentations.
- L-lysine fermentation requires a pH ranging from 5 to 9.

Effect of Temperature
- As like O₂ requirement & pH, temperature plays an important role in L-lysine fermentation.
- Optimum temperature requirement for L-lysine production is between 24°C to 37°C for 2 to 7 days.

Antifoaming
- Antifoams are generally added to control foaming occurring during fermentation.
- Antifoams which are used in L-lysine fermentation include fatty acid polyglycol esters or silicone and polypropylene.

L-lysine Fermentation Technology
- Various fermentations are used for commercial production of amino acids like,
  1. Batch or fed-batch fermentation
  2. Repeated batch or fed-batch fermentation &
  3. The continuous fermentation or chemostat.

Downstream Processes of L-lysine
- Product separation and purification is a very important factor enormously affecting fermentation process effectiveness and production costs.
- L-lysine is recovered from fermentation broth in various ways.
- L-lysine of resultant culture broth can be recovered by known conventional methods such as using ion exchange resins, or by directly crystallization of L-lysine from culture broths.
- For many years, L-Lysine- HCl solid has been produced following various steps such as separation, purification, crystallization and drying.
- After cell separation by cell filtration or centrifugation, L-Lysine may be recovered from fermentation broth by an ion exchange step and thereafter concentrated by evaporation and spray drying.
L-lysine Fermentation

- Cell line (strain) development
  - Classic Mutagenesis
  - Recombinant DNA technology

- Fermentation Technology Dev
  - Medium
  - DO, T, pH, etc

- Purification
  - Metabolic engineering
  - Cell Separation
  - Ion Exchange Chromatography
  - Drying
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