METHODS FOR PRODUCTION OF AMINO ACIDS

The industrial production of amino acids is carried out by one or more of the following three processes:

1. Extraction:

Amino acids are the building blocks in protein structure. The proteins can be subjected to hydrolysis, and the requisite amino acids can be isolated e.g. cysteine, tyrosine, leucine.

2. Chemical synthesis:

Chemical synthesis results in a mixture of D- and L-amino acids. Most of the amino acids required for commercial applications are of L-category. However, for the synthesis of glycine (optically inactive) and some other amino acids which can be used in L- or D-form (D, L-alanine, D, L-methionine) for certain purposes, chemical methods are employed.

3. Microbiological production:

For the large- scale production of amino acids, microbiological methods are employed. There are three different approaches.

(a) Direct fermentation methods:

Amino acids can be produced by microorganisms by utilizing several carbon sources e.g. glucose, fructose, alkanes, ethanol, glycerol, propionate. Certain industrial byproducts like molasses and starch hydrolysate can also be used. Methanol, being a cheap carbon source, is tried for amino acid production, but with limited success.

(b) Conversion of metabolic intermediates into amino acids:

In this approach, the microorganisms are used to carry out selected reactions for amino acid production e.g. conversion of glycine to serine.

(c) Direct use of microbial enzymes or immobilized cells:

Sometimes resting cells, immobilized cells, crude cell extracts or enzyme-membrane reactors can be used for the production of amino acids. Some examples are given below. Amino acid dehydrogenases from certain bacteria (e.g. Bacillus megaterium) can be used for the amination of α-keto acids to produce L-amino acids e.g. alanine (from pyruvate), leucine (from α-ketoisocaproic acid) and phenylalanine (from phenyl pyruvate). Immobilized cells or enzyme- membrane reactors can be used. Enzymes or immobilised cells are also employed for the production of several other amino acids e.g. tryptophan, tyrosine, lysine, valine.

L-Glutamic Acid:

L-Glutamic acid was the first amino acid to be produced by microorganisms. The original bacterium, Corynebacterium glutamicum, that was first used for large scale manufacture of glutamic acid continues to be

successfully used even today. The other important organisms (although used to a lesser extent due to low yield) employed for glutamic acid production belong to genera Micro bacterium, Brevibacterium and Arthrobacter.

All these organisms have certain morphological and physiological characters comparable to C. glutamicum. Biochemically, glutamic acid- producing bacteria have a high activity of glutamate dehydrogenase and a low activity of α-ketoglutarate dehydrogenase. They also require the vitamin biotin.

Improved Production Strains:

Several improvements have been made, particularly in C. glutamicum, for improving the strains to produce and excrete more and more of glutamic acid. These include the strains that can tolerate high concentrations of biotin, and lysozyme-sensitive mutants with high yield.

Biosynthesis of L-glutamic Acid:

The pathway for the synthesis of glutamic acid with glucose as the carbon source is depicted in Fig. 26.1. Glucose is broken down to phosphoenol pyruvate and then to pyruvate. Pyruvate is converted to acetyl CoA. Phosphoenol pyruvate (by the enzyme phosphoenol pyruvate carboxylase) can be independently converted to oxaloacetate. Both these carboxylation reactions are quite critical, and require biotin as the cofactor.

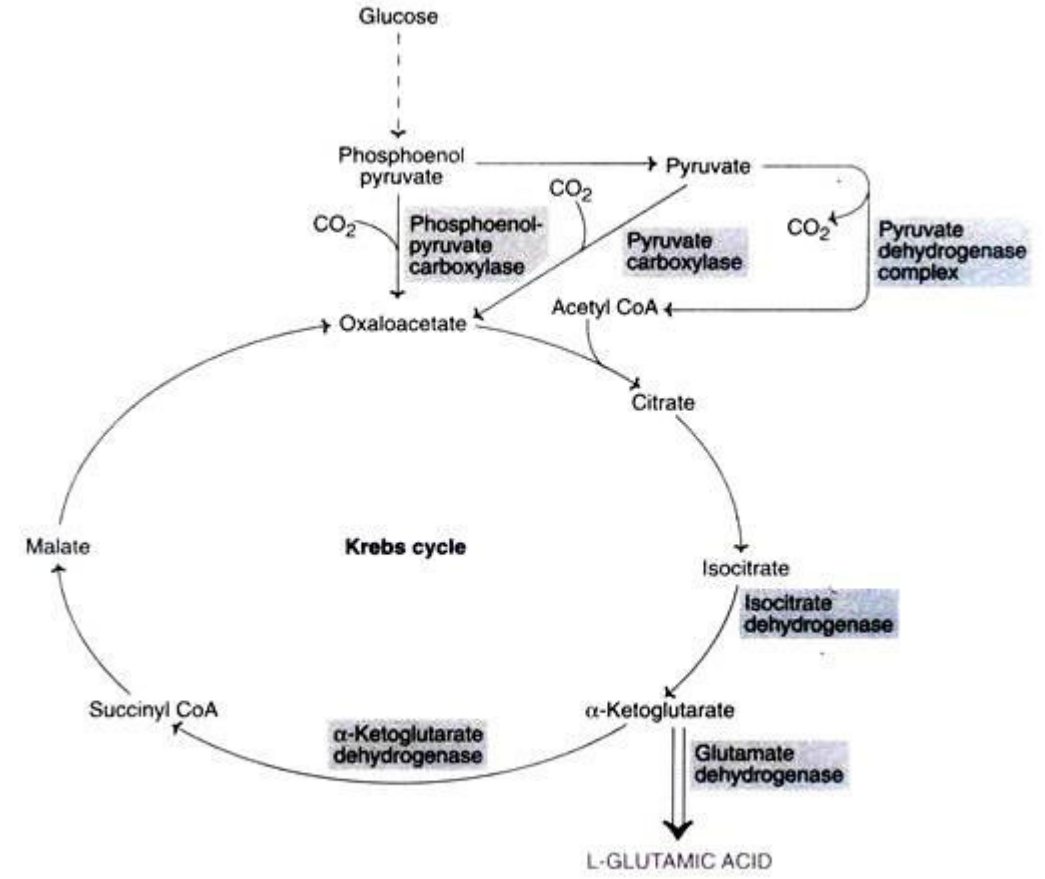

Fig. 26.1 : Biosynthesis of L-glutamic acid in Corynebacterium glutamicum.

The next series of reactions that follow are the familiar citric acid (Krebs) cycle reactions wherein the key metabolite namely α-ketoglutarate is produced. In the routine citric acid cycle, α – ketoglutarate is acted upon by the enzyme α- ketoglutarate dehydrogenase to form succinyl CoA.

For the production of glutamic acid, α-ketoglutarate is converted to L-glutamic acid by the enzyme glutamate dehydrogenase (GDH). This enzyme is a multimer, each subunit with a molecular weight of 49,000. The reducing equivalents, in the form of NADPH + H^+, are required by GDH. They are generated in the preceding reaction of Krebs cycle (catalysed by the enzyme isocitrate dehydrogenase) while converting isocitrate to α-ketoglutarate. The supply and utilization of NADPH + H^+ occurs in a cyclic fashion through the participation of the two enzymes, namely isocitrate dehydrogenase and glutamate dehydrogenase (Fig. 26.2).

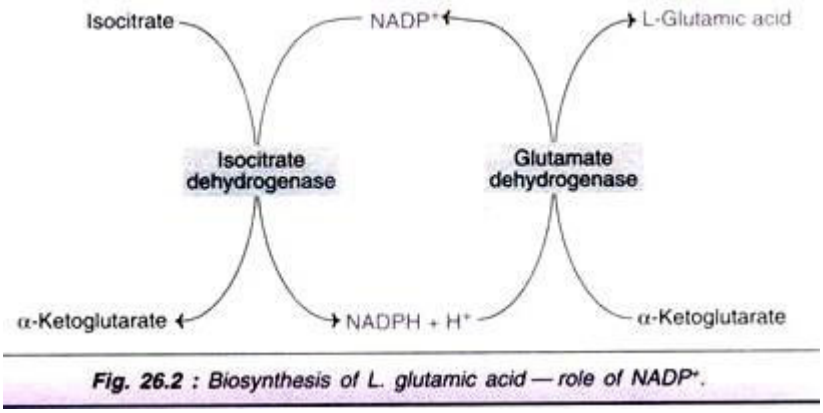

Fig. 26.2 : Biosynthesis of L. glutamic acid — role of NADP+.

Theoretically, one molecule of glutamic acid can be formed from one molecule of glucose. In practice, the conversion efficiency of glucose to glutamic acid was found to be around 70%.

Regulation of Glutamic Acid Biosynthesis:

The essential requirement for glutamic acid production is the high capability for the supply of the citric acid cycle metabolites. This is made possible by an efficient conversion of phosphoenol pyruvate as well as pyruvate to oxaloacetate .Thus, there are two enzymes (phosphoenol pyruvate carboxylase and pyruvate carboxylase) to efficiently produce oxaloacetate, while there is only one enzyme (pyruvate dehydrogenase) for the formation of acetyl CoA.

Certain microorganisms which have either phosphoenol pyruvate carboxylase (e.g., E. coli) or pyruvate carboxylase (e.g. B. subtilis) are not capable of producing glutamic acid to any significant extent. C. glutamicum has both the enzymes and therefore can replenish citric acid cycle intermediates (through oxaloacetate) while the synthesis of glutamic acid occurs.

Another key enzyme that can facilitate optimal production of glutamic acid is α-ketoglutarate dehydrogenase of citric acid cycle. Its activity has to be substantially low for good synthesis of glutamic acid, as is the case in C. glutamicum.

Further, exposing the cells to antibiotics (penicillin) and surfactants reduces the activity of α-ketoglutarate dehydrogenase while glutamate dehydrogenase activity remains unaltered. By this way, oxidation of α-ketoglutarate via citric acid cycle can be minimised, while the formation of glutamic acid is made maximum possible.

Release of Glutamic Acid:

Glutamic acid is synthesized intracellularly, and therefore its release or export is equally important. It now appears that there is a carrier-mediated energy-dependent active process involved for the export of glutamic acid.

There are several ways of increasing the membrane permeability for exporting glutamic acid:

i. Biotin limitation

ii. Addition of saturated fatty acids

iii. Addition of penicillin

iv. Use of oleic acid auxotroph's

v. Use of glycerol auxotroph's

vi. Addition of local anesthetics

vii. Addition of surfactants (Tween 40).

The effect of biotin deficiency in facilitating the release of intracellular glutamic acid has been worked out. Biotin is an essential cofactor (required by the enzyme acetyl CoA carboxylase) for the biosynthesis of fatty acids. Due to a limited supply or deficiency of biotin, fatty acid biosynthesis and consequently phospholipid synthesis is drastically reduced. As a result, membrane formation (protein- phospholipid complex) is defective which alters permeability for an increased export of intracellular glutamic acid.

It is found that there is an alteration in the membrane composition of phospholipids in oleic acid and glycerol auxotroph mutants. This facilitates release of intracellular glutamic acid. The knowledge on the membrane permeability of glutamic acid is successfully exploited for increased industrial production of glutamic acid.

Production of Glutamic Acid-Requirements and Influencing Factors:

The industrial production of glutamic acid is influenced by carbon sources, nitrogen sources, growth factors, pH and O_2 supply. The relevant aspects are briefly described.

Carbon sources:

Either refined (glucose, sucrose, fructose, maltose) or unrefined (sugar beet molasses, sugar cane molasses) carbon sources are used. In countries like Japan, acetate (inexpensive) is utilized. Other substrates like alkanes, ethanol and methanol are less frequently used.

Nitrogen sources:

The concentration of ammonia is very crucial for converting carbon source to glutamic acid. However, high concentration of ammonia inhibits the growth of the organisms. In the beginning of fermentation, ammonium salts and a low concentration of ammonia are added.

During the course of fermentation, ammonia in aqueous solution is continuously fed. In this way, pH can be controlled, besides continuous supply of nitrogen source. Sometimes, urea is also used as a nitrogen source, since glutamic acid-producing bacteria possess urease that can split urea and release ammonia.

Growth factors:

Biotin is an important growth factor and its concentration in the medium is influenced by the carbon source. For instance, a supply 5 µg of biotin per liter medium is recommended if the carbon source is 10% glucose; while for acetate as the carbon source, the biotin requirement is much lower (0.1-1.0 µg/l). Addition of L-cysteine in the medium is recommended for certain strains.

Supply of O_2:

O_2 supply should be adequately and continuously maintained. It is observed that a high O_2 concentration inhibits growth of the organisms while a low O_2 supply leads to the production of lactic acid and succinic acid. In both instances, glutamic acid formation is low.

Process of Production and Recovery:

Some important information on the production of glutamic acid by Brevibacterium divaricatum is given below.
Carbon source – Glucose (12%)
Nitrogen source – Ammonium acetate (0.5%)
pH – 7.8
Temperature – 38°C
Period for fermentation – 30-35 hours
Yield of glutamic acid – 100 g/l medium.

A schematic representation of glutamic acid production plant is shown in Fig. 26.3. As the fermentation is complete, the cells are separated, the culture broth is passed through anion exchanger. The glutamic acid bound to the resins is eluted in NaOH, while the ammonia released can be reused. With NaOH, glutamic acid forms monosodium glutamate (MSG) which can be purified by passing through anion exchanger. MSG can be subjected to evaporation and crystallization.

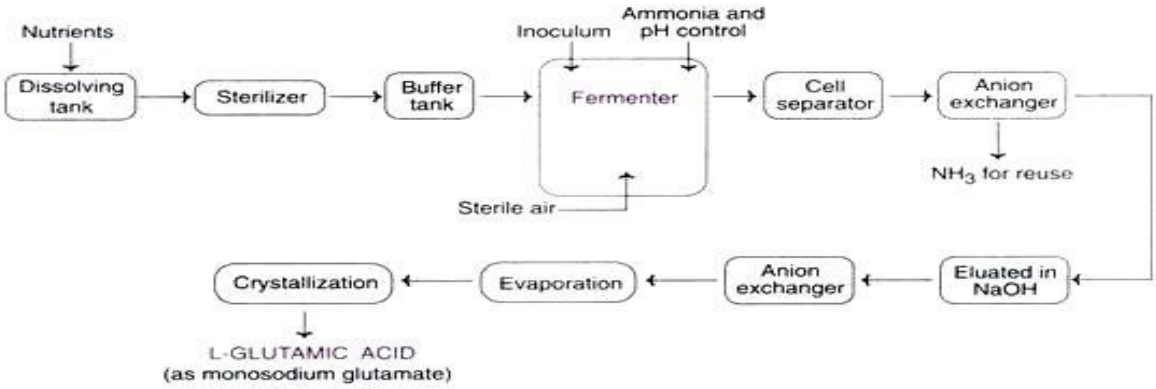

Fig. 26.3 : Diagrammatic representation of glutamic acid production plant.