Lab4

Industrial microbiology

Production of glutamic acid by fermentation

Glutamic acid: is an important flavouring agent, which has the largest commercial demand among various amino acid, which is consider as primary products that was produced from microorganisms at exponential stage. The maximum glutamate yield was noticed after maximum cell, but in order to be useful ,glutamate producers must do two things well: growth

1- They must produce glutamate in excess of their normal metabolic needs.

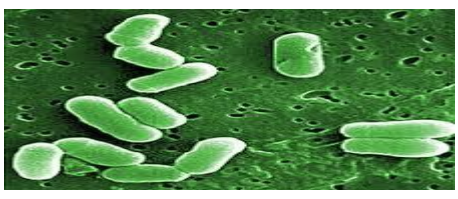
2- They must excrete it into culture broth.

The history of glutamic acid production:

The first industrial production process in 1909, L-glutamic acid was separated from vegetable proteins by **extraction method**, when treated vegetable proteins like wheat gluten, or soybean with hydrochloric acid. L-glutamic acid hydrochloride was then isolated from this material and purified as MSG (Mono Sodium Glutamate). This process continued for 50 years. But because of increasing demand for L-glutamic acid as flavouring enhancer was combined with the discovery of L-glutamic acid producing bacteria named *C. glutamicum* by Kinoshita in 1957, which was produced a large amount of glutamic acid in fermentation media ,this strain was used industrially because of it's high excretion of glutamic acid by **fermentation method**. Now a day's 1.5 million tons L-glutamic acid produced per year using coryneform bacteria.

Uses: glutamic acid can be used as a flavour enhancer and also as a precursor of drug, cosmetics and further pharmaceutical compounds.

(5) Cephalosporium sp



(6) Corynebacterium

(7) ghutamicum

Lab4

Production of glutamic acid by fermentation

- Amino acid
- Flavoring agent with largest commercial demand.
- Primary product of fermentation.
- Produced at exponential phase of microorganism growth.
- Maximum glutamate yield noticed after maximum cell growth.
- Microorganisms can produce useful glutamate under two important conditions:
  1- They must produce glutamate in excess of their normal metabolic needs.
  2- They must excrete the glutamate into culture broth
- L-Glutamic acid was firstly extracted (for 50 years) from vegetable proteins (wheat gluten, soybean) by treating vegetables with HCl to produce L-Glutamic acid hydrochloride. L-Glutamic acid hydrochloride was then isolated and purified as (MSG).
- Producing bacteria of L-Glutamic acid (Corynebacterium glutamicum) was used (for its high excretion of glutamic acid =1.5 ton/year) by Kinoshita in 1957 in fermentation for producing large amount of glutamic acid.
- Glutamic acid used as a flavor enhancer and a precursor in pharmaceutical industry (medicines + cosmetics)

The important microorganisms used for glutamic acid production by direct fermentation were classified into 4 genera:

1- Corynebacterium
2- Brevibacterium
3- Microbacterium
4- Arthrobacter

- morphologically similar
- G^+
- nonspore forming
- nonmotile
- increased activity of glutamate dehydrogenase

Lab4

Other producer of glutamic acid from carbohydrate:

- ***Escherichia coli.***
- ***Bacillus circulans.***
- ***Bacillus cereus.***
- ***Sarcina lutea.***

Biosynthesis of glutamic acid

The precursor of glutamic acid is a-ketoglutarate

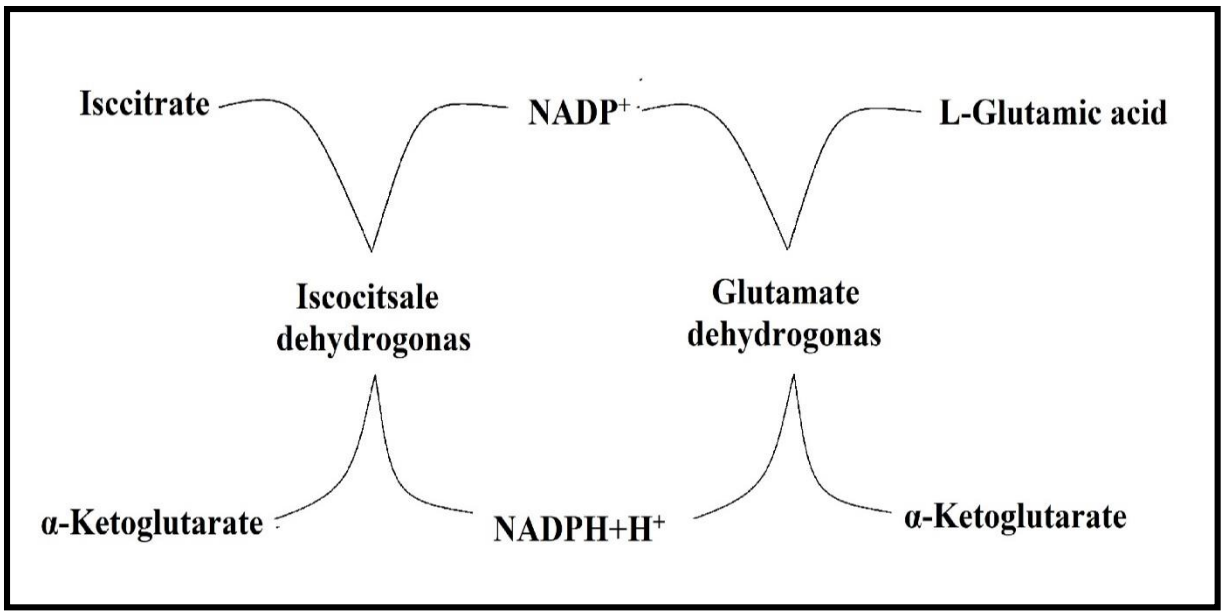
Glutamic acid is produced through the following steps:

1- α-ketoglutarate is formed in Tricarboxylic acid cycle (TCA) by citrate and isocitrate.

2- α -ketoglutarate is then converted into L-Glutamic acid through reductive amination with free (NH_4^+) ions. This step is catalyzed by NADP-dependent glutamic dehydrogenase (NADP-GDH).

3- NADPH2 is then formed through an **oxidative decarboxylation** of isocitrate to α-ketoglutarate by the enzyme isocitrate dehydrogenase.

- In normal TCA cycle a-ketoglutarate is converted to succinyl-Co enzyme A by the α -ketoglutarate dehydrogenase complex (ODHC).
- The strains used for commercial production of glutamic acid lack or have very low activity of α -ketoglutarate dehydrogenase complex (ODHC).
- The interruption in the TCA cycle is recovered by other anaplerotic reactions to synthesize oxaloacetate, which combines with acetyl-CoA to produce isocitrate, which is used for the synthesis of glutamic acid.



Biosynthesis of glutamic acid

- In normal growth conditions, glutamate can't excrete outside the cells of glutamic acid bacteria due to their rigid cell wall.
- Increasing the permeability of bacterial cell for glutamate can be performed by:

1- Preventing the formation of normal phospholipid biosynthesis of cell wall or cell membrane by using biotin deficient media.

2- Using penicillin in case of biotin rich media (beet molasses)

3- Altering the composition of cell wall or cell membrane by using detergents (tween 60, tween 40)

Industrial production of glutamic acid

- Glutamic acid bacteria convert 50-60% of the added carbon source to L-glutamic acid under the optimal conditions.
- The carbon source:

Corn starch	Methanol
Potato starch	Ethanol
cassava	Acetaldehyde
Waste molasses	alkane

Lab4

Acetate palm waste

The fermentation is carried out aerobically at:

- 30-37 Codepending on microorganism used.
- Oxygen supply (under O_2 deficiency leads to lactate and succinate excretion).
- PH at 7-8 which maintained by adding NH_4.

- Accumulation of L-glutamic acid starts at the mid-way of fermentation process.
- Fermentation process lasts 30-35 hours.
- L-glutamic acid is recovered from the fermentation broth by:

1- Separating bacterial cells from culture medium, then the glutamic acid will be crystalized by lowering the broth pH to 3.2 using HCl. Then, the crystals will be washed.

2- Mono Sodium glutamate (MSG) is prepared by adding NaOH to the crystallized L-glutamic acid.

Procedure:

1- Preparation of inoculum:

Inoculum will be prepared by adding a loop-full of corynebacterium spp. to 100 ml of seed culture medium.

Seed culture medium consists of (g/L)

- Glucose (20 g)
- $MgSO_4.7H_2O$ (0.25 g)
- KH_2PO_4 (1 g)
- NaCl (2.5 g) $MnSO_4.H_2O$ (0.1 g)
- Yeast extract (10 g)

pH = 7
incubation at 30-35 C°
shaking at 180 rpm for 48 hours

2- Microorganism cultivation in fermenter:

- **Transfer 4% inoculum into 100 ml of sterile fermentation media consists of: (g/L)**

- Urea (3 g)
- $MnSO_4$. H_20 (0.01 g)
- $FeSO_4.7H_20$ (0.01 g)
- $MgSO_4.7H_20$ (2 g)
- KH_2PO_4 (0.08 g)
- K_2HPO_4 (0.08 g)
- Biotin (10g)
- Date syrup sterilized at 63 C for 30 min. (10%)

incubation at 30-37 C°
shaking at 180 rpm for 96 h

3- Determination of glutamic acid:

- Centrifuge the fermentation broth at 8000 rpm for 20 min. to remove microbial cells.
- Then the clear supernatant is carefully taken for glutamic analysis.

4- Quantitative detection of L-glutamic acid:

Ninhydrin method is used to detect the presence and quantitate the amount of glutamic acid.

Principle:

- Ninhydrin oxidizes the amino acid to aldehyde releasing CO_2 and NH_3.
- The reduced ninhydrin forms condense with NH_3. appear as a complex coloured complex.

Amino acid+ ninhydrin $\longrightarrow$ CO_2+Aldehyde +final complex (blue) +$3H_2O$

Procedure:

- Mix 1 ml of supernatant with 1 ml of ninhydrin reagent.
- Heat the mixture for 5 min using water bath.
- Cool the mixture under running tap water.
- Read the absorbance of the resulted colour solution at 570 nm using spectrophotometer.