ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 8, Issue, 7, pp. 18518-18521, July, 2017

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Review Article

LACTIC ACID PRODUCTION AND APPLICATIONS: A REVIEW

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DOI: http://dx.doi.org/10.24327/ijrsr.2017.0807.0524

ARTICLE INFO

Article History:

Received 16th April, 2017
Received in revised form 25th May, 2017
Accepted 23rd June, 2017
Published online 28th July, 2017

Key Words:

Lactic acid, lactic acid bacteria, homofermentative, heterofermentative L and D isomers.

ABSTRACT

Lactic acid is the common occurring organic acid in nature. Lactic acid bacteria are used for the industrial production of lactic acid by both homofermentative and heterofermentative fermentation. Both L and D isomers of lactic acid can be selectively synthesized by using selective strains of micro organisms. Lactic acid has many important uses such as acidulant/ seasoning/ pH buffering agent. It also prevents the microbial spoilage in many processed food articles.

INTRODUCTION

Lactic acid (2-hydroxy propionic acid) is the most commonly occurring organic acid in nature. Lactic acid was first introduced to the world as a sour component of milk by a Swedish chemist, Scheele in 1780. Scheele is considered as the dicoverer of lactic acid. Lactic acid was considered as a milk component, later on after the discovery of fermentation process by Louis Pasteur in 1857 it was made clear that lactic acid was actually the fermentation product of milk sugar which was converted to lactic acid by microorganisms present in curd. Charles E. Avery at Littleton, Massachusetts, USA started producing lactic acid on a commercial scale in 1881, by microbial fermentation process.

Lactic acid is a naturally occuring carboxylic acid. It can also be easily produced by both biological as well as chemical syntheis methods. Lactate is present in many food articles naturally or it may be produced as a result of *in situ* fermentation like sauerkraut, yogurt, buttermilk, sourdough breads. Lactic acid is also found as a major metabolic intermediate in majority of the living organisms, from prokaryotes to humans. (Datta & Henry, 2006).

Both L(+) and D(-) isomers of lactate are formed in chemical synthesis resulting in the formation of racemic mixture, on the other hand biological fermentation produces optically pure form of lactic acid depending upon the microorganism used, used substrate and the conditions employed during the process. Lactic acid easily dissolves in water and water miscible solvents however, it is sparingly soluble in other organic solvents. Lactic acid shows low volatility. Considering the structure of lactic acid it has on carboxylic group attached to the asymmetric carbon atom. Lactic acid structure shows the presence of one asymmetric carbon atom. It can be found in two natural isomeric forms L(+) and D(-). Since elevated levels of the d-isomer are harmful to humans, l(+)-lactic acid is the preferred isomer for food-related and pharmaceutical industries. Therefore, most of the world's commercial lactic acid is prepared by fermentation of carbohydrates by bacteria. Higher levels of D(-) form of lactic acid is injurious to our health. Hence, L(+) form is preferred in the food articles and pharmaceutics. Biological fermentation is usually the method of choice as it produces the pure form of the desired isomer(L) form (Hecky *et al.*, 2007).

C* - asymmetric carbon atom

Fig 1 Structure of D and L Lactic acid

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Uses of Lactic acid

Lactic acid is commonly used as acidulant/ seasoning/ pH buffering agent. It also prevents the microbial spoilage in many processed food articles. In comparison to the other organic acids lactic acid has a mild acidic taste. It is non-volatile, inodorous and is classified as GRAS (generally regarded as safe) by FDA in the US. It is a good preservative and pickling agent.

Lactic acid is a weak acid and has good solvent properties, these properties of it are used for the synthesis of polylactic acid (PLA) a homopolymer of lactic acid. Lactic acid polymers are biodegradable thermoplastics. Polymers of lactic acid are transparent and their breakdown can be modulated by manipulating the formation, and the molecular weight of the polymer synthesized. These polymers have medical applications such as sutures, orthopaedic prosthetics, restricted drug delivery etc. Lactic acid also has many pharmaceutical and cosmetic applications. In cosmetic industry it is commonly abbreviated as alpha hydroxy acid. Many pharmaceutical products like ointments, lotions and other medicated solution contain lactic acid. Salts of lactate are used in many fields of medicine like sodium lactate ($NaC_3H_5O_3$) which is used in parenteral and kidney dialysis solutions, calcium and magnesium lactates can be used for the treatment of mineral deficiencies. Optically pure forms of methyl, ethyl, and isopropyl lactate esters are used for chiral molecules as pharmaceutical intermediates. Lactic acid is mainly used as moisturizer, pH regulator, anti-wrinkle agent in the cosmetic preparations, along with these applications it also possess many other useful properties such as antimicrobial activity, skin lightening, and skin hydration.

In the dairy industries cheese production is favoured by the direction acidification achieved by the addition of lactic acid over traditional fermentation processes as it produces the desired quality product and also the risk of failure and spoilage is very less as compared to the traditional fermentaion method.

Role of microorganisms in lactic acid production

Lactic acid bacteria (LAB) have the property of producing lactic acid from sugars by a process called fermentation. Lactic acid Bacteria are generally gram positive bacteria, non motile and non spore forming cocci or bacilli. Lactic acid bacteria are facultatively anaerobic or microaerophilic and they normally grow at low oxygen concentration. Regarding temperature range these bacteria are mesophilic and grow within the temperature range of 10-45^{o}C. These bacteria grow easily within a wide pH range of 3.5-9.6. They are distinguished from other organisms because of their capability to ferment hexose sugar like glucose to lactic acid. Most of the lactic acid production is carried out using the genus *Lactobacillus*, *L. delbrueckii* is the most commonly used species. Other species used for the batch fermentation are *L. casei, L. plantarum, L.rhamnosus, L.pentosus* etc. Along with *Lactobacilli* certain species of lactococci like d *L. lactis ssp. Lactis*, *Enterococcus faecalis* has been employed for the lactic acid production.

The genus is divided into three groups based on fermentation patterns:

1. Homofermentative: produce more than 85 % lactic acid from glucose.
2. Heterofermentative: produce only 50 % lactic acid and considerable amounts of ethanol, acetic acid and carbon dioxide.
3. Less well known heterofermentative species which produce DL-lactic acid, acetic acid and carbon dioxide

Organisms used for the production are selected using criteria like yields(grams/ gram of substrate utilized), substrate(carbohydrate) to be fermented, pH tolerance, temperature used for the process, lactic acid tolerance, productivity(grams/litre/ hour) also the phage resistance is considered while selecting the strain for the commercial production. (Litchfield, 2009).

Most of the lactic acid production is aimed at using lactic acid bacteria, filamentous fungi, like *Rhizopus,* utilize glucose to produce lactic acid under aerobic conditions. Starch can be fermented to L(+)-lactic acid by various species of *Rhizopus* like *R. oryzae* and *R. arrhizus*. Although L(+)-lactic acid can be formed from fungal strains it has some disadvantages, as the fungal growth increases it increases the viscosity of the medium which makes it difficult for oxygen supply and the rotation of impeller as fungal mycelia tend to wrap around the impeller. It also causes obstruction in sampling process. Genetically modified strains of yeast, *Saccharomyces cerevisiae*, have been used for large scale lactic acid production on experimental basis. *Saccharomyces cerevisiae* is more resistant to pH fluctuations as compared to lactic acid bacteria, which provides an added advantage to the process.

Industrial strains of lactic acid bacteria/fungi or yeast must have some desired characteristics: Rapid and complete fermentation of the raw material.

Must produce lactic acid from cheap raw materials.
Must require minimum amount of nitrogenous material.
Produce desired stereo isomer of lactic acid in pure form.
Able to tolerate lower pH, higher temperature.
Must produce very small amount of cell mass and other by products.

Biochemistry of Lactic acid production

Lactic acid can be formed as L(+) form or D(-) form depending upon the organism used. Some species of *Lactobacilli* produce L(+) form which is converted to D(-) form by the enzyme racemase which converts L(+) form to D(-) form until the equilibrium is reached.. There are some organisms which produce two types of lactate dehydrogenses(LDH); hence they form both L(+) as well as D(-) form of lactic acid.

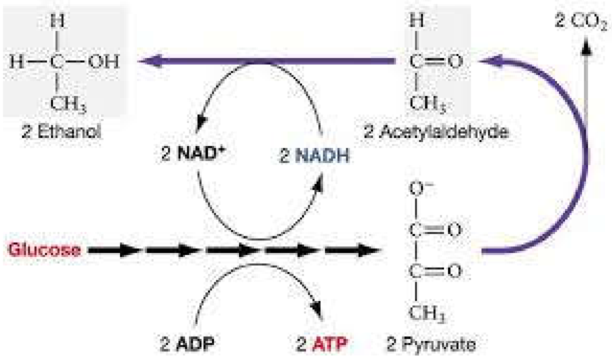

Fig 2 Lactic acid fermentation

Microbial fermentation

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 (Ghaffar *et al*., 2014).

From a metabolic standpoint, they are either homofermentative (produce lactic acid only) or heterofermentative (produce lactic acid and other metabolic products).

Homofermentative Metabolism

The homofermentative LAB usually metabolize glucose via the Embden-Meyerhof pathway (i.e. glycolysis). Since glycolysis results only in lactic acid as a major end-product of glucose metabolism, two lactic acid molecules are produced from each molecule of glucose. Only the homofermentative LAB are available for the commercial production of lactic acid (Wee al., 2006). The terminal electron acceptor in this pathway is pyruvate which is reduced to lactic acid.

Heterofermentative Metabolism

Heterofermentativie process involves the action of lactic acid bacteria *Lecuconostoc mesenteroides* which produces lactic acid, carbon dioxide, ethanol, acetic acid, water and few other products. Bacteria using the heterofermentative pathway use the pentose phosphate pathway. In this pathway, NADPH is generated as glucose is oxidized to ribose 5-phosphate.

Raw materials for the production of lactic acid by LAB

Sustainable cheap raw material with lower levels of contamination is the prime requirement for the industrial scale production of lactic acid. Raw materials must give higher yields, facilitate easy and cheap recovery of the product, must favour optimum biomass production, lower by product formation, cheap and simple pre-treatment requirements, faster rates of product formation, must be cheap and available throughout the year. Selection of the raw material is based on the desired product and the selected organism (Bayitse *et al*., 2015).

Biomass as raw material in the form of starch (corn, wheat, potato, cassava, rice, sweet sorghum) and lignocelluloses (corn cobs, waste paper and woody materials) can be used as a substrate for fermentation of lactic acid. Molasses can also be used for the production but it gives very low product and the recovery of lactic acid is very laborious. Glucoseobatained by starch conversion, mannitol, sucrose obatained from syrups, juices etc., lactose obatained from dairy industry have been commercially employed for the commercial production. Whey, a major by-product of the dairy industry which serves as an inexpensive medium for lactic acid production has have expensive purification processes like molasses. (Narayanan, N *et al*., 2004). Many nitrogenous materials have been studied which are supplemented to carbohydrate sources to give fast and heavy growth like whey permeate, yeast extract, malt sprouts, malt combing nuts, grass extract, peptones, beef extract, casein hydrolysate, corn steep liquor, N-Z-amine, soybean hydrolysate.

Production of lactic acid by fermentation processes

Batch, fed-batch, repeated batch, and continuous fermentations are the most frequently used methods for lactic acid production. Batch and fed-batch cultures may give higher concentrations of lactic acid, but continuous culture gives higher productivity of lactic acid. Second advantage of continuous culture is, it can be continued for a prolonged time period. (Vijaykumar *et al*., 2008).

Commercial scale lactic acid fermentation processes have been operated in a batch fermentation mode, in which the medium is inoculated with cells of lactic acid bacteria and maintained in suspension by agitation or with mycelia of *Rhizopus* spp. and maintained by combined aeration and agitation. The size of inoculum is usually 5-10% of the liquid volume in this fermentor. The fermentation is usually kept at 35^0C-45^0C and at pH 5-6.5 by adding a suitable base, such as ammonium hydroxide. 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. Homofermentative strains such as *Lactobacillus delbrueckii* subsp. and *Lactobacillus helveticus* generally give greater than 90% of the theoretical yield of lactic acid from glucose in both laboratory as well as commercial fermentations (Nagarjun *et al*., 2015).

Process control

Process controls in lactic acid production by fermentation include temperature; pH; and concentrations of microbial cells, substrates, and lactic acid. Temperatures during fermentation can be controlled by cooling water coils immersed in fermenter vessels or by external cooling loops for continuous flow immobilized cell bioreactors. pH is a critical control factor in maximizing lactic acid concentrations. Today, pH can be easily monitored and manipulated by automatic control systems, lactic acid formed during the fermentation reduces the pH which is immediately neutralized to the predefined value. Lactic acid production processes suffer from end-product inhibition. An un-dissociated lactic acid moves through the bacterial membrane and dissociates within the cell. The inhibition mechanism of lactic acid is probably related to the solubility of the un-dissociated lactic acid within the cytoplasmic membrane and the insolubility of dissociated lactate, which results in acidification of cytoplasm and obstructs the formation of proton motive forces. It decreases the amount of energy available to the cell by affecting the trans-membrane pH gradient. Therefore, to alleviate the inhibitory effect of lactic acid during the fermentation, it must be removed selectively in situ from the fermentation broth (Wee *et al*., 2006).

Purification and Recovery of lactic acid

Product recovery and purification are the important steps in the recovery of lactate, it is involved with the separation of lactate 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 purity and avoid lactic acid losses. For its uses, lactic acid must be free of residual sugars, other organic acids, amino acids, proteins, vitamins, complex organic nitrogen compounds, heavy metals, residual organic solvents, and Maillard reaction products between carbohydrates and amino acids. For the recovery of lactic acid, bacterial fermentation processes employed neutralization with calcium carbonate, heating to 82.2 C (180^0F) to kill the lactic acid bacteria, filtering to separate calcium lactate, acidifying with sulphuric acid, filtering to remove calcium sulphate, concentrating the lactic acid solution by multiple vacuum evaporation, decolorizing with activated carbon, and precipitating heavy metals with sodium sulfide. This process resulted in the production of calcium sulphate (gypsum), which had little or no resale value and create disposal problems (Kucharczyk *et al*., 2010).

a) Fermentation and neutralization fermentation

$C_6H_{12}O_6$ + Ca $(OH)_2$ ---------2$(CH_3CHOHCOO^-)$ Ca^{2+} + $2H_2O$

Carbohydrate Calcium hydroxide Calcium lactate

b) Hydrolysis by H_2SO_4

2$(CH_3CHOHCOO^-)$ Ca^{2+} + H_2SO_4--------- 2 $CH_3CHOHCOOH$ + $CaSO_4$ +

Calcium lactate

Lactic acid

Figure 3 Microbial production of lactic acid

Various processes are employed for the recovery and purification of the lactic acid. In one procedure, the heated and filtered fermentation broth is concentrated to allow crystallization of calcium lactate, followed by addition of sulphuric acid to remove the calcium as calcium sulphate. The lactic acid is then re-crystallized as calcium lactate, and activated carbon is used to remove coloured impurities. As an alternative to the latter step, the zinc salts of lactic acid are sometimes prepared because of the relatively lower solubility of zinc lactate. In other procedure, the free lactic acid is solvent extracted with isopropyl ether directly from the heated and filtered fermentation broth (Vijaykumar *et al*., 2008).

Lactic acid can be purified by distillation of methyl, isobutyl, or 2-methyl isobutyl lactate esters, followed by hydrolysis of the esters. Continuous counter current solvent extraction with isopropyl ether has been practiced on a commercial scale. A batch reactive distillation system has been investigated for recovering lactic acid in which esterification, distillation, and hydrolysis take place in a single unit. This system could reduce equipment costs. However, a large quantity of water would still have to be removed from dilute lactic acid solutions with the attendant energy costs. Ion exchange chromatography is used among the variety of downstream operations to recover and purify the L(+)- lactic acid produced from the microbial fermentation media. More recent methods for lactic acid recovery include centrifugation, or membrane filtration using either microfiltration (0.2 mm pore size) or ultrafiltration to separate the cells and higher-molecular weight residues such as peptides in the spent production medium. Heavy metals are removed using cation exchange resins. Lactic acid can be extracted from the fermentation medium using a strong anion exchanger, such as Amberlite IRA-400 in the chloride form, followed by elution with 1.0 M H_2SO_4 at pH 5.0 or with water at pH 2.0 to give high recoveries in the 86-92% range. Lactic acid can be concentrated by reverse osmosis membrane systems or electrodialysis as an alternative to effect evaporation. Extractive fermentation systems include a water immiscible phase in the fermenter to continuously remove lactic acid and reduce inhibition of the fermentation. Tertiary amines, such as Alamine 336, and solid-phase polymers having tertiary amine groups have been evaluated for extractive fermentation recovery of lactic acid. It is important to consider the potential toxicity to lactic acid bacteria of solvents used in extractive fermentation processes. Imadazolium ionic liquids are of interest as 'green' low-toxicity replacements for organic solvents (Litchfield, 2009).

CONCLUSIONS

Lactic acid can be obtained from renewable resources like refined sugars, whey, lignocelluloses, molasses and ray starchy materials. Lactate is one of the most important organic acid produced by microbial fermentation, but still there is a huge scope for researchers to develop methods which are more economical and promising than the current ones, and also find new microbes or genetically modify the available microbes to produce lactate of desired quality and in desired quantity.

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