

INDUSTRIAL PRODUCTION OF LACTIC ACID

Hello dear students welcome for the lecture series on Industrial microbiology, the topic for today's discussion is Industrial Production of Lactic Acid

The topic can be studied under the subsequent subheadings,

- 1. HISTORY OF LACTIC ACID**
- 2. USES OF LACTIC ACID**
- 3. COMMON ASPECTS IN THE SYNTHESIS OF LACTIC ACID**
- 4. PRODUCTION OF LACTIC ACID BY FERMENTATION PROCESSES**
- 5. PURIFICATION OF LACTIC ACID**

1. HISTORY OF LACTIC ACID

Lactic acid, also known as milk acid, is a chemical compound that plays a role in various biochemical processes. Lactic acid is a carboxylic acid with the chemical formula $C_3H_6O_3$. It has a hydroxyl group adjacent to the carboxyl group, making it an alpha hydroxyl acid (AHA). It is classified as GRAS (generally regarded as safe) by Food and Drug Authority (FDA) in the USA and its annual consumption is estimated to be 30000 tones. Lactic acid is produced by many organisms: animals including man produce the acid in muscle during work. Lactic acid was among the earliest materials to be produced commercially by fermentation and the first organic acid to be produced by fermentation. Lactic acid was refined for the first time by the Swedish chemist Carl Wilhelm in 1780 from sour milk. In 1808 Jöns Jacob Berzelius discovered that lactic acid (actually L-lactate) also is produced in muscles during exertion. Its structure was established by Johannes Wislicenus in 1873. In 1856, Louis Pasteur discovered Lactobacillus and its role in the making of lactic acid. Lactic acid started to be produced commercially by the German pharmacy Boehringer Ingelheim in 1895. In 2006, global production of lactic acid reached 275,000 tones with an average annual growth of 10%.

From the histological point of view lactic acid has a long history of uses for fermentation and was first discovered in 1780 by Swedish chemist, Carl Wilhelm Scheele, who isolated the lactic acid from sour milk as impure brown syrup and gave it a name based on its origins: 'Mjölksyra'. After nine years around in 1789, Lavoisier named this milk component "acide lactique", which became the core origin of the current terminology for lactic acid. For a very long time until 1857 it was being considered a milk component while later on that year Pasteur discovered another phenomenon and postulated lactic acid as a fermentation metabolite generated because of the involvement of certain microorganisms. In support with Pasteur's discovery a French scientist Frémy produced

lactic acid by fermentation and this gave rise to first industrial production of lactic acid in the United States by a microbial process in 1881. From that time it has Wide applications in food, pharmaceutical, cosmetic and chemical industries etc. The worldwide demand for lactic acid is estimated roughly to be 130 000 to 150 000 tons per year. However, the global consumption of lactic acid is expected to increase rapidly in the near future.

2. USES OF LACTIC ACID

1.It is used in the baking industry. Originally fermentation lactic acid was produced to replace tartarates in baking powder with calcium lactate. Later it was used to produce calcium stearoyl 2- lactylate, a bread additive.

2.In medicine it is sometimes used to introduce calcium in to the body in the form of calcium lactate, in diseases of calcium deficiency.

3. Esters of lactic acid are also used in the food industry as emulsifiers.

4. Lactic acid is used in the manufacture of rye bread.

5. It is used in the manufacture of plastics.

6.Lactic acid is used as acidulant/ flavoring/ pH buffering agent or inhibitor of bacterial spoilage in a wide variety of processed foods. It has the advantage, in contrast to other food acids in having a mild acidic taste.

7.It is non-volatile odorless and is classified as GRAS (generally regarded as safe) by the FDA.

8.It is a very good preservative and pickling agent. Addition of lactic acid aqueous solution to the packaging of poultry and fish increases their shelf life.

9.The esters of lactic acid are used as emulsifying agents in baking foods (stearoyl-2-lactylate, glyceryl lactostearate, glyceryl lactopalmitate). The manufacture of these emulsifiers requires heat stable lactic acid, hence only the synthetic or the heat stable fermentation grades can be used for this application.

10.Lactic acid has many pharmaceutical and cosmetic applications and formulations in topical ointments, lotions, anti acne solutions, humectants, parenteral solutions and dialysis applications, for anti caries agent.

11.Calcium lactate can be used for calcium deficiency therapy and as anti caries agent.

12.Its biodegradable polymer has medical applications as sutures, orthopaedic implants, controlled drug release, etc.

3. COMMON ASPECTS IN THE SYNTHESIS OF LACTIC ACID

Lactic acid can be synthesized industrially by two means either through chemically or by microbial fermentation. However, the least one (fermentation through microbes) has some potential advantages e.g. pure lactic acid can be attained whereas, chemical synthesis of lactic acid always give a raceme mixture.

One of the most expanding uses of lactic acid is its use in polymerization of lactic acid to form polylactic acid (PLA), a polymer of great interest because it can be produced from renewable means which is biodegradable in nature. Many PLA-based products are already available in the market, where they are used to replace the petroleum-based consumables.

Lactic acid is the simplest hydroxy acid which has an asymmetric carbon atom and is present in two optically active forms. Therefore, most of the world's commercial lactic acid is prepared by fermentation of carbohydrates by bacteria, using homolactic microbes such as a variety of modified or optimized strains the genus *Lactobacilli*, which especially produce lactic acid. Commercially pure lactic acid can be synthesized by microbial fermentation of the following carbohydrates such as glucose, sucrose, lactose, and starch/maltose derived from feed-stocks such as beet sugar, molasses, whey, and barley malt. The preference of feedstock entirely depends on its price, availability, and on the respective costs of lactic acid recovery and purification. Biomass of lignocelluloses is a low-cost and extensively available renewable carbon source as an alternative to these conventional feed-stocks that has no challenging food value. Other biological agents capable of producing lactic acid are also used such as strains of *Rhizopus*, *Escherichia*, *Bacillus* and *Saccharomyces*.

Widely used method for the production of lactic acid is Batch fermentation. Conditions for Fermentation are different for each industrial method but are usually in the range of 45–60 °C having a pH of 5.0–6.5 for *Lactobacillus delbrueckii* and 43°C with a pH of 6.0–7.0 for *Lactobacillus bulgaricus*. The acid synthesized is neutralized by calcium hydroxide or calcium carbonate. Fermentation takes 1–2 days under optimal lab conditions. The yield of lactic acid after the fermentation stage is 90–95 wt% based on the initial sugar or starch concentration.

Rate of fermentation depends mainly on the parameters such as pH, temperature, initial substrate concentration and concentration of nitrogenous nutrients. There has been a great interest in solving the issues such as PLA weakens at high temperature for the purpose to enhance the use of this renewable plastic. Hydrolysis reaction of methyl lactate is use to enhance the performance of batch reactive distillation to produce lactic acid. Lactic acid bacteria are conventionally particular microbes that have complex nutrient necessities industrial wastes of food which have high moisture and loaded in

carbon source have been considered as an eye-catching nutrient source for industrial production of lactic acid. Product recovery is an important step in lactic acid production that is associated to separation and purification of lactic acid from fermentation broth. A conventional procedure for lactic acid production by lactose fermentation involves the purification steps that are necessary to attain the pure lactic acid. Alternatives to this industrialized procedure are being studied. Numerous studies on lactic acid purification have been conducted by using several different techniques for separation such as ion exchange, reactive extraction, membrane technology, distillation and electro-dialysis.

4. PRODUCTION OF LACTIC ACID BY FERMENTATION PROCESSES

Fermentation is an energy yielding process in which organic molecules play role as both electron donors and electron acceptors. The molecule which is metabolized does not possess its whole potential energy extracted from it. Therefore, lactic acid bacteria are widely used as a cheap method for food maintenance by fermentation and usually no or little heat is required in fermentation. In batch fermentation process the culture is first grown in a series of inoculum vessels and after that transferred to the fermentor. The size of inoculum is usually 5–10% of the liquid volume in this fermentor. The fermentation is usually kept at 35–45 °C and at pH 5–6.5 by adding a suitable base, such as ammonium hydroxide. Other fermentations for lactic acid production are, fed-batch, repeated batch, and continuous batch.

But the higher concentration of lactic acid has achieved in batch and fed-batch cultures than in others, whereas higher productivity has obtained by continuous cultures. Another advantage of the continuous batch over batch culture is that the process can be run for a long period of time.

Raw material for lactic acid production

The commercial production of lactic acid using fermentation technology mainly depends on the cost of raw material used. Therefore, it is compulsory to select a raw material for industrial production of lactic acid with a number of characteristics such as low cost, rapid rate of fermentation, lowest amount of contaminants, high yields of lactic acid production, little or no formation of by-products and availability for whole year.

E. faecalis has been used to hydrolyze Agricultural resources such as wheat, barley, and corn by commercial amylolytic enzymes RKY1 and fermented into lactic acid. Lactic acid productivities obtained were at >0.8 g/L h although no added nutrients were provided to those resources, using barley and wheat. Attempts have been also made for lactic acid production from sugar cane molasses as a cheap raw substrate through fermentation by using indigenous bacterial culture. The conditions are optimized for

fermentation taking into consideration fermentation time, substrate level and temperature as factors for main process.

Lignocellulosic biomass was used for the production of lactic acid as an alternate to above conventional feed-stocks. Lignocellulose is composed of cellulose and hemicellulose that is made up of hexose and pentose sugar surrounded in phenolic polymer lignin matrix. The main procedure relies on hydrolysis by cellulolytic and hemicellulolytic enzymes to obtain sugars from lignocelluloses which are fermentable. A pretreatment, either chemical or mechanical is required of the lignocellulose to reduce the size of particle, to remove the lignin or to modify it and to improve the convenience of the polysaccharides for the purpose of enzymatic hydrolysis. Supplementation of fermentation media is very necessary for the fast production of lactic acid with adequate nutrients. Yeast extract is used as the most general nutrient for production of lactic acid, but this may cause of increase in production costs considerably. A by-product from the process of corn steeping has been utilized effectively for lactic acid production as an alternate. Since the corn steep liquor is derived from corn, 85% of its nitrogen content consists of amino acids, peptides and proteins. For production of optically pure d-lactic acid from raw glycerol, five technical schemes have been planned. These were pretended and assessed economically based on five fermentative scenarios by using engineered strains of *E. coli*.

Optimization of lactic acid fermentation

A range of procedure variables have been optimized in the production of lactic acid using wastes raw materials as a substrate. It has been found that productivity is affected by temperature, fermentation time and the level of substrate. The highest yield has been obtained after 7 days of fermentation in media possessing 18% substrate level having a mean value of 7.76 ± 0.08 g/100 mL (77.6 g/L) at temperature of 42 °C. The maximum recovery was 78.30%.of lactic acid with respect to initial whole sugar contents of the media. *R. oryzae* NRRL 395 which immobilized in polyurethane foam has been used in Lactic acid production by using response surface methodology. Three independent variables; pH, glucose concentration, and rate of agitation has been explained by a 23 full-factorial central composite design.

Maximum production of lactic acid was achieved 93.2 g/L by using a glucose concentration of 150 g/L, pH of 6.39 and rate of agitation 147 rpm. Agitation rate and concentration of glucose have found to be as limiting factors. So, any variation in these parameters can alter the production of lactic acid.

Lactic acid production is not affected by the Initial pH due to neutralizing agent added. Production of lactic acid under optimum conditions using immobilized whole cells was calculated about 55% that is higher than the lactic acid production from suspension

culture systems. In another study, calcium alginate has been utilized to immobilize *L. delbrueckii* bacteria by using pineapple waste. Various important factors such as temperature, pH, calcium alginate concentration, beads diameter and inoculum size have to be considered systematically for the successful production of lactic acid.

Yeast strains having heterologous l-lactate dehydrogenases can be used for lactic acid production. As these microbes can survive in acidic environments, it was identified that at low pH, cells are stressed by lactic acid. Two low-cost nitrogen sources such as yeast autolysate (YA) and corn steep liquor (CSL) have been used for the production of d(-) lactic acid with *Lactobacillus* LMI8 sp.

5. PURIFICATION OF LACTIC ACID

The recovery of lactic acid must be improved in order to reduce lactic acid losses and to increase purity. Purification or product recovery is an important step in production of lactic acid that is associated with separation and purification of lactic acid from fermentation broth. Fermentation broth contains a number of impurities such as residual sugars, color, nutrients and other organic acids, as part of cell mass.

These impurities must be removed from the broth in order to achieve more pure lactic acid. To recover and purify the l(+)- lactic acid produced from the microbial fermentation media economically and efficiently, ion exchange chromatography is used among the variety of downstream operations. It is extremely selective and gives product recovery at very low cost within a short period of time. The other purposes were to analyze the end product purity, to check adsorption or desorption behaviors of lactic acid and to examine the applicability of this method for industrial usage. Process strengthening and monomer grade lactic acid has been achieved in high purity by advancement of a new membrane-integrated technology. It has lesser the processing steps, chemical requirement and energy expenditure. The fastidious modular design provides a great elasticity in action of the system which the modern industrialized sector is looking for dreadfully in this era of shrunken profit edge.

Commercial production of pure lactic acid has also been carried out in many areas using strong-acid cation-exchange resins as solid catalysts. Surface active molecules such as enzymes and proteins are separated in aqueous solution by a simple and low cost method known as foam separation. Applicability and efficiency of foam separation technique has been studied by lactic acid broth, yeast extract and spent brewer's residual beer was used to examine the partial purification of products and recovery of important components from industrial waste stream. Investigation has been carried out to check the usability of nanofiltration in a definite process of lactic acid production

based on old bipolar electrodialysis operations. DK nanofiltration membrane was used for recovery rate and purification of lactic acid efficiency.

Magnesium and calcium ions are removed by nanofiltration efficiently from a sodium lactate fermentation broth before its conversion and concentration by electrodialysis. Maximum removal of impurities and lactic acid recovery has been achieved at maximum pressures of transmembrane. Phosphate and Sulfate ions are also partially removed. Lactic acid can also be extracted from aqueous solution using n-butanol as an extractant. Factors such as pH, mixing time, initial concentration of lactic acid, and volume ratio between the organic and the aqueous phase affect the extraction of lactic acid. Degree of lactic acid extraction and distribution coefficient increases when the pH of aqueous solution is decreased. The pH effect is considerably marked when the pH of the aqueous solution is less than 1. Initial concentrations of lactic acid and organic-to-aqueous volume ratio appear to have positive effect on the degree of extraction and distribution coefficient. As the n-butanol is miscible partially in water, so integration of aqueous phase into organic phase in the extraction has a great organic-to-aqueous volume ratio. Lactic acid can be separated and substantially purified from fermentation broths by several membrane-based unit operations.

Conclusions

Due to the growing demand of l(+) lactic acid for a wide range of applications in addition with the production of biodegradable plastic (PLA), it is necessary to make improvement in the conventional fermentation-based lactic acid production processes with efficient and sustainable method. There is still a big need for the researches to be carried out in order to produce lactic acid biotechnologically and commercially within the lowest cost, lowering the cost of the raw materials and improvement of high-performance microorganisms producing lactic acid.