INDUSTRIAL PRODUCTION OF AMINO ACIDS BY FERMENTATION TECHNOLOGY

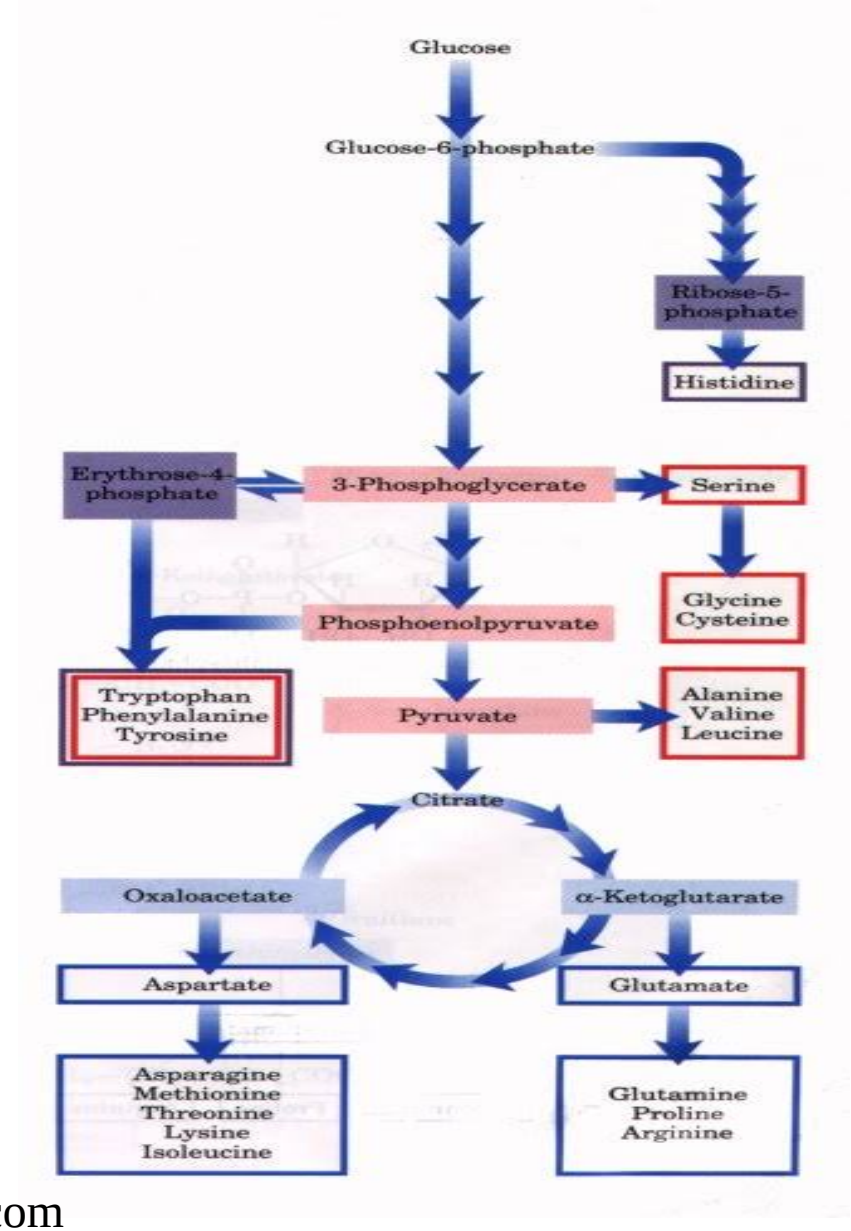
Presented by:
Yogita Salgar
Akshada Gaikwad
Jui Panse
MSC.II Biotechnology
Modern College,Shivajinagar

INTRODUCTION:

- Amino acids plays important role in biology of life, biochemistry and industrial chemistry.
- Amino acids are important as nutrients, flavoring, starting materials, cosmetics and other chemical production.
- There are 20 a.a. classified as,

1. Essential Amino Acids
2. Non-essential Amino Acids

BIOSYNTHESIS OF AMINO ACIDS

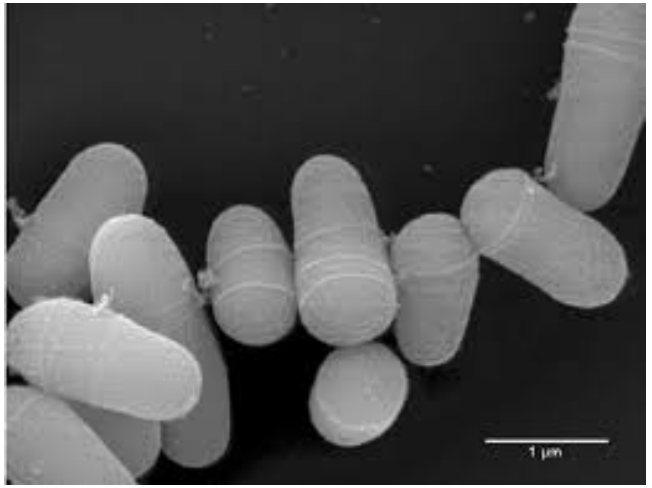


- The production of amino acids through fermentation process was initiated by Kinoshita and his co-workers in 1956.
- Certain microorganisms are capable of producing certain a.a. such as glutamic acid, lysine,etc.
- Artificially derived auxotrophic or regulatory mutants which are lack of formation of regulatory end product are used for production of a.a.
- The intermediates of metabolic pathways get accumulate in medium.

MICROORGANISM :

Corynebacterium glutamicum-

- Gram positive bacterium
- Soil bacterium
- Non – motile
- Rod shape
- Non – sporing
- Non pathogenic bacterium



- Production of amino acids using various auxotrophic and analogue resistant mutants:

Table 14.1. Productivities of amino acids using various auxotrophic and analogue resistant mutants.

Amino Acid	*Micro-organism*	*Mutation specificity*	*Concentration of carbo-hydrate, %*	*Yield, (g./Litre)*
L-lysine	*C. glutamicum*	*Amino acid requirement* homoserine	15	45
L-threonine	*E. coli*	lysine	15	24
L-leucine	*C. glutamicum*	phenylalanine & histidine	12	16
L-homoserine	*C. glutamicum*	threonine	10	15
L-valine	*C. glutamicum*	isoleucine	7·5	11
L-ornithine	*C. glutamicum*	arginine	10	36
L-citrulline	*C. glutamicum*	arginine	10	10·7
L-proline	*C. glutamicum*	base	15	31
L-isoleucine	*Serratia marcescens*	*Analogue resistance*[b] isoleucinehydroxamate & a-aminobutylic acid	12	12
L-arginine	*Bacillus subtilis*	argininehydroxamate & 6-azauracil	8	28
L-histidine	*C. glutamicum*	TRA, PUA & PYA	15	15
L-tyrosine	*C. glutamicum*	PheA and TyrA	10	17·6
L-Phenylalanine	*C. glutamicum*	PheA	10	9·5
L-tryptophan	*C. glutamicum*	PheA, TyrA & TryA	10	12

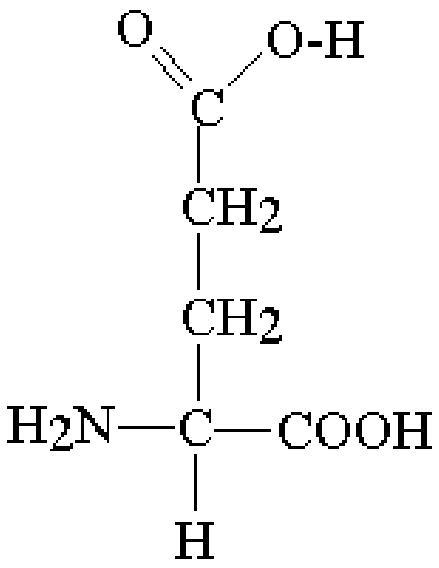
- Large scale chemical and microbial production processes have been commercialized for number of essential amino acids.
- Amino acids can be produced by

1. Isolation form natural materials
2. Fermentation

L-GLUTAMIC ACID

- L-glutamic acid was the first amino acid which produced by fermentation using micro-organisms by Kinoshita his co-workers.
- It is non-essential amino acid in humans,

means body can synthesize it.



glutamic acid

FERMENTATIVE PRODUCTION OF L-GLUTAMIC ACID:

- L-glutamic acid may be produced in many ways:

1. By hydrolysis of wheat gluten, soya bean cake or other proteineceous materials
2. By the cleavage of the pyrrolidone carboxylic acid found in Steffen's molasses
3. By one–stage fermentation process involving single micro-organism
4. By two stage fermentation process where α-ketoglutaric acid is produced by one microbe and then it is converted to L-glutamic acid by another microbe.

PARAMETERS REQUIRED IN BIOREACTOR

Sr.no	Requirements	
1.	Production strain	Corynebacterium glutamicum
2.	Production medium	Glucose and starch hydrolysates
3.	Aeration and agitation	Aerobic
4.	Temperature	30 degree C
5.	Fermentation time	40hrs.
6.	Yield	38 g/lit (38%)
7.	ph	alkaline

- Biotin (1 to 5µg/lit)- growth of microorganism
- Urea or ammonia- source of nitrogen

FEMENTATIVE PRODUCTION L-GLUTAMIC ACID USING VARIOUS CARBON SOURCES:

Table 14.2. Fermentative production of L-glutamic acid using various carbon sources.

Carbon source	Micro-organism	Yield	
		g./Litre	per cent
Glucose	(i) **Corynebacterium glutamicum*	38	38
	(ii) **Brevibacterium flavum*	50	50
Acetic acid	(i) *Brevibacterium flavum*	98	48
	(ii) *Brevibacterium thiogentalis*	51	51
Ethanol	*Brevibacterium* sp.	59	66
Propylene-glycol	*Bacillus megaterium*	27	27
Benzoic acid	*Brevibacterium* sp.	80	80
Xylose	*Brevibacterium pentosaaminoacidicum*	5	10

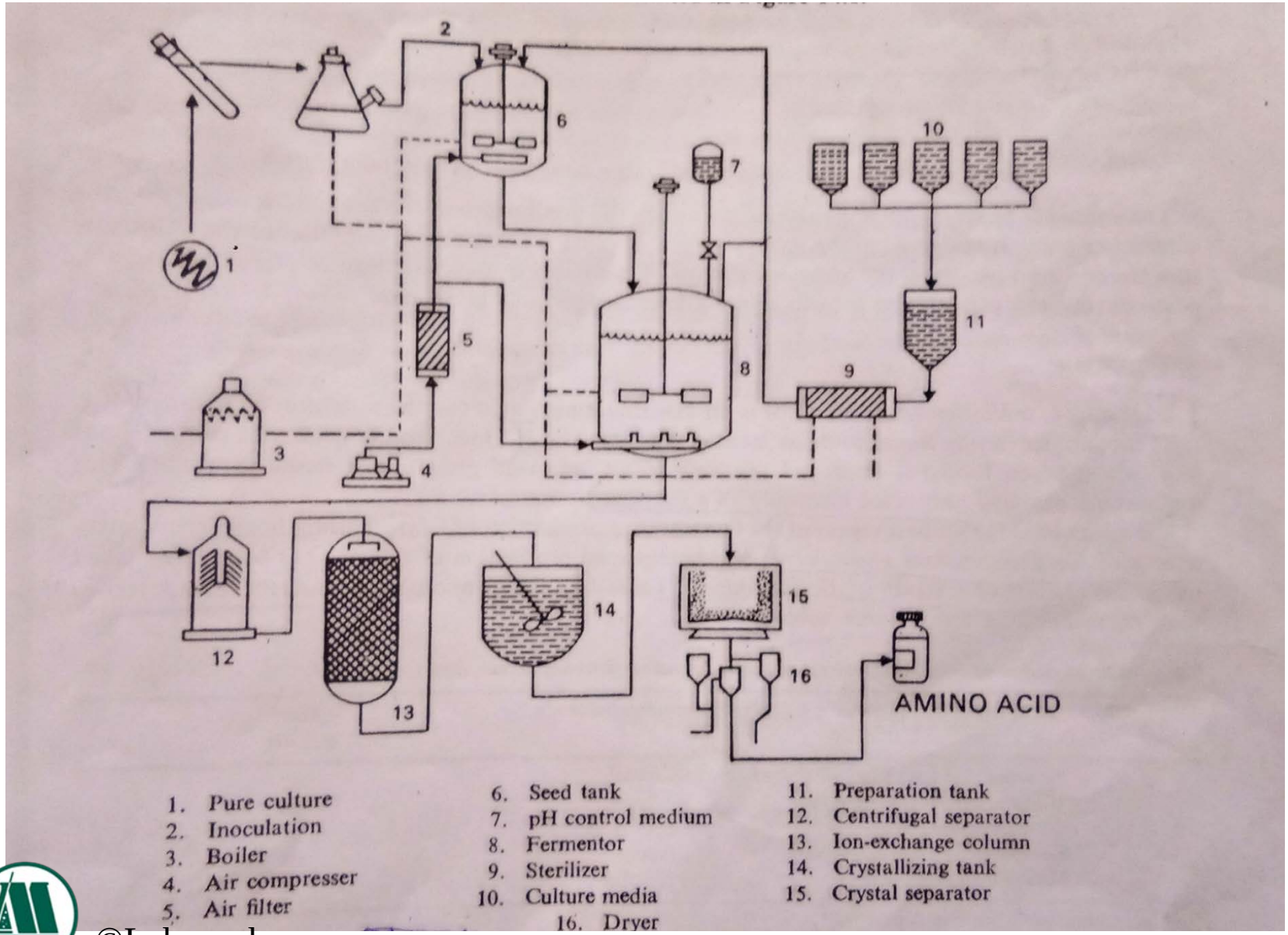
*indicates industrialization in Japan.

INDUSTRIAL PRODUCTION OF GLUTAMIC ACID:

- The manufacturing process of glutamic acid by fermentation comprise:

1. Fermentation
2. Crude isolation
3. purification

DOWNSTREAM PROCESSING OF AMINO ACIDS



DOWNSTREAM PROCESSING:

- After accumulation of glutamate into medium excretion of glutamate occurs in absence of biotin.
- If molasses is used addition of penicillin or detergents during exponential phase allows excretion of glutamate.
- Separation of bacterial cells from medium carried out by:

1. Centrifugation
2. Filtration

- The extraction method of amino acid from filtrate, depends on the level of purity desired in the production.
- Two methods generally used for purification of amino acid:

1. Ion exchange
2. Crystallization

ION EXCHANGE:

- Widely use for extraction and purification of a.a. from fermentation broth.
- Adsorption of a.a. strongly get affected by pH of solution.
- Anion exchange resins binds to glutamic acid which carries negative charge.
- Extraction of bound amino acid is done by introducing a solution containing counter ion of the resin.

CRYSTALLIZATION:

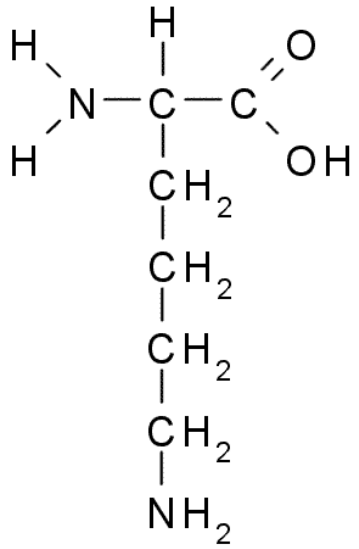
- The glutamic acid eluted from ion-exchange column is mixed with mother liquor from the product filtration step and concentrated by evaporation.
- Glutamic acid crystal added to sodium hydroxide solution and converted into monosodium glutamate(MSG).
- MSG is more soluble in water, less likely absorb moisture.
- Clean MSG solution is concentrated by heating and monosodium glutamate crystal is formed.
- Crystal produce are dried with hot air in close system.
- Then, the crystal is packed in the packaging and ready to be sold.

APPLICATION:

- Amino acid are used as animal feed additives (lysine, methionine, threonine)
- Flavor enhancers (monosodium glutamic, serine, aspartic acid) in food production and beverage
- As specialty nutrients in medical field glutamate is medically used as a neurotransmitter.
- In cosmetics for hair restorer in treatment of hair loss and wrinkle treatment to preventing aging.
- Other industries as intermediate in manufacturing of various organic chemicals.

LYSINE (2,6-DIAMINOHEXANOIC ACID)

- Essential amino acid for the nutrition of humans and other animals
- It is used for the supplementing the cereal proteins lacking this amino acid
- It is used medically as a nutrient in form of supplements and medicines
- Lysine is present in two forms, L - form and D – form
- Its major commercial form is L-Lysine-



HISTORY…

- In 1889, first it was isolated from casein.
- In 1956, Lysine was commercially introduced as a feed.
- In 1978, first fermented L-Lysine was produced by Japanese company “kyowa Hokko Kogyo”
- Recent method of lysine production is based on fermentation of carbohydrates.

LYSINE BIOSYNTHESIS

- Synthesis of lysine
  - 80% by fermentation,
  - 20% by chemical synthesis.
- Production of lysine naturally, takes places by two biosynthetic pathways-

1. The DAP (Diaminopimelic acid) pathway found in bacteria(e.g. *Corynebacterium glutamicum)*, algae, higher plants
2. The AAA (α amino adipate) pathway found in fungi and euglenoids

Acetate + α-ketoglutarate → AAA → Lysine

FERMENTATIVE PRODUCTION OF L-LYSINE:

- Fermentation process:
  - submerged fermentation
  - Aerobic fermentation
- Mode of operation:
  - Batch process
  - Fed – batch process
- Fermenter type:
  - Stirred tank reactor
  - Air - lift bioreactor

FERMENTATION MEDIA:

- Carbon source – cane molasses
- Nitrogen source – corn steep liquor, soybean meal
- Minerals and salts – KH_2PO_4/ K_2HPO_4, $CaCo_3$
- Trace elements - corn steep liquor
- Anti foaming agents – PEG-2000, silicon based oils

PROCESS PARAMETER:

- Optimum pH- 7.2
- Optimum temperature – 35-37^{o}C
- Time – 100 hrs production cycle

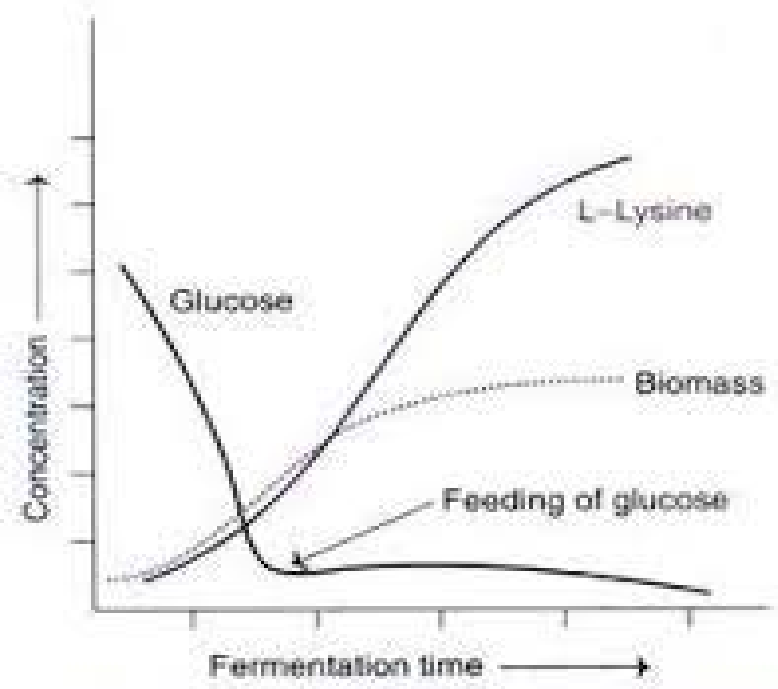


Fig. 25.5 : Production of L-lysine in relation to substrate (glucose) and biomass concentration.

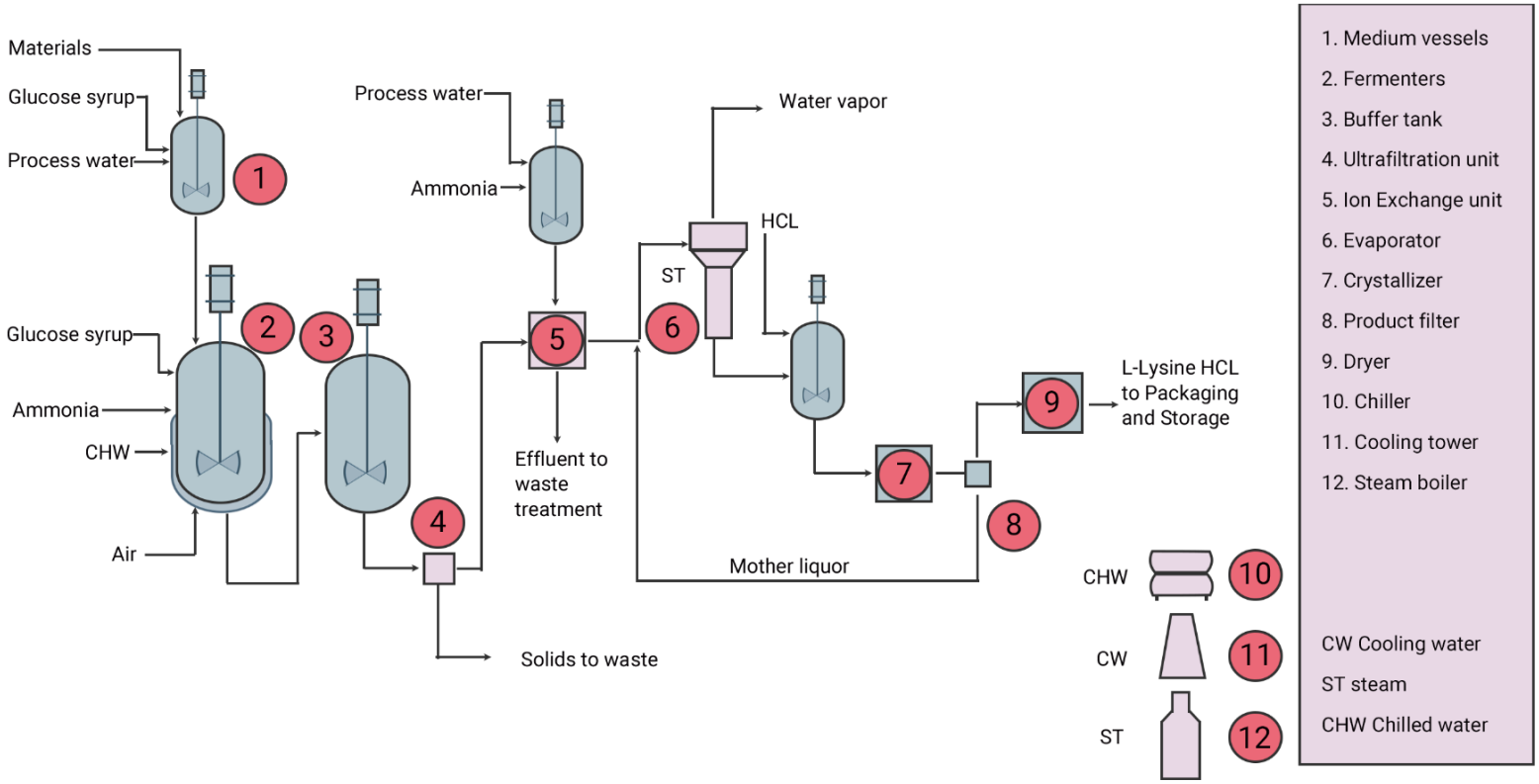
FERMENTATION PROCESS:

- Media preparation for growth of culture and inoculum development

The typical medium composition includes:

Glucose 100 g/L
Urea 5g/L
Biotin 0.01g/L
Leucine 0.4g/L
$MgSO_4$ 2.85G/L
KH_2PO_4/K_2HPO_4 0.5g/L
$MnSO_4$ 0.016g/L
$CaCO_3$ 20g/L
$(NH_4)_2SO_4$ 46g/L
Distilled water 1000L

DOWNSTREAM PROCESSING OF LYSINE



PRODUCT RECOVERY:

- The fermentation broth is sent to an ultrafiltration system for the removal of cell debris and other suspended solids.
- Subsequently, the liquor from ultra filtration is fed to ion-exchange columns, where L-Lysine is selectively adsorbed.
- The adsorbed L-Lysine is eluted from the ion-exchange resins by washing with an aqueous ammonia solution.

PRODUCT CONCENTRATION, DRYING AND PACKAGING:

- The L-Lysine eluted from ion-exchange column is mixed with mother liquor from the product filtration step and concentrated by evaporation.
- The concentrated lysine solution is acidified with hydrochloric acid and free lysine is converted to lysine HCL
- The L-lysine HCl solution is then sent to the crystallizer, and lysine salt is filtered. The mother liquor is recycled to the evaporator and the wet cake is conveyed to dryers.
- Final dry L-lysine HCl (98.5 wt.%) is obtained and sent to a packaging line before being stored in bags.

APPLICATION OF LYSINE:

- Food and dietary supplement(66%)
- Medicine, cosmetic, chemicals(4%)
- Feed essential amino acid for most mammals(30%)
- In industries - as intermediate to produce other organic chemicals
- In beverages – nutritional sports drink
- In pharmaceutical L-lysine is widely used as nervous system drugs and nutritional therapy.

REFRENCES

- Industrial Microbiology by H. A. Patel
- Fermentation Technology by Stanbury and whittaker 2 nd edition.
- www.downstreamprocessingofaminoacids.co.in

THANK YOU !! □□