Industrial Production of L-Glutamine

Isao Kusumoto

Ajinomoto Co., Inc., Kawasaki, Japan

ABSTRACT The industrial production of L-glutamine (L-Gln) started with its fermentation in the late 1960s. Currently, it is manufactured for use as pharmaceuticals and health foods at the worldwide annual production of ~2000 metric tons. To manufacture high quality L-Gln at a low cost, it is of prime importance to obtain a strain of microorganism with good production efficiency and minimum by-products. Furthermore, to obtain the final crystalline powder product, the efficient removal of impurities contained in the fermentation broth becomes paramount. Therefore, the industrial process is designed to take into account characteristics of the fermentation broth as well as chemical, physical and biological properties of L-Gln. Points that should be considered in the process design and typical industrial production of L-Gln are described in this report. J. Nutr. 131: 2552S–2555S, 2001.

KEY WORDS: glutamine • amino acid • ion exchange resin • resin chromatography • crystallization • polymorphism

The industrial production of amino acids is briefly reviewed before discussing the production technology of L-glutamine (L-Gln). Historically, the industrial production of amino acids started with the availability of monosodium glutamate (MSG) in 1909. MSG was discovered in 1908 by Dr. Kikunae Ikeda as a basic taste substance of kelp that is a traditional seasoning in Japan. Currently, MSG is used worldwide as a flavor enhancer. Originally, MSG was manufactured by extraction from acid hydrolysate of plant protein. Small-scale production of various amino acids followed but they were produced by the same extraction method used in MSG production. In the late 1950s, fermentation technology was established and used for the commercial production of MSG. This was the beginning of modern amino acid production. Since then, fermentation technologies for various amino acids have been established. Production of L-Gln by fermentation started in the late 1960s.

Current total annual worldwide consumption of amino acids is estimated to be over 2 million tons (Ajinomoto, estimate). The annual demand for amino acids as MSG-based flavor enhancers, and as feed additives comprised mainly of L-lysine hydrochloride, DL-methionine and L-threonine, is estimated to be 1 million tons for each amino acid (Ajinomoto, estimate). The annual demand for amino acids used in pharmaceutical products mainly for intravenous and enteral nutrition is ~15,000 tons. The annual demand for L-Gln as a pharmaceutical ingredient, such as for the treatment of gastric ulcer, and as a health food ingredient is estimated to be ~2000 tons.

General manufacturing process for amino acids

The manufacturing methods of amino acids are categorized as: 1) extraction from hydrolysates of animal or plant protein, 2) chemical synthesis, 3) fermentation, and 4) enzymic. Although DL-methionine for feed additives use and glycine, without an asymmetric carbon, are manufactured in a large scale by chemical synthesis, most amino acids are produced by fermentation. The fermentation process for L-Gln involves the following steps: growth of the microorganism, synthesis of L-Gln, removal of impurities and production of the final crystalline powder product.

FIGURE 1 Schematic of the fermentation process.
acids are manufactured by fermentation and enzymic methods. Several amino acids including L-leucine, hydroxy L-proline, L-tyrosine and L-cystine are still being manufactured by extraction in addition to fermentation and chemical synthesis. L-Gln is manufactured by several manufacturers throughout the world, all using fermentation.

The manufacturing process of an amino acid by fermentation comprises fermentation, crude isolation and purification processes. In the fermentation process, the desired amino acid is specifically produced by the fermentation microorganism. In the crude isolation process, most impurities contained in the fermentation broth are removed by combining various technologies. Final purification is performed to ensure the required quality for the intended use. The final product is obtained as a crystalline powder. The product is released only after quality tests have verified that the product meets specific requirements, and the normal functioning of each process step has been verified. All manufacturing processes for the production of amino acids for medical use must comply with current good manufacturing practice requirements.

As stated, most impurities are removed by the crude isolation process. The purification process is relatively simple and performed frequently. Careful performance of the fermentation and isolation processes, as well as the combination of these two processes, is critical to manufacture a high quality product with high productivity.

**Manufacturing method of L-Gln**

The following describes production of L-Gln by the fermentation process. It is essential to the outcome of the fermentation process to maintain a clean and sterile fermentation tank. Compared with wild-type strains, L-Gln-producing strains are weak and are compromised in a contaminated environment. Furthermore, it is important to maintain the tank under positive pressure by aeration during fermentation to prevent contamination by other microorganisms and external materials. The fermentation medium consists of glucose as a carbon source, ammonia as a nitrogen source, a small amount of minerals and vitamins as growth factors. Control factors during fermentation are pH, temperature and dissolved oxygen (Fig. 1).

An L-Gln-producing bacterium is shown in Figure 2. This strain is derived from an improved strain of L-glutamic acid.
acid producing bacteria capable of high quality and high productivity L-Gln, while minimizing the formation of by-products. Figure 3 illustrates the fermentation profile of L-Gln. The amount of fed glucose is decreased with proliferation of the fermentation bacteria. L-Gln begins to accumulate when the growth of the bacteria reaches a certain level. When the initial glucose concentration decreases, more glucose is added to the batch to improve productivity and to increase the accumulation of L-Gln. Each step of fermentation is controlled by optimizing pH, temperature and dissolved oxygen.

Crude isolation process

Figure 4 illustrates a typical process for the isolation of crude L-Gln. The purpose of the isolation process is to obtain crude L-Gln with adequate purity from the fermentation broth. The broth is centrifuged or filtered through a membrane filter to separate cells and debris. It is desirable that crude crystals are harvested through the direct crystallization of the supernatant or filtrate. If it is difficult to harvest crude crystals with adequate purity, then preparatory steps are required usually involving repeated ion exchange resin treatment, chromatographic treatment and crystallization.

The main points to be considered in designing processes for amino acid production are characteristics of the desired amino acid and impurity levels in the fermentation broth. The chemical, physical and biological properties of the amino acid are all important.

L-Gln is stable around the isoelectric point (pH 5.65), but if the pH shifts from the isoelectric point to either acid or alkaline conditions, L-Gln is easily hydrolyzed to L-glutamic acid and ammonia. Figure 5 shows stability of L-Gln in aqueous solution.

The solubility of several amino acids, including L-Gln, is shown in Figure 6. The solubility of L-glutamic acid and L-histidine HCl increases with increasing temperature. In contrast, the solubility of L-Gln is barely affected by temperature as shown by the flat solubility curve. Consequently, cooling crystallization is not applicable for harvesting L-Gln.

Purification of amino acids by crystallization is an effective means to produce polymorphism. For example, as shown in Figure 7, two crystal forms can be used. After crystallization of L-glutamic acid in the α-form, the crystals are dissolved, and

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<th>Amino acid</th>
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<td>Glutamic acid</td>
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FIGURE 7  Amino acids crystal forms.

FIGURE 6  Solubility of several amino acids, including L-Gln.

FIGURE 8  Decomposition of L-Gln in aqueous solution at 35°C.

FIGURE 9  Simplified production flow chart of the L-Gln manufacturing process.
then recrystallized in the β-form. In this manner, it is possible to remove impurities based on their different affinities for the two crystal forms.

Unfortunately, L-Gln occurs only as one crystal form. Therefore, to use crystallization for purification, there is no way other than the inefficient simple repetitive crystallization of the one crystal form of L-Gln.

Figure 8 shows the biodegradability of L-Gln in aqueous solution at 35°C. Because L-Gln is easily degraded by microorganisms, proper control of the process as well as aspects of process design is imperative.

The intrinsic properties of L-Gln need to be considered when designing its isolation and purification processes. Compared with other amino acids, L-Gln is an amino acid that is difficult to design. Therefore, the purity of the fermentation broth is of vital importance to obtain high purity L-Gln with high productivity.

The flow diagram depicted in Figure 9 briefly illustrates the entire process of L-Gln production.

CONCLUSION

The industrial production of L-glutamine has been reviewed with a historical background of the industrial production of amino acids. From the standpoint of the manufacturing industry of glutamine, it is hoped that basic and clinical research on glutamine will continue and further contribute to the improvement of human health.