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LITERATURE STUDY OF L-CARNITINE PREPARATION METHODS FOR 1000 TONS OF ANNUAL PRODUCTION

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ABSTRACT

L-carnitine is a gamma-hydroxy amino acid and also known as vitamin B_{γ}. L-carnitine has many benefits as well as uses. Supplements with L-carnitine content are much needed in the field of health. L-carnitine is obtained using a variety of methods. They are the chemical optic resolution method, the biological method, and those that use chiral materials from natural sources. The purpose of this literature study is to determine the best method to be used in the design of the L-carnitine production plant with a capacity of 1000 tons per year by comparing various existing methods from various aspects. Based on the results of the comparison, it was found that the production of L-carnitine from (S)-3-activated hydroxybutyrolactone is the best method to be used in the production of L-carnitine capacity of 1000 tons per year for 300 days. This method produces a lot-more L-carnitine products at a lower cost than other methods that have a higher yield.

Keywords: *Annual Production, L-carnitine, Selection Process*

1. INTRODUCTION

Over the past few years, there have been various advertisements for supplements in various media. This happens because of the high public awareness of the importance of maintaining health. There are many people who invest their money not only in health insurance services but also in supplement products [1]. The high use of supplements affects the community, especially for people who need and have high activity. One of the nutritional supplements used by the public today is L-carnitine. L-carnitine has gotten a lot of attention in the supplement market because of its bio functionality over the last two decades [2]. The use of L-carnitine, which can be synthesized in either the liver or kidney or be obtained from certain foods, is a potential intervention [3].

Carnitine (β-hydroxy-γ-trimethyl-amino butyric acid) is a quarter nary ammonium compound bio-synthesized from the amino acid lysine and methionine [4]. There are two stereo isomers of carnitine. L-Carnitine is the bioactive form, while its enantiomer D-carnitine is inactive. L-carnitine is ordinarily known as a hydroxy amino acid that advances the mitochondrial oxidation of fatty acids to energy at the level of the muscular system [5]. L-Carnitine is known to be obtained through synthetic chemical processes that begin with products that are not optically active or from mixtures of race mates. The role of L-carnitine in the body is to convert fat in the body into energy for the body.

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L-carnitine is normally present in the body where it expert the function of a carrier of activated long chain free fatty acids through the mitochondrial membrane [6]. L-carnitine exists naturally in human cells and tissues, and its availability in the physiological system primarily depends on dietary conditions, lean body mass, and age [7]. L-carnitine (L-b-hydroxy-g-N-trimethyl amino butyric acid) is an essential nutrient that plays a pivotal role in fatty acid metabolism [8].

L-carnitine has many benefits for human needs. One of these benefits will be the production of L-carnitine in large quantities. L-carnitine is an important nutritional supplement when used as an additive to fermentation media, it promotes growth in yeasts and bacteria [9]. This biologically active L-carnitine enantiomer is in high demand for use in these. The other applications have led to a worldwide search for ways to synthesize this betaine in an optically pure form. This is because the chemically synthesized race mate cannot be used. This happens because of the inhibition of the carnitine acetyltransferase and also of the carnitine carrier protein.

Aside from the previously mentioned benefits, L-Carnitine has a wide range of other benefits. It can be used for digestion, promoting appetite, reducing blood fat from carnitine deficiency, fat-reducing and treatment of vascular disease. It can also be used to treat acute ammonia poisoning, stupor, and nervous system disorders. L-carnitine can also be used as a functional food addictive in infant formula to promote infant growth and development. In addition, it is used in sports drinks to improve movement stamina and explosive power.

L-carnitine is for improving cognitive function. Cognitive functions refer to higher brain functions that encompass senses, perceptions, recognition, judgment, and action or suppression. Information about the surrounding circumstances is continuously transmitted to the brain through the use of sense organs, which select the necessary information. At that time, the ability of a person to pay attention to a plurality of pieces of information is important. As a result, L-carnitine is widely used in the art to treat individuals suffering from diseases such as cancer or Alzheimer's disease, or individuals whose metabolism is impaired as a result of aging or extreme physical exercise [10]. In addition, combinations of multiple active substances, including L-carnitine, have been proposed for other uses.

Numerous methods for producing optically pure L-carnitine have been discovered. The methods applied include the chemical optical resolution method, the biological method, and the means of using chiral material from natural sources [11]. Each method has its own advantages and disadvantages. It will affect the selectivity of the methods used to produce L-carnitine in the desired production. According to one study, numerous chemical procedures can be found in the literature involving asymmetric synthesis; chemical multi-step racemization; resolution through diastereomers derivatives; microbiological or enzymatic techniques and the use of chiral starting materials [12]. Another study reports that adding chromium to the biofuel waste contributed by Y. lipolytica biomass increases the production of L-Carnitine [13].

Based on existing exposure, a literature study is needed on comparisons between each of the existing methods. Through the literature study, it will be able to choose the most appropriate method used to produce L-carnitine with a capacity of 1000 tons per year. The purpose of this study is to determine the best method to be used in the design of the L-

carnitine production plant with a capacity of 1000 tons per year by comparing various existing methods from various aspects.

2. RESEARCH METHOD

This research is a literature review that was conducted by collecting various theories and data. Existing data are derived from previously conducted studies. This is a qualitative research method that entails collecting data on L-carnitine preparation methods and then comparing them until an analysis is completed to lead to a conclusion.

The existing data will be analyzed to determine the best method of producing L-carnitine for a 1000 tons annual production. The selection of L-carnitine preparation methods influenced the results of the data recapitulation from the literature research. Data are gathered by comparing information from previous journals that include the L-carnitine preparation process. Furthermore, data analysis is used to determine the advantages and disadvantages of each existing method.

3. RESULTS AND DISCUSSION

3.1 The Chemical Optical Resolution Method

According to the chemical optical resolution methods, D, L-carnitine or the race mate of its derivatives is reacted with an optically pure chiral optical resolution agent to yield diastereomers. Then, the target diastereomers are obtained by resolution using the difference in solubility in an appropriate solvent. The above compound is again hydrolyzed so as to yield the target L-carnitine. The commonly used optical resolution agents include D-camphoric acid, L-tartaric acid, dibenzoyl-D-tartaric acid, dibenzoyl-L-tartaric acid, D-mandelic acid, and N-acetyl-D-glutamic acid [14].

Various chemical procedures have been proposed for the industrial-scale production of carnitine. Typically, the D, L-racemic mixture is reacted with an optically active acid (e.g. D-(-)-tartaric acid, D-(+)-camphor-sulfonic acid, (+)-dibenzoyl-D-(-)-tartaric acid, N-acetyl-L-(+)-glutamic acid and D-(+)-camphoric acid to obtain two diastereomers which can be separated from each other. In the classic method described in U.S. Patent 4,254,053, D-(+)-camphoric acid is used as a resolution agent in a racemic mixture of D and L-carnitine, yielding D-(+)-carnitinamide as a by-product and L-(-)-carnitinamide, which is hydrolyzed to yield L-(-)-carnitine [15]. However, many resolution procedures are complex and costly, and in all cases result in the production of equimolar quantities of L-(-)-carnitine and D-(+)-carnitine or a precursor thereof as a by-product, having configuration opposite to that of L-(-)-carnitine [15].

3.2 Biological Method

There is a biological method for preparing L-carnitine using microorganisms or enzymes. L-carnitine is produced from butyrobetaine as a raw material by means of stereo-selective hydroxylation with pertinent enzymes or from crotonobetaine as a raw material by means of stereo selective hydration with appropriate enzymes.

Crotonobetaine is transformed into L-carnitine in an aqueous medium by the action of microorganism competent to transform crotonobetaine into L-carnitine. By a microorganism competent to transform crotonobetaine into L-carnitine is meant a microorganism which, when brought in to with crotonobetaine under the conditions

hereinafter described, produces L-carnitine by the action of enzymes contained in the microorganism on the substrate crotonobetaine [16].

A microorganism of the strain DSM number 3225 (HK 1331b) type is cultivated in a bioreactor with crotonobetaine and/or γ-butyrobetaine in the presence of growth substrate [17]. The culture fluid is guided outside the bioreactor in a circulation in which a cell separation is carried out, where a quantity of cell-free solution, which is as large as the amount fed to the bioreactor as a substrate, is withdrawn from the bioreactor and L-carnitine is separated from the cell-free solution. The advantages of the invention method of operation are used and as a result, biomass retention, higher productivity, and greatly improved long-term stability of the continuous process are achieved.

Another biological method for preparing L-carnitine is via the reaction between (R)-3,4-epoxybutyric acid and trimethylamine. (R)-3,4-epoxybutyric acid as a main raw material undergoes a chemical reaction to yield a racemic 3,4-epoxybutyric acid ester. Based on the biological method, such an ester undergoes optical resolution to yield an (R)-3,4-epoxybutyric acid ester selectively, which is again hydrolyzed to produce the target compound. This method has proven to have excellent stereo-selectivity, although it requires careful modulation of the reaction with a prolonged reaction time of approximately 24 hours.

3.3 Use Chiral Material from The Natural Source

There is a method of preparing L-carnitine by means of use chiral material from a natural source. According to such a method, D-mannitol is employed as a raw material. Then, via various steps, L-carnitine is prepared. Another method of preparing L-carnitine from (S)-3-activated hydroxybutyrolactone has been disclosed. According to this method, (S)-3-activated hydroxybutyrolactone of 1.0 equivalent and a 25% trimethylamine solution of 2.0 equivalent are mixed. Then, the mixture in a closed container is stirred at room temperature for one hour. In addition, the mixture is reacted at 100 °C for 16 hours to produce pure L-carnitine, but the yield is not mentioned.

L-carnitine is obtained by (S)-3-activated hydroxybutyrolactone. (S)-3-activated hydroxybutyrolactone (S-HGB), also known as (S)-3-hydroxybutyrolactone (S-HGB), is a critical building block in the synthesis of other chiral intermediates using classical chemistry [18]. (S)-3-hydroxyl-gamma-butyrolactone is a kind of very important organic synthesis intermediate. It is also a very important chiral source (Chira Pool) [19].

(S)-3-hydroxy-γ butyrolactone (S-HGB) is a precursor to various enantiopure intermediates for chiral drugs such as cholesterol-lowering drugs and can be used in the synthesis of linezolid and ezetimibe. Enantiopure (S)-3-hydroxy-γ-butyrolactone and its structural related C3-C4 chemicals are an important target for chiral building blocks in synthetic organic chemistry [20]. Optically pure HGBs have both alcohols and carboxylic acids amenable to further derivation. Among others, S-HGB can be converted to (S)-4-Chloro-3-hydroxybutyrate (S-CHB) and further to hydroxy-nitrile (HN) for the synthesis of intermediates of hydroxy-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitors, 4-hydroxy-2-pyrrolidone, oxazolidinones, and L-carnitine.

3.4 Selection of Processes for The Design of an L-carnitine Production Plant

Based on the description and data that have been described above, a comparison is obtained in the selection process in L-carnitine plant planning, as can be seen in Table 1.

Table 1. Comparison of Each L-Carnitine Preparation Method

Chemical Optical Resolution Method (D, L-carnitine)	Biological Method (Crotonobetaine)	Use Chiral Material from Natural Source ((S)-3-activated hydroxybutyrolactone)
The process of recovering such an agent would be essential, and the optical resolution is extremely difficult during the re-crystallization step for the formation on the diastereomers[a]	Maximum L-carnitine Production 40 g/l, molar yield 50%[b]	Pure L-carnitine may be obtained with a high yield[a]
39°C with pH 8.5[b]	30°C with pH 6.5 for 2-3 days[b]	100°C for 16 hours at room temperature[a]
High priced optical resolution agent[b]	High priced[b]	Inexpensive and facile method[a]
A much waste product that needs to be disposed[b]	25% less waste water than chemical process[b]	Less organic solvent is use and purity of the final product without separate purification processes[a]

Source: a. Byun et al., 2002 [11] ; b. Naidu et al., 2000 [21]

The chemical synthesis of L-carnitine usually starts with the cheap raw materials such as epichlorohydrin and trimethyl-amine [22]. Followed by a separation of the race mate by, e.g., fractional crystallization [23]. The optical resolution is extremely difficult during this step.

In terms of process, the biotechnological process for L-carnitine production outperforms the chemical process. According to reports, biotechnological processes generate approximately 50% less total organic waste, 25% less waste water, and 90% less waste for incineration than chemical process [24]. As a result, microbial production of L-carnitine has gained prominence.

Based on existing comparisons of each method, researcher choose to use the L-carnitine preparation method of (S)-3-activated hydroxybutyrolactone because a very inexpensive and facile method of preparing (S)-3-activated hydroxybutyrolactone through oxidation and successive cyclization of inexpensive natural D-carbohydrates and hydrogen peroxide has been developed. As a raw material, (S)-3-activated hydroxybutyrolactone should have a specific condition. With this method, it will be seen that the use of inexpensive compounds in aqueous solutions will yield L-Carnitine that is very pure with high yields.

The first reaction step is a ring-opening reaction of (S)-3-activated hydroxybutyrolactone. The next reaction step, the ring-opened compound of 4-hydroxy-3-activated hydroxybutyric acid, undergoes an epoxidation reaction in which the chiral center is stereo selectively reversed in the presence of a base. Then, an optically pure salt of 3,4-epoxybutyric acid is prepared from it. The last step of the reaction process is the manufacture of L-carnitine through the reaction between (R)-3,4-epoxybutyric acid and trimethylamine. The sodium 3,4-epoxybutyrate, which was formed from the above reaction, was not separated while an aqueous solution of 25% trimethylamine equivalent

was immediately added to the reagent solution and stirred at 45°C for two hours to produce L-carnitine.

A method of separating and purifying L-carnitine from reagent solution uses a commonly known method, preferably a cation exchange resin. According to the method of separation and purification of L-carnitine via cation exchange resin, the optical purity of L-carnitine obtained is more than 95%, with a yield of about 55% or more. Further, similar results can be obtained if sodium 3,4-epoxybutyrate, which is formed during epoxybutyric acid, is reacted with trimethylamine.

L-carnitine making has four process units. The process units namely: preparation of (S)-3-methanesulfonylhydroxybutyrolactone, preparation of (S)-4-hydroxy-3-methanesulfonyl hydroxybutyric acid, preparation of sodium (R)-3,4-epoxybutyrate, and preparation of L-carnitine [11].

4. CONCLUSION AND SUGGESTION

There are various methods for the manufacture of L-carnitine, including chemical optical resolution methods, biological methods, and use of chiral material from natural sources like (S)-3-activated hydroxybutyrolactone. Based on the selection of processes that have been done by comparing each method, the utilization of chiral material from natural sources like (S)-3-activated hydroxybutyrolactone was chosen as the method used in the design of the L-carnitine plant with a capacity of 1000 tons per year. The selection is based on aspects of the process, operations, and economy, as well as the environmental impact.

The literature study in this study has several things that are necessary if you are using this research as a reference for L-carnitine production research, such as the capacity to be produced, the materials to be used, and the estimated cost. For further research on L-carnitine production, it can be innovated or developed into more effective materials for the production of L-carnitine plants in large quantities.

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