

PRODUCTION OF GLUTAMIC ACID

Introduction:

Amino acids, like vitamins, form an important class of compounds. They are required by living cells as growth factors. The production of amino acids through the fermentation process was initiated by Kinoshita et al., (1956). They produced L- glutamic acid as the first amino acid by the fermentation method. Thereafter, various L-amino acids were economically produced by the use of suitable micro-organisms. Thus L- α - amino acids were made available at low cost to be used in many areas as economical raw materials. Patents have been produced, particularly in Japan, describing the production of most of the naturally occurring amino acids. But only glutamic acid and lysine are produced in large quantities. These amino acids are used in the food industry.

Fermentation production of L-Glutamic acid: A derivative of glutamic acid, mono-sodium glutamate, is used to develop a flavour in food products (e.g. soups). The commercial production of this acid in the world exceeds 1,00,000 tons per annum. L- Glutamic acid may be produced in many ways:

- By the hydrolysis of wheat gluten, soyabean cake or other proteinaceous material;
- By the cleavage of the pyrrolidone carboxylic acid found in Steffen's molasses;
- By a one-stage fermentation process involving a single micro-organism;
- By a two-stage fermentation process where α -ketoglutaric acid is produced by one microbe and α -ketoglutaric acid, in turn, is converted to L- α -glutamic acid by another microbe.

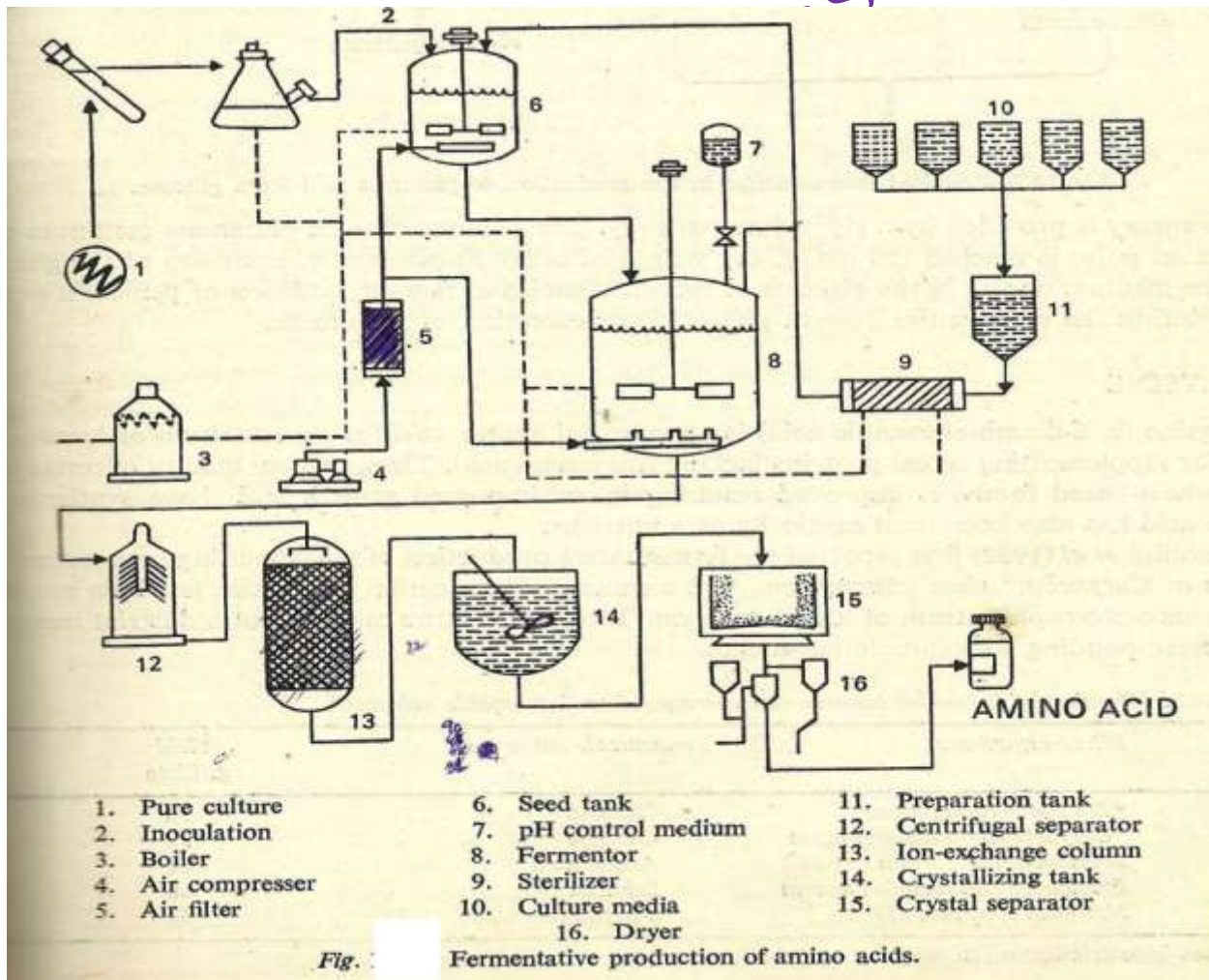
Nowadays, a one-stage process utilizing *Corynebacterium glutamicum* is mainly adopted to produce L- glutamic acid on large scale. Japanese workers have obtained high-yielding mutants of this microbe.

Glucose and starch hydrolysates are the principal raw materials for the fermentative production of amino acids. More efforts have been made to replace these materials with such cheaper and more easily available ones as molasses, ethanol, pentose, acetic acid and other petrochemicals. The fermentative production of L-glutamic acid from various carbon sources is given in following table:

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

*indicates industrialization in Japan.

Industrial production of L-glutamic acid using glucose as carbon source was initiated in 1957 by Kyowa Hakko Kogyo Co. It is necessary to add a limited amount of biotin to the production medium. The quality of biotin needed is in the range of 1 to 5 µg/ltr. The accumulated glutamic acid gets excreted through the cell-wall by the mechanism involving biotin. With inexpensive raw materials, fine control of biotin in glutamic acid fermentation is not possible. Glutamic acid formation and its excretion occur at higher biotin concentrations, provided the fermentation medium is supplemented by penicillin or detergents. Moreover, the use of antibiotics, penicillin, allows us to use sugar cane waste molasses in place of pure glucose as carbon substrate. Urea or ammonia may be used as nitrogen sources. Also, phosphates and other ordinary salts are added to the production medium in the manufacture of glutamic acid. The production medium is slightly alkaline in reaction. The temperature to be maintained in *Corynebacterium glutamicum* – L-glutamic acid fermentation is 30°C. The duration of the fermentation period is 40 hours. The carbon source may be converted to glutamic acid in 50% of the yield under optimum conditions. The general flow-diagram for the production of amino acids is illustrated in **Fig 1**.



Biochemistry of L-glutamic acid fermentation:

In the case of *Corynebacterium glutamicum*, the sugars are metabolized via the glycolysis. Therefore, formation of glutamic acid does not occur if classical inhibitors of glycolytic pathway (e.g. fluoride and iodoacetate) are present. Owing to the absence of α -ketoglutarate dehydrogenase in the mutant, accumulation of glutamate occurs. Thus, the citric acid cycle is not complete (Fig 2).

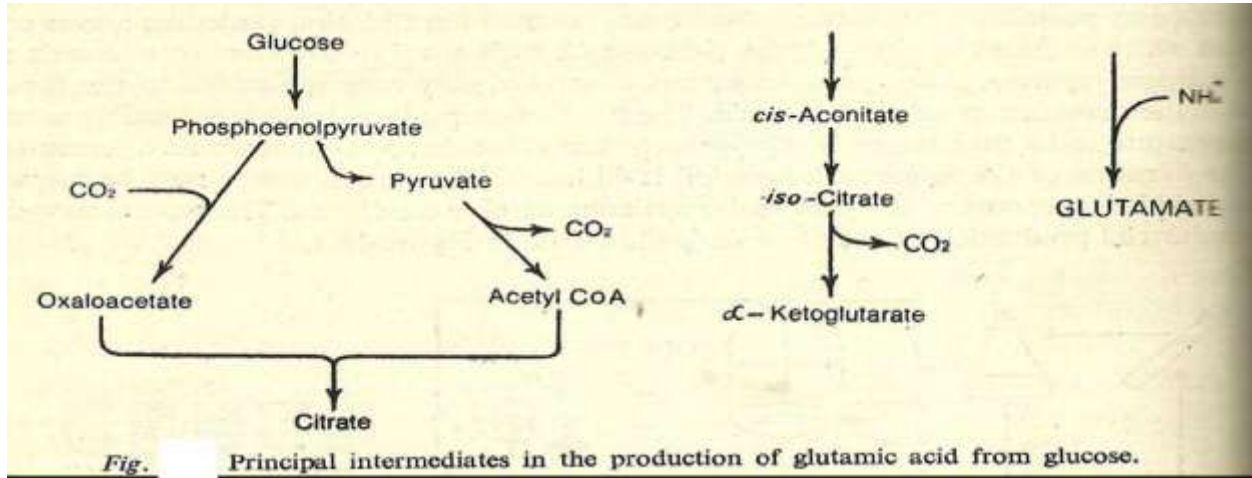


Fig. Principal intermediates in the production of glutamic acid from glucose.

The energy is provided by glyoxylate pathway. The accumulation of glutamate continues until a saturation point is reached (50 mg/g dry weight of cells). Subsequently, excretion of the glutamate into the medium occurs in the absence of biotin. If molasses is used, addition of penicillin or detergents during the exponential growth phase allows excretion of glutamate.

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