L-Glutamic acid Production

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History

- The history of the first amino acid production dates back to 1908 when Dr. K. Ikeda, a chemist in Japan, isolated glutamic acid from kelp, a marine alga, after acid hydrolysis and fractionation.
- He also discovered that glutamic acid, after neutralization with castic soda, developed an entirely new, delicious taste.
- This was the birth of the use of monosodium glutamate (MSG) as a flavour-enhancing compound.
- The breakthrough in the production of MSG was the isolation of a specific soil-inhabiting gram-positive bacterium, *Corynebacterium glutamicum*, by Dr. S. Ukada and Dr. S. Kinoshita in 1957.
- The successful commercialization of monosodium glutamate (MSG) with this bacterium provided a big boost for amino acid production and later with other bacteria like *E. coli* as well.

Biosynthetic Pathway

- The glucose is broken down into C_3 and C_2 fragments by glutamic acid producing microorganisms through the Embden Meyerhof-Parnas (EMP) pathway and the pentose-phosphate pathway and the fragments are channeled into the tricarboxylic acid (TCA) cycle.
- The reactions of EMP pathway are more common under conditions of glutamic acid production.
- The key precursor of glutamic acid is α – ketoglutarate, which is formed in the TCA cycle via citrate, isocitrate and α-ketoglutaric acid, which is then converted into L-glutamic acid through reductive amination with free NH_4^+ ions.
- The last step is catalysed by the NADP dependent glutamate dehydrogenase.
- The $NADPH_2$ required at this stage of the reaction is furnished through the preceeding oxidative decarboxylation of isocitrate dehydrogenase.
- The $NADPH_2$ is then used by the reductive amination of α-ketoglutarate.

Effect of Permeability on Glutamic Acid Production

- Production and excretion of glutamic acid is dependent on cell permeability.
- **Increased permeability in glutamic acid producing bacteria can be accomplished by one of the following ways:**

  (a) Through biotin deficiency.

  (b) Through the addition of penicillin.

  (c) Through the addition of saturated fatty acids or fatty acid derivatives.

  (d) Through the oleic acid deficiency in oleic acid auxotrophs.

  (e) Through the glycerol deficiency in glycerol auxotrophs.

Conditions of Production

Carbon Source:

- Glucose and sucrose are frequently used.
- However, starch hydrolysates, fructose, maltose, ribose and xylose are also used less frequently.
- Moreover sucrose, sugarcane molasses, sugar beet molasses can also be used.
- Both the molasses contain high biotin content (0.4-1.2 mg kg^{-1} in cane molasses and 0.02-0.08 mg kg^{-1} in beet molasses).
- Penicillin or fatty acid derivatives (e.g. Tween-66) must be added to the fermentation medium, when these molasses are used in the medium preparation.
- For industrial production, generally cane molasses or starch hydrolysate are used.

Nitrogen Source:

- Ammonium sulphate, ammonium chloride, ammonium phosphate, aqueous ammonia, ammonia gas and urea have been used as nitrogen source.
- Although large amount of ammonium ions are necessary, a high concentration of it inhibits the growth of the microorganism as well as the yield of L-glutamic acid.
- Therefore, suitable amount of ammonia is added, as the fermentation progresses.
- These salts also help in the pH control.

... Conditions of Production

Growth Factors:

- The important growth factor is biotin.
- Its optimal concentration depends upon the carbon source used.
- In media with 10% glucose, its requirement is 5 mg liter^{-1}.
- In media with lower glucose concentration, it is considerably lower.
- Some strains require L-cystine as an additional growth factor.

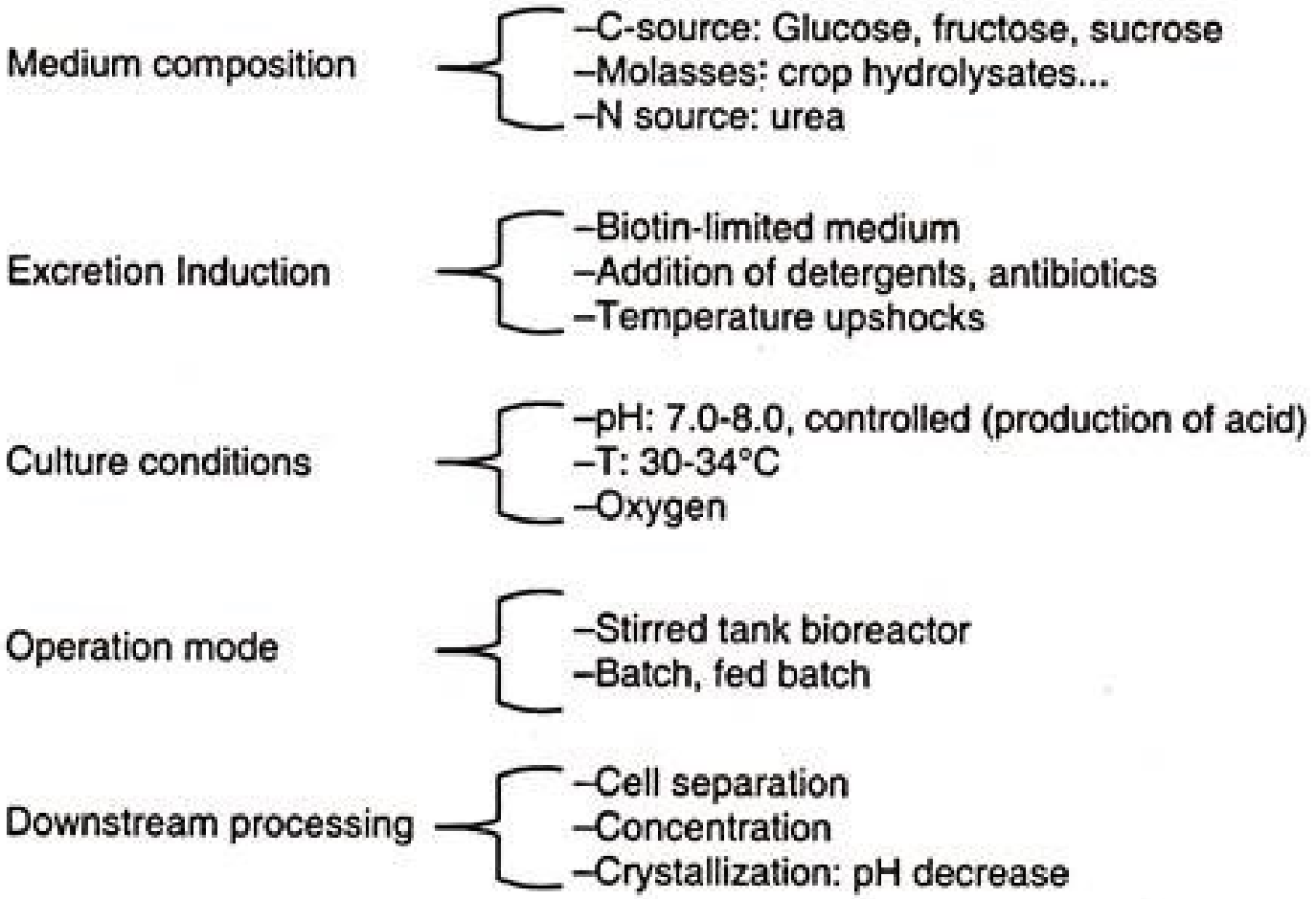
Oxygen Supply:

- The oxygen concentration should neither be too low nor too high.
- Excretion of lactate and succinate occurs under oxygen deficiency.
- Whereas excess oxygen under ammonium ions deficiency causes growth inhibition and production of α-ketoglutarate.
- In both the cases, glutamic acid yields are low.

pH:

- Optimum pH for growth and glutamic acid production is 7.0-8.0 and it is controlled by the addition of ammonium salts.

Commercial Production of Glutamic Acid



Important features of L-glutamic acid production by fermentation.

...Commercial Production of Glutamic Acid

- **Inoculum preparation:** A suitable strain of *C. glutamicum* is selected and is inoculated into the sterilized medium.
- The culture is incubated upto 16 hours at 35°C.
- After sufficient growth occurs, approximately 6% by volume of inoculum is added to the production fermenter.
- **Fermentation:** The fermentation is carried out, approximately, for 40-48 hours at 30°C temperature.
- The pH is adjusted to 7.0-8.0.
- The urea is added intermittently during the fermentation.
- Approximately 50% of the supplied carbohydrate is converted into L-glutamic acid.
- The broth contains glutamic acid in the form of its ammonium salt.

Recovery

- In a typical downstream process, the bacterial cells are separated and the broth is passed through a basic anion exchange resin.
- Glutamic acid anions get bound to the resin and ammonia is released.
- This ammonia can be recovered via distillation and reused in the fermentation.
- Elution is performed with NaOH to directly form monosodium glutamate (MSG) in the solution and to regenerate the basic anion exchanger.
- From the elute, MSG may be crystallized directly followed by further conditioning steps like decolourization and serving to yield a food-grade quality of MSG.

Flow Diagram of L-Glutamic acid Production