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Recent Development of Lactic Acid Production using Membrane Bioreactors

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Abstract. Lactic acid has been widely used as flavour and preservative in the food, pharmaceutical, leather and textile industries. It can be produced by fermentation process of the substrates with high lactose content, such as cheese whey, soybean milk, corn, and potatoes. Among various existing technologies, membrane bioreactor is one of the promising methods to achieve high productivity of lactic acid. In addition, membrane bioreactor allows integration of fermentation and separation steps, thus it able to simultaneously maintain high cell density, recycle the cells for further use, and continuously remove lactic acid from the fermenter.

Keywords: lactic acid, membrane bioreactor, production yield

1. Introduction

Lactic acid (2-hydroxypropionic acid), CH_3-CHOHCOOH, is a simple organic compound containing both the hydroxyl and carboxylic acid groups. It was first isolated from sour milk by CW Scheele in 1780 and commercially produced by CE Avery in Littleton, MA, USA in 1881 [1-3]. The production demand of lactic acid has been increased over years due to due to its high potential of application in a wide range of fields [4-6]. In 2012, the production of lactic acid was around 259,000 metric tons with the worldwide growth 12–15% per year [7, 8]. In recent years, lactic acid manufacture is mostly based on carbohydrate fermentation. The major manufacturers are Archer Daniels Midland Company (USA), NatureWorks LLC (USA), Purac (The Netherlands), and Galactic S.A. (Belgium).

Lactic acid has been mainly used for food and food-related applications. It is due to the mild acidic taste of lactic acid. In addition, lactic acid is non-volatile, odourless, and classified as GRAS (generally recognized as safe) for use as a general purpose food additive [2]. Therefore, many industries choose lactic acid as a safety flavour and preservative in the food. Lactic acid also has been utilized in the cosmetic industry such as in the manufacture of hygiene and aesthetic products due to its moisturizing, antimicrobial and rejuvenating effects on the skin, as well as of oral hygiene products [8]. The other promising application of lactic acid lies on its polymer, the poly-lactic acid (PLA). It offers tremendous advantages like biodegradability, thermos-plasticity, high strength etc. [9]. PLA is considered as an environment-friendly alternative to substitute plastics derived from petrochemicals [10]. PLA can be applied in medical applications for filling the gaps in bones, producing sutures (stitching material), and joining membranes or thin skins in humans [11].

Another potential growth area for lactic acid derivatives is environmentally friendly solvents, particularly lactate esters of low molecular weight alcohols such as ethyl, propyl and butyl lactate. Oxygenated chemicals such as propylene glycol, propylene oxide, acrylic acid, acrylate esters, and other chemical intermediates such as lactate ester plasticizers also can be made from lactic acid [2, 3]. The schematic diagram of potential products from lactic acid derivatives can be seen in Figure 1.

The production of lactic acid has been dominated by carbohydrates fermentation processes. Various studies aimed to improve lactic acid productivity using membrane bioreactors. In membrane bioreactor, membrane module is integrated with conventional fermenters, thus permit simultaneous production and purification of lactic acid in the same unit. Membrane bioreactor can be operated in various modes, such as batch, semi-continuous, continuous, immobilized, and membrane recycle.

Membrane bioreactor offers advantages of great flexibility in scale of production depending on market demand as well as high levels of separation and purification. In addition, membrane bioreactor eliminates the requirement of separate purification units and results in compact design with reduced capital investment [12]. For better understanding, this paper then aims to give a brief review of recent development in lactic acid production using membrane bioreactors. The configuration and performance of membrane bioreactors are discussed.

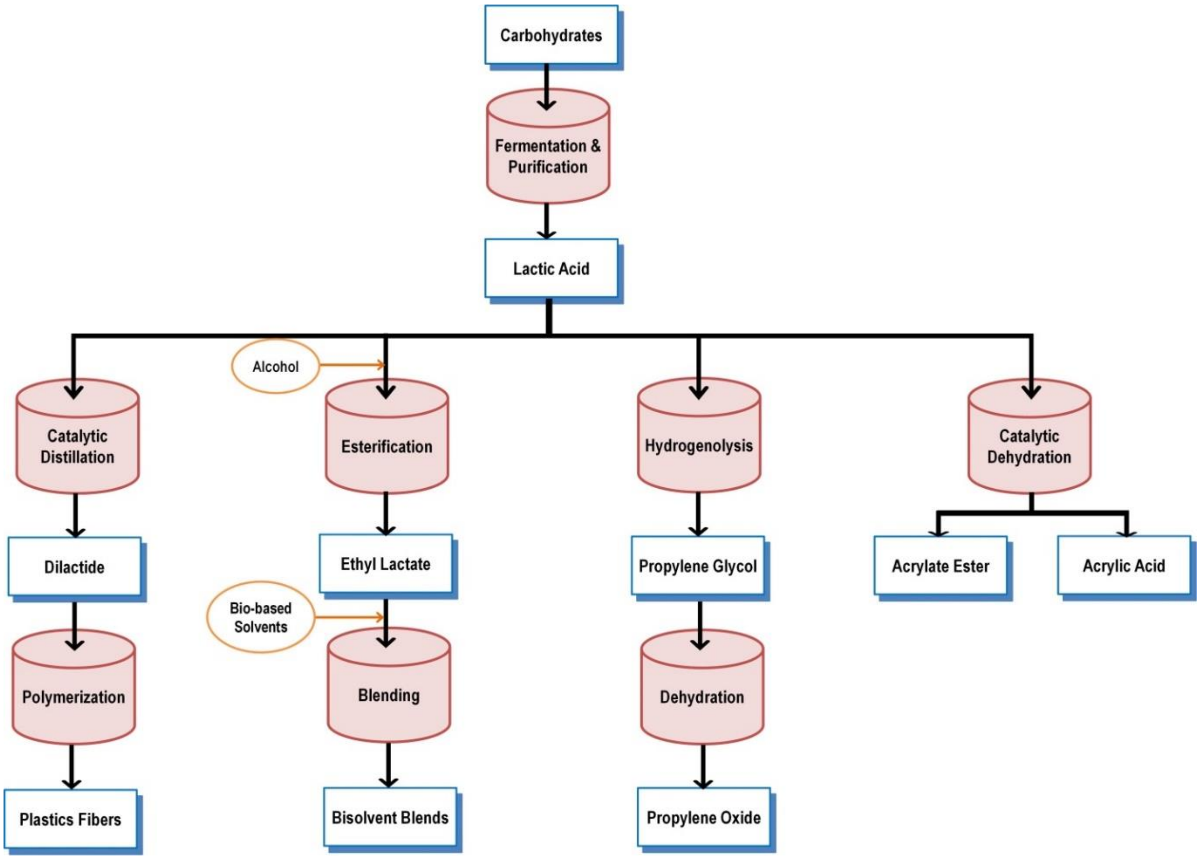

Figure 1. schematic diagram of potential products from lactic acid derivatives

2. Experimental

In general, lactic acid can be manufactured by either chemical synthesis or carbohydrates fermentation. The chemical synthesis is mainly based on the hydrolysis of lacto nitrile by a strong acid and produces only a racemic mixture of lactic acid [13]. Meanwhile, a fermentation process is able to produce a stereoisomer of lactic acid [7]. Fermentation process is more attractive in terms of its environmental impact, low production cost, decreased fossil-based feedstock dependency, reduction of CO_2 emission, and high product specificity [14]. Therefore, fermentation process is preferred for the production of lactic acid, approximately 90% from the total lactic acid production worldwide [13, 15].

Table 1. Comparison of different strains and substrates for lactic acid production

Substrate	Organism	Yield (g/g)	Ref.
Glucose	*Lactobacillus casei*	0.91	[16]
	Lactobacillus rhamnosus	0.74-0.93	[16-18]
	Lactobacillus delbrueckii	0.57-0.83	[19]
	Lactobacillus salivarius	0.92	[18]
	Lactobacillus zeae	0.71-0.98	[17, 20]
	Lactobacillus coryniformis	0.98	[20]
Lactose	*Lactobacillus rhamnosus*	0.38	[21]
	Lactococcus Lactis	1.50	[21]
Molasses	*Lactobacillus delbrueckii*	0.45-0.58	[22]
	Lactobacillus rhamnosus	0.40	[22]
Hydrolized barley flour	*Lactobacillus amylovorus*	0.52	[23]
	Lactobacillus casei	0.67	[23]
	Lactobacillus delbrueckii	0.85	[24]
	Lactobacillus rhamnosus	0.74	[24]
Hydrolized wheat flour	*Lactococcus Lactis*	0.76	[25]
	Lactobacillus delbrueckii	0.11-0.82	[25]
Paneer whey	*Lactobacillus kefir*	0.20	[26]
	Lactobacillus acidophilus	0.17	[26]
Whey	*Lactobacillus casei*	0.32	[27]
	Leuconostoc Lactis	0.22	[27]
Whey permeate	*Streptococcus Thermophilus*	0.35-0.50	[28-30]
	Lactobacillus delbrueckii	0.18-0.41	[28-30]
	Lactococcus Lactis	0.20-0.88	[28, 29, 31]
	Lactobacillus rhamnosus	0.71	[31]
Solid waste	*Lactobacillus plantarum*	0.42-0.46	[32]
	Lactobacillus pentosus	0.43-0.51	[32]
	Lactococcus Lactis	0.16	[32]

2.1. Carbohydrate Resources

Lactic acid can be produced from various substrates, either sugar in pure form such as glucose, sucrose, lactose etc. or sugar-containing materials such as whole-wheat powder [33], starch [13, 34], cucumber juice [35], cheese whey [36, 37], molasses [38], and sugarcane juice [39]. The selection of carbohydrate feedstock depends on the price, availability and its purity. The purified sugars are mostly expensive as the feedstock for lactic acid production. Therefore, many researchers utilized agricultural by-products such as corn starch, cassava starch, cottonseed hulls, wheat bran, sugarcane press mud, barley starch, carrot processing waste, corn fiber hydrolyzates, and potato starch as potential substrates for lactic acid production [9]. Meanwhile, proteinaceous and other complex nutrients that required by the organisms can be provided from corn steep liquor, yeast extract, soy hydrolysate, etc. [3, 39, 40].

2.2. Microorganisms

The characteristic of the produced lactic acid depends much on the type of organism. In general, microorganisms for lactic acid production can be classified into homofermentative strain and heterofermentative strain. A homofermentative strain produces a single product, lactic acid only, while heterofermentative strain produces other products such as ethanol, diacetyl, formate, acetoin or acetic acid, and carbon dioxide along with lactic acid [9]. The comparisons of homofermentative strain and heterofermentative strain organisms for lactic acid production are shown in Table 2. The existing commercial lactic acid production processes mainly use homofermentative organisms, especially from the genera *Lactobacillus, Streptococcus, Enterococcus, and Pediococcus*. These organisms exhibit maximum productivity only within a very narrow pH range, preferably in the range of 5.5 to 6.5 [41].

The choice of microorganisms is mainly determined by the resource of carbohydrate. For substrate from glucose, any homofermentative strains of the genus *Lactobacillus* can be used, however the preferred organism is *Lactobacillus delbrueckii* [42]. For lignocellulosic substrates, *Lactobacillus pentosus* is required to maximize the yield [32]. Meanwhile, starch is able to be both hydrolyzed and fermented by certain amylase-producing *Lactobacillus* strains, such as *Lactobacillus amylovorus* [43]. *Lactobacillus bulgaricus* is the most used organism for fermentation of whey. This organism is able to produce lactic acid with yields in the range of 80-90% [44, 45]. Besides, fungal strains such as *Mucor, Monilia*, and *Rhizopus* can also be utilized to produce lactic acid. The best-known fungal source of lactic acid is *Rhizopus oryzae* that able to obtain 63–69% yields of lactic acid from chemically defined media containing 15% glucose [46, 47].

2.3. Methods

Lactic acid is a metabolic product of simple carbohydrates produced by many species of organisms mainly through the fermentative metabolic pathway. After supplementation of nutrients, sugar contained substrates are inoculated with the selected microorganism, and the fermentation takes place [8]. The stoichiometry for homofermentative fermentation of lactic acid from hexose and pentose can be expressed as equation (1) and (2), respectively [48].

$$C_6H_{12}O_6 \rightarrow 2\ C_3H_6O_3 \quad (1)$$

$$C_5H_{10}O_5 \rightarrow C_3H_6O_3 + C_2H_4O_2 \quad (2)$$

The carbohydrate fermentation operates most efficiently and effectively at near neutral pH, which requires neutralization and produces the salt of the acid instead of the acid itself. Meanwhile, pH of the fermentation broth goes on lowering as lactic acid is formed and accumulated. Therefore, pH of the fermentation must be maintained, usually by addition of lime, thus lactic acid (partly) is converted to be calcium lactate. The precipitation and acidification of calcium lactate then generates huge quantity of calcium sulphate (gypsum). Approximately one ton of gypsum by-product is produced for every ton of lactic acid produced by the conventional fermentation, which poses an environmental problem [2]. In addition, the fermentation broths of lactic acid also contain impurities such as a residual sugars, nutrients, and microorganisms, and other organic acids [7, 41]. Therefore, the complex separation steps to recover and purify the lactic acid from the fermentation broths become one of the major economic hurdle and process cost of conventional carbohydrate fermentation process.

Table 2. Comparison of different strains and substrates for lactic acid production

Characteristic	Homofermentative strain	Heterofermentative strain
Products	• Lactic acid	• Lactic acid • Ethanol • Diacetyl • Formate • Acetic acid • Carbon dioxide
Genera	• *Lactococcus* • *Streptococcus* • *Pediococcus* • *Enterococcus* • *Lactobacillus* • *Sporolactobacillus*	• *Leuconostoc* • *Oemococcus* • *Bifidobacterium* • *Lactobacillus*
Selectivity of lactic acid	High selectivity	Low selectivity (high by-product formation)
Availability for commercial production	Available	Not available

Various technologies have been introduced for lactic acids recovery from the fermentation broth, such as precipitation, extraction, adsorption, ion-exchange system, membrane, etc. [51-59]. However, those existing processes still have many obstacles that need to be addressed. Precipitation and liquid-liquid extraction need a large number of chemical agents and energy. They produce a large amount of water effluent as well as solid residue and involve phase changes that lead to quality degradation of citric acid [60-62]. Meanwhile, adsorption has a short lifetime of adsorbents and low capacity [54, 60]. In addition, fouling still becomes the most significant hurdle in membrane processes [63, 64].

3. Results and discussion

The increasing demand of lactic acid and increasing concern over environmental impact of gypsum accumulation as a by-product leads to development of alternative technologies for lactic acid production. Batch, fed-batch, repeated batch, and continuous fermentations have been developed to increase production efficiency of lactic acid. Various studies showed that higher lactic acid concentration could be obtained in batch and fed-batch fermentations, however higher productivity was achieved by the use of continuous fermentations [15]. It is due to the low concentration of microbes in the batch system.

To solve the problem of production efficiency as well as gypsum formation, membrane bioreactor can be a promising alternative. Membrane bioreactor integrates the fermentation and separation step using membrane such as microfiltration, ultrafiltration, Nano filtration, and electro dialysis, thus simultaneously maintaining high cell density, recycling the cells for further use, and continuously removing lactic acid from the fermenter [65-68]. In continuous fermentation processes using membrane bioreactor, components like microbial cells, proteins, nutrients (yeast extract, salts of ammonium, potassium, phosphorus, etc.), unconverted carbon sources, and water are separated by a filtration unit and returned to the fermenter , while the lactic acid is concentrated in the permeate [4, 12, 69]. Therefore, the use of membrane bioreactor in lactic acid production does not produce any harmful bio-product. The detail comparison of membrane bioreactor and conventional technologies for lactic acid production are shown in Table 3.

In general, both polymer (i.e. polysulfone [65] and polyacrylonitrile [70]) and ceramic (alumina [71]) can be used as membrane bioreactor materials to produce lactic acid. Ceramic membrane tends to have better mechanical and chemical resistance than polymer membrane. Meanwhile, polymer membrane offers advantages of flexible configuration and low production cost [72, 73]. There are only a few researchers who mention the material of membrane bioreactor for lactic acid production. They mostly focused on the operation mode and product efficiency.

Table 3. Comparison of membrane bioreactor and conventional technologies for lactic acid production [12, 50, 74]

Characteristic	Membrane bioreactor	Conventional technology
Unit operations	• Fermentation tank • Membrane module	• Fermentation tank • pH adjustment through hydrated lime • Rotary drum filtration • Carbon bleaching • Plate and frame filtration • Calcium lactate evaporation • Crystallization • Extraction • Distillation unit • Dilution
Flexibility parameters • Modules • Production capacity • Steps of operations	 Flexible in size and numbers Flexible Few steps	 Fixed configuration Fixed Large number of steps
Effect to the environment and economic aspects	• Does not produce any harmful bio-product • Free from pre- and post-treatment • Low energy consumption • Use no harsh chemicals	• Generates million tons of gypsum/year • Heat generation due to exothermic reaction • Highly energy intensive • Use harsh chemicals, especially acids and alkalis

In lactic acid production, the operation mode of membrane bioreactor can be divided into batch, semi-continuous, continuous, immobilized, and membrane recycle. Among those modes, membrane recycle bioreactor has the highest efficiency in production of lactic acid, as shown in Table 4. However, the high productivity is not the only requirement for the economic feasibility of the process. Timmer and Kromkamp [44] investigated that the process might be primarily influenced by production capacity and product concentration and to a lesser extent by the volumetric productivity when annual lactic acid production capacity rose to as high as 4540 metric tons. When lactic acid concentration is significantly low, the energy cost for water removal in the downstream process offsets the benefits of the increased productivity.

In 1986, Mehaia and Cheryan [37] produced lactic acid from acid whey permeate in a membrane recycle bioreactor. Whey permeate was obtained by ultrafiltration of cottage cheese whey and supplemented with yeast extract. The lactose in the permeate was converted into lactic acid by Lactobacillus bulgaricus in a high-performance membrane bioreactor configured in the cell recycle mode. At a cell concentration of 10 g/L, optimum productivity of lactic acid was only 35 g/L.h. Meanwhile, lactic acid productivity of 80 g/L.h only could be obtained at cell concentration of 60 g/L. Other studies [89-93] also showed that lactic acid concentration produced by membrane bioreactor was still below 60 g/L.

To enhance the economic advantage of the membrane bioreactor process, methods that increase the lactic acid concentration along with the high-cell density are required. Ohashi et al. [84] studied continuous production of lactic acid by retaining cells at a high density of Lactococcus lactis in a stirred ceramic membrane reactor (SCMR). The mass concentration and productivity of lactic acid reached 40 g/L and 10.6 g/L.h, respectively. In 1998, Kamoshita et al. [94] developed the improved SCMR system. Using the improved SCMR system, a cell mass concentration of 178 g/L and viability of 98 % were obtained after 198 h of fermentation. They demonstrated that the improved permeability of the SCMR with the use of a membrane cleaning system influenced a rapid increase in the concentration and viability of cells, and accordingly, the increased production rate of lactic acid in proportion to the concentration of viable cells.

Table 4. Comparison of bioreactors for lactic acid production

Bioreactor	**Substrate**	**Organism**	**Lactic acid (g/L)**	**Productivity (g/L.h)**	**Ref.**
Batch	Banana wastes	*Lactobacillus casei*	-	0.13	[75]
	Cassava bagasse	*Lactobacillus delbrueckii*	81.9	1.36	[76]
	Sugar cane baggage	*Lactococcus lactis*	10.9	0.17	[77]
	Cheese whey	*Lactobacillus bulgaricus*	9.6	4.8	[78]
Semi-continuous	Whey permeate	*Lactobacillus helveticus*	55	3.54	[69]
Continuous	Whey permeate	*Lactobacillus helveticus*	64	22	[79]
	Glucose	*Lactobacillus lactis*	210	2.2	[80]
	Cheese whey	*Lactobacillus helveticus*	38	19-22	[81]
Immobilized	Glucose	*Lactobacillus lactis*	115	2.25	[82]
	Liquid distillery stillage	*Lactococcus rhamnosus*	42.2	1.22	[83]
Membrane recycle	Whey permeate	*Lactobacillus bulgaricus*	43	85	[37]
	Molasses	*Lactococcus lactis*	40	10.6	[84]
	Glucose	*Lactococcus rhamnosus*	88	35.2	[85]
	Glucose	*Lactococcus paracasei*	91	31.5	[86]
	Glucose	*Lactococcus paracasei*	120	150	[87]
	Glucose	*Lactobacillus delbrueckii*	35	76	[88]

Another effort to increase lactic acid productivity was developed by combining the advantage of both membrane bioreactor and multi-staged bioreactor. Kulozik et al. [95] investigated the performance of a seven-staged cascade reactor with cell recycle. In comparison with a single-stage membrane bioreactor, the cascade reactor resulted 4 times higher productivity, 28 g/L.h, in which the cell concentrations were maintained at 20 g/L and the lactic acid concentrations were around 72 g/L. In 2001, Kwon et al. [96] produced lactic acid by a two-stage cell-recycle culture of Lactococcus rhamnosus. The membrane cell-recycle bioreactors were arranged in a series and successfully obtained 92 g/L of lactic acid with a productivity of 57 g/L.h. Meanwhile, Xu et al. [86] developed a membrane cell-recycle bioreactor that equipped with a diaphragm pump and tangential flow-rate controller to produce lactic acid by Lactococcus paracasei. The maximum productivity of this system was 31.5 g/L.h.

Later, Danner et al. [97] developed UF membrane bioreactor coupled with on-line monopolar electro dialysis to recover, pre-purify, and concentrate lactic acid. The results showed that the volumetric productivity was low (1.38 g/L h). The lactic acid concentration was 35 g/L with lactic acid yield on consumed glucose appeared stable at around 80%.

4. Conclusion

Fermentative production of lactic acid has roused interest among researchers in the recent years due to its high potential of application in a wide range of fields. High purity lactic acid could be produced and separated continuously in a fully membrane-integrated fermentation process using a cheap and renewable carbon source. Various types of substrate such as whole-wheat powder, starch, cheese whey, molasses, and sugarcane juice have been investigated to produce lactic acid with the help of organisms, especially from the genera *Lactobacillus*.

In general, the efficiency of lactic acid production by membrane bioreactor is higher than conventional technologies. However, several studies showed that membrane bioreactor was only able to produce lactic acid with concentration below 60 g/L. Therefore, further development of methods that increase the lactic acid concentration was required. The improvement in continuous fermentation could be done by increasing the cell density in the bioreactor, thus enhances the substrate-to-product conversion rate and resulting in higher productivity. The increase of cell densities in the fermentation process can be conducted either by the use of cell immobilization or by cell-recycle using membrane

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