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review

Biotechnological Production of Lactic Acid and Its Recent Applications

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Summary

Lactic acid is widely used in the food, cosmetic, pharmaceutical, and chemical industries and has received increased attention for use as a monomer for the production of biodegradable poly(lactic acid). It can be produced by either biotechnological fermentation or chemical synthesis, but the former route has received considerable interest recently, due to environmental concerns and the limited nature of petrochemical feedstocks. There have been various attempts to produce lactic acid efficiently from inexpensive raw materials. We present a review of lactic acid-producing microorganisms, raw materials for lactic acid production, fermentation approaches for lactic acid production, and various applications of lactic acid, with a particular focus on recent investigations. In addition, the future potentials and economic impacts of lactic acid are discussed.

Key words: lactic acid, poly(lactic acid), lactic acid bacteria, fermentation, biodegradable polymer

Introduction

Lactic acid has a long history of uses for fermentation and preservation of human foodstuffs (*1*). It was first discovered in sour milk by Scheele in 1780, who initially considered it a milk component. In 1789, Lavoisier named this milk component »acide lactique«, which became the possible origin of the current terminology for lactic acid. In 1857, however, Pasteur discovered that it was not a milk component, but a fermentation metabolite generated by certain microorganisms (*2*).

Lactic acid can be produced by either microbial fermentation or chemical synthesis (Fig. 1). In the early 1960s, a method to synthesize lactic acid chemically was developed due to the need for heat-stable lactic acid in the baking industry (*3*). There are two optical isomers of lactic acid: L(+)-lactic acid and D(–)-lactic acid. Lactic acid is classified as GRAS (generally recognized as safe) for use as a food additive by the US FDA (Food and Drug Administration), but D(–)-lactic acid is at times harmful to human metabolism and can result in acidosis and decalcification (*4*). Although racemic DL-lactic acid is always produced by chemical synthesis from petrochemical resources, an optically pure L(+)- or D(–)-lactic acid can be obtained by microbial fermentation of renewable resources when the appropriate microorganism that can produce only one of the isomers is selected (*5*). The optical purity of lactic acid is crucial to the physical properties of poly (lactic acid) (PLA), and an optically pure L(+)- or D(–)-lactic acid, rather than racemic DL-lactic acid, can be polymerized to a high crystalline PLA that is suitable for commercial uses (*6*,*7*). Therefore, the biotechnological production of lactic acid has received a significant amount of interest recently, since it offers an alternative to environmental pollution caused by the petrochemical industry and the limited supply of petrochemical resources.

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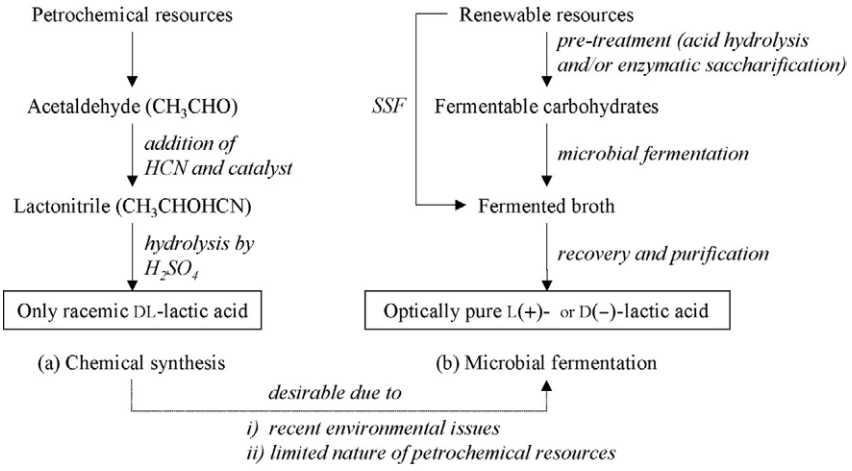

Fig. 1. Overview of the two manufacturing methods of lactic acid; chemical synthesis (a) and microbial fermentation (b). SSF represents simultaneous saccharification and fermentation

Lactic acid is now considered to be one of the most useful chemicals, used in the food industry as a preservative, acidulant, and flavouring, in the textile and pharmaceutical industries, and in the chemical industry as a raw material for the production of lactate ester, propylene glycol, 2,3-pentanedione, propanoic acid, acrylic acid, acetaldehyde, and dilactide (*8,9*). Recently, lactic acid consumption has increased considerably because of its role as a monomer in the production of biodegradable PLA, which is well-known as a sustainable bioplastic material (*4,10*). The worldwide demand for lactic acid is estimated roughly to be 130 000 to 150 000 (metric) tonnes per year (*11*). However, the global consumption of lactic acid is expected to increase rapidly in the near future. NatureWorks LLC, a major PLA manufacturer established in the US, expects that the global PLA market may increase to 500 000 (metric) tonnes per year by 2010 (*12*).

Biotechnological processes for the production of lactic acid usually include lactic acid fermentation and product recovery and/or purification. There have been numerous investigations on the development of biotechnological processes for lactic acid production, with the ultimate objectives to enable the process to be more efficient and economical. This article presents a review of recent advances in the biotechnological production of lactic acid, as well as its recent applications and the future prospects of biologically-derived lactic acid.

Lactic Acid-Producing Microorganisms

Microorganisms that can produce lactic acid can be divided into two groups: bacteria and fungi (*10*). The microorganisms selected for recent investigations of the biotechnological production of lactic acid are listed in Table 1 (*13–24*). Although most investigations of lactic acid production were carried out with lactic acid bacteria (LAB), filamentous fungi, such as *Rhizopus*, utilize glucose aerobically to produce lactic acid (*13,25,26*). *Rhizopus* species such as *R. oryzae* and *R. arrhizus* have amylolytic enzyme activity, which enables them to convert starch directly to L(+)-lactic acid (*27,28*). Fungal fermentation has some advantages in that *R. oryzae* requires only a simple medium and produces L(+)-lactic acid, but it also requires vigorous aeration because *R. oryzae* is an obligate aerobe (*25*). In fungal fermentation, the low production rate, below 3 g/(L·h), is probably due to the low reaction rate caused by mass transfer limitation (*14*). The lower product yield from fungal fermentation is attributed partially to the formation of by-products, such as fumaric acid and ethanol (*25*).

Table 1. Microorganisms used for recent investigations of the biotechnological production of lactic acid

Organism	γ(lactic acid) g/L	η(yield) g/g	Productivity g/(L·h)	Reference
Rhizopus oryzae ATCC 52311	83.0	0.88	2.6	(*13*)
Rhizopus oryzae NRRL 395	104.6	0.87	1.8	(*14*)
Enterococcus faecalis RKY1	144.0	0.96	5.1	(*15*)
Lactobacillus rhamnosus ATCC 10863	67.0	0.84	2.5	(*16*)
Lactobacillus helveticus ATCC 15009	65.5	0.66	2.7	(*17*)
Lactobacillus bulgaricus NRRL B-548	38.7	0.90	3.5	(*18*)
Lactobacillus casei NRRL B-441	82.0	0.91	5.6	(*19*)
Lactobacillus plantarum ATCC 21028	41.0	0.97	1.0	(*20*)
Lactobacillus pentosus ATCC 8041	21.8	0.77	0.8	(*21*)
Lactobacillus amylophilus GV6	76.2	0.70	0.8	(*22*)
Lactobacillus delbrueckii NCIMB 8130	90.0	0.97	3.8	(*23*)
Lactococcus lactis ssp. *lactis* IFO 12007	90.0	0.76	1.6	(*24*)

Several attempts have been made to achieve higher cell density, lactic acid yield, and productivity in fungal fermentation. Tay and Yang (*25*) immobilized *R. oryzae* cells in a fibrous bed to produce lactic acid from glucose and starch. Kosakai *et al.* (*26*) cultured *R. oryzae* cells with the use of mycelial flocs formed by the addition of mineral support and poly(ethylene oxide). They observed that cotton-like mycelial flocs were the optimal morphology in the culture of *R. oryzae*. Park *et al.* (*14*) reported that lactic acid production was enhanced in a culture of *R. oryzae*, by the induction of mycelial floc morphology. Their results also suggested that cotton-like mycelial flocs were the optimal morphology for use in the air-lift bioreactor culture of *R. oryzae*. Although there have been persistent attempts to produce lactic acid through fungal fermentation, LAB have been commonly used for the production of lactic acid due to the aforementioned disadvantages of fungal fermentation.

Lactic acid bacteria can be classified into two groups: homofermentative and heterofermentative. While the homofermentative LAB convert glucose almost exclusively into lactic acid, the heterofermentative LAB catabolize glucose into ethanol and CO_2 as well as lactic acid (Fig. 2) (*5,29*). 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 with a yield of more than 0.90 g/g (*30,31*). Only the homofermentative LAB are available for the commercial production of lactic acid (*5,15*).

Recently, strains used in the commercial production of lactic acid has become almost proprietary, and it is believed that most of the LAB used belong to the genus *Lactobacillus* (*4,5*). Berry *et al.* (*16*) attempted to produce lactic acid by batch culture of *L. rhamnosus* in a defined medium. Schepers *et al.* (*17*) used *L. helveticus* for the production of lactic acid from lactose and concentrated cheese whey, and Burgos-Rubio *et al.* (*18*) reported the kinetic investigation of the conversion of different substrates into lactic acid with the use of *L. bulgaricus*. Hujanen and Linko (*19*) investigated the effects of culture temperature and nitrogen sources on lactic acid production by *L. casei*, and Roukas and Kotzekidou (*32*) also used this strain for lactic acid production from deproteinized whey by mixed cultures of free and coimmobilized cells. Fu and Mathews (*20*) investigated the kinetic model of lactic acid production from lactose by batch culture of *L. plantarum*, and Bustos *et al.* (*21*) used *L. pentosus* for the production of lactic acid from vine-trimming wastes. The strains of amylase-producing *L. amylophilus* were used often for the direct conversion of starch into lactic acid (*22,33,34*).

However, among the genus *Lactobacillus*, *L. delbrueckii* has appeared commonly in many investigations on the production of lactic acid. Kotzanmanidis *et al.* (*23*) used *L. delbrueckii* NCIMB 8130 for lactic acid production from beet molasses. Monteagudo *et al.* (*35*) and Göksungur and Güvenç (*36*) also attempted to produce lactic acid from beet molasses with *L. delbrueckii*. In addition to lactobacilli, strains of lactococci were often used for lactic acid production. Roble *et al.* (*24*) co-cultured *Lactococcus lactis* ssp. *lactis* cells with *Aspergillus awamori* for lactic acid production from cassava starch, and Åkerberg *et al.* (*37*) used *L. lactis* ssp. *lactis* for modeling the kinetics of lactic acid production from whole wheat flour. Moreover, Yun *et al.* (*15*) and Wee *et al.* (*38*) reported the production of lactic acid by batch culture of a newly isolated species, *Enterococcus faecalis*.

Efforts have been made to improve the production of lactic acid through metabolic engineering approaches. Kylä-Nikkilä *et al.* (*39*) attempted to express L-lactate de-

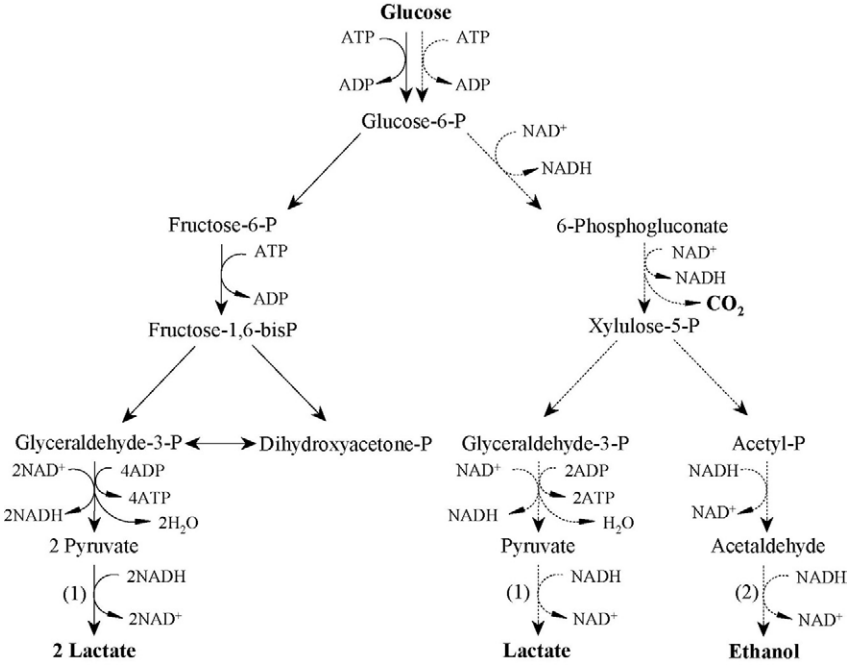

Fig. 2. Metabolic pathways of homofermentative (solid line) and heterofermentative (dotted line) lactic acid bacteria: P, phosphate; ADP, adenosine 5'-diphosphate; ATP, adenosine 5'-triphosphate; NAD^+, nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide (reduced form); (1), lactate dehydrogenase; (2), alcohol dehydrogenase

hydrogenase and D-lactate dehydrogenase genes in *L. helveticus* for the production of pure D(–)- and L(+)-lactic acids. They constructed two D-lactate dehydrogenase gene--negative *L. helveticus via* a gene replacement method for the production of pure L-lactic acid. Each L-lactate dehydrogenase activity of two D-lactate dehydrogenase-deficient *L. helveticus* was 53 or 93 % higher than that of the wild type strain. Dien *et al.* (*40,41*) constructed recombinant *Escherichia coli* for the conversion of hexose sugar, as well as pentose sugar, into L(+)-lactic acid, and they metabolically engineered the *E. coli* for the construction of carbon catabolite repression mutants. Similarly, Chang *et al.* (*42*) constructed recombinant *E. coli* for the production of optically pure D(–)- or L(+)-lactic acid. They introduced L-lactate dehydrogenase genes from *L. casei* into a *pta ldhA* strain, which lacked phosphotransacetylase and D-lactate dehydrogenase. Their results suggested that the central fermentation metabolism of *E. coli* can be reoriented to the production of D(–)- or L(+)-lactic acid. Recent advances in metabolic engineering of microorganisms may provide more opportunities for selective and efficient production of optically pure lactic acid through the improvement of future strains.

Lactic acid bacteria typically have complex nutritional requirements, due to their limited ability to synthesize their own growth factors such as B vitamins and amino acids. They require some elements for growth, such as carbon and nitrogen sources, in the form of carbohydrates, amino acids, vitamins, and minerals (*29,43,44*). There are several growth-stimulation factors that have a considerable effect on the production rate of lactic acid. The mixture of amino acids, peptides, and amino acid amides usually stimulates the growth of LAB, and the resulting growth rates are much higher than those obtained with free amino acids (*43*). Fatty acids also influence LAB growth, and phosphates are the most important salt in lactic acid fermentation. Ammonium ions cannot serve as the sole nitrogen source, but they seem to have some influence on the metabolism of certain amino acids. Since minerals do not seem to be essential to LAB growth, the amount found in commercial complex media is usually sufficient (*29,45*). Temperature and pH are also important factors influencing LAB growth and lactic acid production (*5*). In general, the desirable characteristics for industrial LAB are the abilities to rapidly and completely convert cheap raw materials into lactic acid with minimal nutritional requirements and to provide high yields of preferred stereoisomer without by-product formation.

Raw Materials for Biotechnological Production of Lactic Acid

In order for the biotechnological production of lactic acid to be feasible, cheap raw materials are necessary, because polymer producers and other industrial users usually require large quantities of lactic acid at a relatively low cost. Raw materials for lactic acid production should have the following characteristics: cheap, low levels of contaminants, rapid production rate, high yield, little or no by-product formation, ability to be fermented with little or no pre-treatment, and year-round availability (*3*). When refined materials are used for production, the costs for product purification should be significantly reduced. However, this is still economically unfavourable because the refined carbohydrates are so expensive that they eventually result in higher production costs (*5*). Therefore, there have been many attempts to screen for cheap raw materials for the economical production of lactic acid. Reports in the literature of recent investigations are listed in Table 2 (*23,38,46–61*).

Table 2. Reports in the literature about recent investigations on the biotechnological production of lactic acid from cheap raw materials

Raw material	Organism	γ(lactic acid) g/L	Productivity g/(L·h)	Reference
Molasses	*Lactobacillus delbrueckii* NCIMB 8130	90.0	3.8	(*23*)
	Enterococcus faecalis RKY1	95.7	4.0	(*38*)
Rye	*Lactobacillus paracasei* No. 8	84.5	2.4	(*46*)
Sweet sorghum	*Lactobacillus paracasei* No. 8	81.5	2.7	(*46*)
	Lactobacillus paracasei No. 8	106.0	3.5	(*47*)
Wheat	*Lactococcus lactis* ssp. *lactis* ATCC 19435	106.0	1.0	(*48*)
	Enterococcus faecalis RKY1	102.0	4.8	(*49*)
Corn	*Enterococcus faecalis* RKY1	63.5	0.5	(*49*)
	Lactobacillus amylovorus ATCC 33620	10.1	0.8	(*50*)
Cassava	*Lactobacillus amylovorus* ATCC 33620	4.8	0.2	(*50*)
Potato	*Lactobacillus amylovorus* ATCC 33620	4.2	0.1	(*51*)
Rice	*Lactobacillus* sp. RKY2	129.0	2.9	(*51*)
Barley	*Lactobacillus casei* NRRL B-441	162.0	3.4	(*52*)
	Lactobacillus amylophilus GV6	27.3	0.3	(*53*)
Cellulose	*Lactobacillus coryniformis* ssp. *torquens* ATCC 25600	24.0	0.5	(*54*)
Corncob	*Rhizopus* sp. MK-96-1196	24.0	0.3	(*55*)
Waste paper	*Lactobacillus coryniformis* ssp. *torquens* ATCC 25600	23.1	0.5	(*56*)
	Rhizopus oryzae NRRL 395	49.1	0.7	(*57*)
Wood	*Lactobacillus delbrueckii* NRRL B-445	108.0	0.9	(*58*)
	Enterococcus faecalis RKY1	93.0	1.7	(*59*)
Whey	*Lactobacillus helveticus* R211	66.0	1.4	(*60*)
	Lactobacillus casei NRRL B-441	46.0	4.0	(*61*)

Cheap raw materials, such as starchy and cellulosic materials, whey, and molasses, have been used for lactic acid production (*5*). Among these, starchy and cellulosic materials are currently receiving a great deal of attention, because they are cheap, abundant, and renewable (*9,46,62*). The starchy materials used for lactic acid production include sweet sorghum (*46,47*), wheat (*9,37,48, 49*), corn (*49,50*), cassava (*50*), potato (*50,63*), rice (*49,51*), rye (*46*), and barley (*49,52,53*). These materials have to be hydrolyzed into fermentable sugars before fermentation, because they consist mainly of α(1,4)- and α(1,6)-linked glucose (*47–49*). This hydrolysis can be carried out simultaneously with fermentation (*52*). Amylase-producing *L. amylophilus* and *L. amylovorus* are often used for the direct fermentation of starchy materials into lactic acid (*50,53,63*).

Cellulosic materials have been used for lactic acid production in similar ways as starchy materials (*5*). These materials consist mainly of β(1,4)-glucan, and often contain xylan, arabinan, galactan, and lignin (*5,10*). Venkatesh (*62*) and Yáñez *et al.* (*54*) have previously attempted to produce lactic acid from pure cellulose through simultaneous saccharification and fermentation (SSF). The utilization of corncob (*55,64*), waste paper (*56,57*), and wood (*58,59*), has been reported as well. Sreenath *et al.* (*65*) investigated the production of lactic acid from agricultural residues such as alfalfa fiber, wheat bran, corn stover, and wheat straw. They suggested that, during SSF of alfalfa fiber, lactic acid production was enhanced by adding pectinase and cellulase together. Garde *et al.* (*66*) used hemicellulose hydrolyzate from wheat straw for lactic acid production by co-culture of *L. brevis* and *L. pentosus*. This study demonstrated that complete substrate utilization was achieved with a mixed culture of two LAB. Fermentation of lignocellulosic hydrolyzate is inhibited usually by inhibitory compounds, such as furfural, 5-hydroxymethyl furfural, and acetic acid, which are generated during pre-treatment of lignocellulose (*67*). Most studies on methods to decrease this inhibition have been focused on the chemical and physical detoxification of the hydrolyzate (*68*). Wee *et al.* (*59*), however, reported that the inhibition of fermentation caused by wood hydrolyzate was reduced to a slight degree by direct adaptation of LAB to the wood hydrolyzate-based medium.

Some industrial waste products, such as whey and molasses, are of interest for common substrates for lactic acid production. Whey is a major by-product of the dairy industry, and it contains lactose, protein, fat, and mineral salts. For complete utilization of whey lactose, it is necessary to supplement whey with an additional nitrogen source (*5*). Amrane and Prigent (*69*), Kulozik and Wilde (*70*), and Schepers *et al.* (*60*) supplemented whey with yeast extract for rapid production of lactic acid with *L. helveticus*. According to Fitzpatrick and O'Keeffe (*71*), the addition of whey protein hydrolyzate to whey medium would make the fermentation more economically viable and would also reduce the amount of unused nutrients left during fermentation. Also, there have been several attempts to produce lactic acid from whey by batch culture of *L. casei* (*61,72,73*). Molasses is a waste product from the sugar manufacturing process, and it usually contains a large amount of sucrose (*5*). *L. delbrueckii* and *E. faecalis* have recently been used for lactic acid production from molasses (*23,35,36,38*). Shukla *et al.* (*74*) also reported D(–)-lactic acid production from molasses with recombinant *E. coli* strain.

It is necessary to supplement the fermentation media with sufficient nutrients for rapid lactic acid production. The most common nutrient for lactic acid production is yeast extract, but this may contribute significantly to an increase in production costs (*3,5*). As an alternative to yeast extract, corn steep liquor, a by-product from the corn steeping process, has been used successfully for lactic acid production (*49*). The nitrogen content of corn steep liquor is dependent on the steeping process used. Since it is derived from corn, 85 % of its total nitrogen content is composed of proteins, peptides, and amino acids (*75*). Yun *et al.* (*51*) suggested that rice bran and wheat bran play important roles as effective nutrients for lactic acid production, because they usually contain several nutritional factors as well as fermentable carbohydrates. Kurbanoglu and Kurbanoglu (*76*) demonstrated that ram horn waste was an effective supplement for lactic acid production. Similarly, Bustos *et al.* (*77*) proposed that vinification lees could be used for the formulation of low-cost media for lactic acid production. According to Wee *et al.* (*78*), wastewater from electrodialyzed fermentation broth still contained some nutrients that could be available to LAB. Their result indicated that, if small amounts of other nutrients were supplemented to electrodialysis wastewater, then the efficiencies of fermentation would be improved significantly.

Fermentation Approaches to Lactic Acid Production

Batch, fed-batch, repeated batch, and continuous fermentations are the most frequently used methods for lactic acid production. Higher lactic acid concentrations may be obtained in batch and fed-batch cultures than in continuous cultures, whereas higher productivity may be achieved by the use of continuous cultures (*5*). Another advantage of the continuous culture compared to the batch culture, is the possibility to continue the process for a longer period of time. Reports in the literature of recent studies on the biotechnological production of lactic acid by different fermentation approaches are listed in Table 3 (*32,79–86*).

The cell-recycle system, together with repeated batch and continuous processes, enables the achievement of a higher cell concentration and product productivity in the process (*79,80*). Oh *et al.* (*79*) produced lactic acid at a rate of 6.4 g/(L·h) through cell-recycle repeated batch fermentation. Their results also indicated that only 26 % of the yeast extract dosage, compared with conventional batch fermentation, should be required to produce the same amount of lactic acid, which might result in a considerable reduction of production costs. The maximum cell concentration in their experiment was greater than 28 g/L, which might contribute to the improvement of the productivity and reduction of nutrient supplementation. A successful approach to continuous production of lactic acid with cell retention has been reported by Kwon *et al.* (*80*), who recently attempted to produce lactic acid by a two-stage cell-recycle culture of *L. rhamno-*

Table 3. Reports in the literature of recent investigations on the biotechnological production of lactic acid by different fermentation approaches

Organism	Fermentation mode	γ(lactic acid) g/L	Productivity g/(L·h)	Reference
Lactobacillus casei SU No 22 + *Lactobacillus lactis* WS 1042	fed-batch, coimmobilization	47.0	2.0	(*32*)
Enterococcus faecalis RKY1	batch	95.7	4.0	(*79*)
	repeated batch, cell-recycle *via* membrane	93.2	6.4	(*79*)
Lactobacillus rhamnosus ATCC 10863	batch	~ 120.0	2.1	(*80*)
	continuous, cell-recycle *via* membrane	92.0	57.0	(*80*)
Lactobacillus casei ssp. *rhamnosus* ATCC 11443	continuous, cell-recycle *via* immobilization	22.4	9.0	(*81*)
Lactobacillus delbrueckii NRRL B445	fed-batch, *in situ* removal *via* solvent extraction	~ 23.1	0.2	(*82*)
Lactococcus lactis IO-1 JCM 7638	batch, *in situ* removal *via* electrodialysis	~ 39.0	0.9	(*83*)
Lactobacillus rhamnosus IFO 3863	batch	98.0	1.9	(*84*)
	continuous, *in situ* removal *via* electrodialysis	~ 20.0	8.2	(*84*)
Lactobacillus helveticus CNRZ 303	continuous, cell-recycle *via* membrane	55.0	7.1	(*85*)
Lactobacillus delbrueckii CECT 286	continuous, *in situ* removal *via* ion-exchange resin	26.1	10.4	(*86*)

sus. They connected the membrane cell-recycle bioreactors in a series, and obtained 92 g/L of lactic acid with a productivity of 57 g/(L·h).

Immobilization of cells has been one of the means for high cell retention in the bioreactor (*87*). Several materials, such as Ca-alginate gels, poly(ethyleneimine), and plastic composite support, have been used for immobilization of LAB in order to produce lactic acid (*36,81,87*). Senthuran *et al.* (*87*) reported the production of lactic acid by continuous culture of *L. casei* immobilized in poly(ethyleneimine). This system was coupled with a cell-recycle bioreactor, and the authors observed that the most important factor for operational stability was the bead size of the matrix. Cotton *et al.* (*81*) tested the immobilized-cell biofilm reactor for continuous production of lactic acid. For biofilm formation, they used a plastic composite support composed mainly of polypropylene.

Lactic acid production processes traditionally suffer from end-product inhibition. An undissociated lactic acid passes through the bacterial membrane and dissociates inside the cell. The inhibition mechanism of lactic acid is probably related to the solubility of the undissociated lactic acid within the cytoplasmic membrane and the insolubility of dissociated lactate, which causes acidification of cytoplasm and failure of proton motive forces. It eventually influences the transmembrane pH gradient and decreases the amount of energy available for cell growth (*29,88*). Therefore, to alleviate the inhibitory effect of lactic acid during the fermentation, it must be removed selectively *in situ* from the fermentation broth.

Recently, various attempts have been carried out to remove the lactic acid simultaneously as it is formed. Hano *et al.* (*89*) studied the reactive extraction of lactic acid from the fermented broth. They indicated that *in situ* extraction was possible with the use of di-*n*-octylamine and with adjustment of the fermentation broth to a pH=5.0 by ammonia. Iyer and Lee (*82*) attempted to extract lactic acid simultaneously with the use of a two-zone fermentor-extractor system. The system was operated under a fed-batch mode with *in situ* removal of lactic acid by solvent extraction. Electrodialysis fermentation with ion exchange membranes was often used for *in situ* removal of lactic acid (*83,90*). Min-Tian *et al.* (*84*) had previously developed a continuous electrodialysis fermentation system for the production of lactic acid. In their study, the system of electrodialysis fermentation with a level meter was the most efficient system and a higher yield could be obtained if the glucose concentration in the broth could be controlled to remain at a lower level. Nanofiltration membranes and ion exchange resins were occasionally coupled with the bioreactor for *in situ* removal of lactic acid (*85,86*).

Current Uses and Applications of Lactic Acid

Lactic acid has received a significant amount of attention as a chemical with many potential applications. There are four major categories for the current uses and applications of lactic acid: food, cosmetic, pharmaceutical, and chemical applications. The potential applications of lactic acid are illustrated in Fig. 3. Since lactic acid is classified as GRAS for use as a food additive by the US FDA (*4*), it is widely used in almost every segment of the food industry, where it serves in a wide range of functions, such as flavouring, pH regulation, improved microbial quality, and mineral fortification. Moreover, lactic acid is used commercially in the processed meat and poultry industries, to provide products with an increased shelf life, enhanced flavour, and better control of food-born pathogens. Due to the mild acidic taste of lactic acid, it is also used as an acidulant in salads and dressings, baked goods, pickled vegetables, and beverages. Lactic acid is used in confectionery, not only for flavour, but also to bring the pH of the cooked mix to the correct point for setting. The advantages of adding lactic acid in confectionery include its low inversion rate, ease of handling, and ability to produce clear candies. Another potential application of lactic acid in the food industry is the mineral fortification of food products (*91,92*).

Lactic acid offers natural ingredients for cosmetic applications. Although primarily used as moisturizers and pH regulators, they possess multiple other properties such as antimicrobial activity, skin lightening, and skin

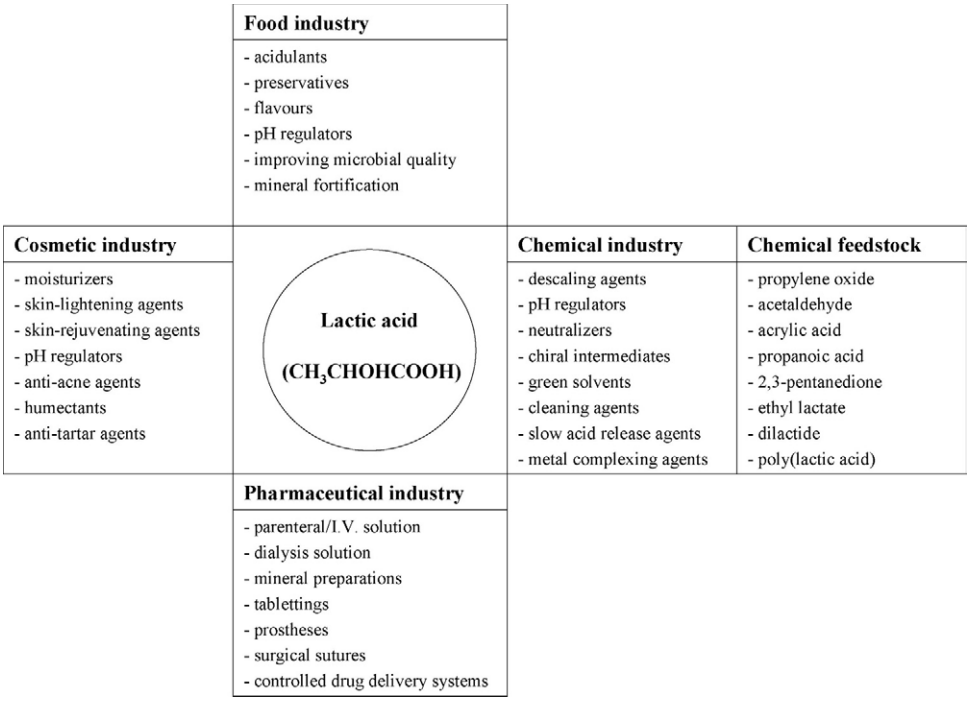

Fig. 3. Diagram of the commercial uses and applications of lactic acid and its salt

hydration. The moisturizing effect is related directly to lactate's water retaining capacity, and the skin-lightening action of lactic acid is produced by the suppression of the formation of tyrosinase. Since they are natural ingredients of the human body, lactic acid and its salt fit perfectly into the modern trend towards natural and safer formulations, and they produce such effects as skin lightening and rejuvenation, which makes them very useful as active ingredients in cosmetics (*91,92*).

Lactic acid is also used in the pharmaceutical industry as an electrolyte in many parenteral/I.V. (intravenous) solutions that are intended to replenish the bodily fluids or electrolytes. Examples include Lactated Ringer's or Hartmann's solutions, CAPD (continuous ambulatory peritoneal dialysis) solution, and dialysis solution for conventional artificial kidney machines. Moreover, lactic acid is used in a wide variety of mineral preparations, which include tablets, prostheses, surgical sutures, and controlled drug delivery systems (*91,92*).

Lactic acid and its salt are used increasingly in various types of chemical products and processes. In this category of applications, lactic acid functions as a descaling agent, pH regulator, neutralizer, chiral intermediate, solvent, cleaning agent, slow acid-release agent, metal complexing agent, antimicrobial agent, and humectant. Natural lactic acid has an emerging use as an excellent and safe solvent, which is alternative in many fine mechanical cleaning applications. Due to the high solvency power and solubility of lactic acid, it is an excellent remover of polymer and resins. It is available with an isomeric purity greater than 98 %, and is suitable as a starting material in the production of herbicides or pharmaceuticals. Since lactic acid offers better descaling properties than conventional organic descalers do, it is often used in many decalcification products, such as bathroom cleaners, coffee machines, and toilets. Ethyl lactate is used in many anti-acne preparations, because it combines excellent solvency power against oils and polymeric stains, with no environmental impact and toxicological effects (*4,91,92*).

Currently, lactic acid is considered the most potential feedstock monomer for chemical conversions, because it contains two reactive functional groups, a carboxylic group and a hydroxyl group. Lactic acid can undergo a variety of chemical conversions into potentially useful chemicals, such as propylene oxide (*via* hydrogenation), acetaldehyde (*via* decarboxylation), acrylic acid (*via* dehydration), propanoic acid (*via* reduction), 2,3-pentanedione (*via* condensation), and dilactide (*via* self-esterification) (*8*). Lactic acid has recently received a great deal of attention as a feedstock monomer for the production of PLA, which serves as a biodegradable commodity plastic. The optically pure lactic acid can be polymerized into a high molecular mass PLA through the serial reactions of polycondensation, depolymerization, and ring-opening polymerization (*7*). The resultant polymer, PLA, has numerous uses in a wide range of applications, such as protective clothing, food packaging, mulch film, trash bags, rigid containers, shrink wrap, and short shelf-life trays (*93,94*). The recent huge growth of the PLA market will stimulate future demands on lactic acid considerably (*4,6*).

Conclusions and Future Potentials

The current major markets for lactic acid are food--related industries, but the emerging markets for PLA polymer would cause a significant increase in growth of lactic acid consumption (*4,10*). Currently, the worldwide consumption of lactic acid is estimated to be 130 000––150 000 (metric) tonnes per year, and the commercial prices of food grade lactic acid range between 1.38 US$/kg

(for 50 % purity) and 1.54 US$/kg (for 88 % purity). Technical grade lactic acid with 88 % purity has been priced as much as 1.59 US$/kg (*11*,*95*). Lactic acid consumption in chemical applications, which include PLA polymer and new »green« solvents, such as ethyl lactate, is expected to expand 19 % per year (*96*).

There are several major manufacturers of fermentative lactic acid, including Purac (Netherlands), Galactic (Belgium), Cargill (USA), and several Chinese companies (*91*,*92*). In late 1997, Cargill joined forces with Dow Chemical and established a Cargill-Dow PLA polymer venture, NatureWorks LLC, which exists today as a stand-alone company. In early 2002, NatureWorks LLC completed the construction of a PLA plant that has the capacity of producing 140 000 (metric) tonnes of PLA per year. Moreover, NatureWorks LLC has recently constructed a major lactic acid facility in Blair, Nebraska, USA, which has the capacity of producing 180 000 (metric) tonnes of lactic acid per year, and it began operating in late 2002 (*96*,*97*). NatureWorks LLC has stated publicly its belief that the PLA market will reach 500 000 (metric) tonnes per year worldwide by 2010, and the construction of two additional PLA plants are being considered presently (*12*,*97*,*98*).

On an industrial scale, the manufacturing cost of lactic acid monomer will be targeted to less than 0.8 US$/kg, because the selling price of PLA should decrease roughly by half from its present price of 2.2 US$/kg. According to the cost analysis by Datta *et al.* (*4*), although their analysis was sensitive to various factors such as plant size, raw material cost, and capital investment, the base manufacturing cost of lactic acid was estimated to be 0.55 US$/kg. However, there are still several issues that need to be addressed in order to produce lactic acid biotechnologically within the targeted cost, such as the development of high-performance lactic acid-producing microorganisms and the lowering of the costs of raw materials and fermentation processes. The biotechnological processes for the production of lactic acid from cheap raw materials should be improved further to make them competitive with the chemically-derived one.

References

1. B.E. Davison, R.L. Llanos, M.R. Cancilla, N.C. Redman, A.J. Hillier, Current research on the genetics of lactic acid production in lactic acid bacteria, *Int. Dairy J. 5* (1995) 763–784.
2. H. Benninga: *A History of Lactic Acid Making*, Kluwer Academic Publishers, Dordrecht, Netherlands (1990) pp. 1–61.
3. T.B. VickRoy: Lactic Acid. In: *Comprehensive Biotechnology*, *Vol. 3*, M. Moo-Young (Ed.), Pergamon Press, New York, USA (1985) pp. 761–776.
4. R. Datta, S.P. Tsai, P. Bonsignore, S.H. Moon, J.R. Frank, Technological and economic potential of poly(lactic acid) and lactic acid derivatives, *FEMS Microbiol. Rev. 16* (1995) 221–231.
5. K. Hofvendahl, B. Hahn-Hägerdal, Factors affecting the fermentative lactic acid production from renewable resources, *Enzyme Microb. Technol. 26* (2000) 87–107.
6. J. Lunt, Large-scale production, properties and commercial applications of polylactic acid polymers, *Polym. Degrad. Stabil. 59* (1998) 145–152.
7. A. Södergård, M. Stolt, Properties of lactic acid based polymers and their correlation with composition, *Prog. Polym. Sci. 27* (2002) 1123–1163.
8. S. Varadarajan, D.J. Miller, Catalytic upgrading of fermentation-derived organic acids, *Biotechnol. Progr. 15* (1999) 845–854.
9. C. Åkerberg, G. Zacchi, An economic evaluation of the fermentative production of lactic acid from wheat flour, *Bioresour. Technol. 75* (2000) 119–126.
10. J.H. Litchfield, Microbiological production of lactic acid, *Adv. Appl. Microbiol. 42* (1996) 45–95.
11. F. Mirasol, Lactic acid prices falter as competition toughen, *Chemical Market Reporter*, *255* (1999) 16.
12. Technical report on Process Evaluation/Research Planning Program PERP00-S3: Biotech routes to lactic acid/polylactic acid, Chem Systems (2002) (*http://www.chemsystems.com/search/docs/abstracts/00-S3-abs.pdf).*
13. Y. Zhou, J.M. Domínguez, N. Cao, J. Du, G.T. Tsao, Optimization of L-lactic acid production from glucose by *Rhizopus oryzae* ATCC 52311, *Appl. Biochem. Biotechnol. 78* (1999) 401–407.
14. E.Y. Park, Y. Kosakai, M. Okabe, Efficient production of L(+)-lactic acid using mycelial cotton-like flocs of *Rhizopus oryzae* in an air-lift bioreactor, *Biotechnol. Progr. 14* (1998) 699–704.
15. J.S. Yun, Y.J. Wee, H.W. Ryu, Production of optically pure L(+)-lactic acid from various carbohydrates by batch fermentation of *Enterococcus faecalis* RKY1, *Enzyme Microb. Technol. 33* (2003) 416–423.
16. A.R. Berry, C.M.M. Franco, W. Zhang, A.P.J. Middelberg, Growth and lactic acid production in batch culture of *Lactobacillus rhamnosus* in a defined medium, *Biotechnol. Lett. 21* (1999) 163–167.
17. A.W. Schepers, J. Thibault, C. Lacroix, *Lactobacillus helveticus* growth and lactic acid production during pH-controlled batch cultures in whey permeate/yeast extract medium. Part I. Multiple factor kinetic analysis, *Enzyme Microb. Technol. 30* (2002) 176–186.
18. C.N. Burgos-Rubio, M.R. Okos, P.C. Wankat, Kinetic study of the conversion of different substrates to lactic acid using *Lactobacillus bulgaricus*, *Biotechnol. Progr. 16* (2000) 305–314.
19. M. Hujanen, Y.Y. Linko, Effect of temperature and various nitrogen sources on L(+)-lactic acid production by *Lactobacillus casei*, *Appl. Microbiol. Biotechnol. 45* (1996) 307–313.
20. W. Fu, A.P. Mathews, Lactic acid production from lactose by *Lactobacillus plantarum*: Kinetic model and effects of pH, substrate, and oxygen, *Biochem. Eng. J. 3* (1999) 163–170.
21. G. Bustos, A.B. Moldes, J.M. Cruz, J.M. Domínguez, Production of fermentable media from vine-trimming wastes and bioconversion into lactic acid by *Lactobacillus pentosus*, *J. Sci. Food Agric. 84* (2004) 2105–2112.
22. C. Vishnu, G. Seenayya, G. Reddy, Direct conversion of starch to L(+)-lactic acid amylase producing *Lactobacillus amylophilus* GV6, *Bioprocess Eng. 23* (2000) 155–158.
23. C. Kotzanmanidis, T. Roukas, G. Skaracis, Optimization of lactic acid production from beet molasses *Lactobacillus delbrueckii* NCIMB 8130, *World J. Microbiol. Biotechnol. 18* (2002) 442–448.
24. N.D. Roble, J.C. Ogbonna, H. Tanaka, L-lactic acid production from raw cassava starch in a circulating loop bioreactor with cell immobilized in loofa (*Luffa cylindrica*), *Biotechnol. Lett. 25* (2003) 1093–1098.
25. A. Tay, S.T. Yang, Production of L(+)-lactic acid from glucose and starch by immobilized cells of *Rhizopus oryzae* in a rotating fibrous bed bioreactor, *Biotechnol. Bioeng. 80* (2002) 1–12.

26. Y. Kosakai, Y.S. Park, M. Okabe, Enhancement of L(+)-lactic acid production using mycelial flocs of *Rhizopus oryzae*, *Biotechnol. Bioeng. 55* (1999) 461–470.

27. P. Yin, N. Nishiya, Y. Kosakai, K. Yahira, Y.S. Park, M. Okabe, Enhanced production of L(+)-lactic acid from corn starch in a culture of *Rhizopus oryzae* using an air-lift bioreactor, *J. Ferment. Bioeng. 84* (1997) 249–253.

28. Y. Oda, K. Saito, H. Yamauchi, M. Mori, Lactic acid fermentation of potato pulp by the fungus *Rhizopus oryzae*, *Curr. Microbiol. 45* (2002) 1–4.

29. L.T. Axelsson: Lactic Acid Bacteria: Classification and Physiology. In: *Lactic Acid Bacteria*, S. Salminen, A. Wright (Eds.), Marcel Dekker, New York, USA (1993) pp. 1–63.

30. T.D. Thomas, D.C. Ellwood, M.C. Longyear, Change from homo- and heterolactic fermentation by *Streptococcus lactis* resulting from glucose limitation in anaerobic chemostat cultures, *J. Bacteriol. 138* (1979) 109–117.

31. J.S. Smith, A.J. Hiller, G.J. Lees, The nature of stimulation of the growth of *Streptococcus lactis* by yeast extract, *J. Dairy Res. 42* (1975) 123–138.

32. T. Roukas, P. Kotzekidou, Lactic acid production from deproteinized whey by mixed cultures of free and coimmobilized *Lactobacillus casei* and *Lactococcus lactis* cells using fedbatch culture, *Enzyme Microb. Technol. 22* (1998) 199–204.

33. M. Altaf, B.J. Naveena, G. Reddy, Screening of inexpensive nitrogen sources for production of L(+)-lactic acid from starch by amylolytic *Lactobacillus amylophilus* GV6 in single step fermentation, *Food Technol. Biotechnol. 43* (2005) 235–239.

34. B.J. Naveena, M. Altaf, K. Bhadrayya, G. Reddy, Production of L(+)-lactic acid by *Lactobacillus amylophilus* GV6 in semi-solid state fermentation using wheat bran, *Food Technol. Biotechnol. 42* (2004) 147–152.

35. J.M. Monteagudo, L. Rodríguez, J. Rincón, J. Fuertes, Kinetics of lactic acid fermentation by *Lactobacillus delbrueckii* grown on beet molasses, *J. Chem. Technol. Biotechnol. 68* (1997) 271–276.

36. Y. Göksungur, U. Güvenç, Production of lactic acid from beet molasses by calcium alginate immobilized *Lactobacillus delbrueckii* IFO 3202, *J. Chem. Technol. Biotechnol. 74* (1999) 131–136.

37. C. Åkerberg, K. Hofvendahl, G. Zacchi, B. Hahn-Hägerdal, Modeling the influence of pH, temperature, glucose and lactic acid concentrations on the kinetic of lactic acid production by *Lactococcus lactis* ssp. *lactis* ATCC 19435 in whole-wheat flour, *Appl. Microbiol. Biotechnol. 49* (1998) 682–690.

38. Y.J. Wee, J.N. Kim, J.S. Yun, H.W. Ryu, Utilization of sugar molasses for economical L(+)-lactic acid production by batch fermentation of *Enterococcus faecalis*, *Enzyme Microb. Technol. 35* (2004) 568–573.

39. K. Kylä-Nikkilä, M. Hujanen, M. Leisola, A. Palva, Metabolic engineering of *Lactobacillus helveticus* CNRZ32 for production of pure L(+)-lactic acid, *Appl. Environ. Microbiol. 66* (2000) 3835–3841.

40. B.S. Dien, N.N. Nichols, R.J. Bothast, Recombinant *Escherichia coli* engineered for production of L-lactic acid from hexose and pentose sugars, *J. Ind. Microbiol. Biotechnol. 27* (2001) 259–264.

41. B.S. Dien, N.N. Nichols, R.J. Bothast, Fermentation of sugar mixtures using *Escherichia coli* catabolite repression mutants engineered for production of L-lactic acid, *J. Ind. Microbiol. Biotechnol. 29* (2002) 221–227.

42. D.E. Chang, H.C. Jung, J.S. Rhee, J.G. Pan, Homofermentative production of D- or L-lactate in metabolically engineered *Escherichia coli* RR1, *Appl. Environ. Microbiol. 65* (1999) 1384–1389.

43. E.W.J. van Niel, B. Hahn-Hägerdal, Nutrients requirements of lactococci in defined growth media, *Appl. Microbiol. Biotechnol. 52* (1999) 617–627.

44. A. Amrane, Evaluation of lactic acid bacteria autohydrolyzate for the supplementation of lactic acid bacteria fermentation, *World J. Microbiol. Biotechnol. 16* (2000) 207–209.

45. V. Arasaratnam, A. Senthuran, K. Balasubramaniam, Supplementation of whey with glucose and different nitrogen sources for lactic acid production by *Lactobacillus delbrueckii*, *Enzyme Microb. Technol. 19* (1996) 482–486.

46. K. Richter, C. Berthold, Biotechnological conversion of sugar and starchy crops into lactic acid, *J. Agric. Eng. Res. 71* (1998) 181–191.

47. K. Richter, A. Träger, L(+)-lactic acid from sweet sorghum by submerged and solid-state fermentations, *Acta Biotechnol. 14* (1994) 367–378.

48. K. Hofvendahl, B. Hahn-Hägerdal, L-lactic acid production from whole wheat flour hydrolysate using strains of *Lactobacilli* and *Lactococci*, *Enzyme Microb. Technol. 20* (1997) 301–307.

49. H. Oh, Y.J. Wee, J.S. Yun, S.H. Han, S. Jung, H.W. Ryu, Lactic acid production from agricultural resources as cheap raw materials, *Bioresour. Technol. 96* (2005) 1492–1498.

50. W. Xiaodong, G. Xuan, S.K. Rakshit, Direct fermentative production of lactic acid on cassava and other starch substrates, *Biotechnol. Lett. 19* (1997) 841–843.

51. J.S. Yun, Y.J. Wee, J.N. Kim, H.W. Ryu, Fermentative production of DL-lactic acid from amylase-treated rice and wheat brans hydrolyzate by a novel lactic acid bacterium, *Lactobacillus* sp., *Biotechnol. Lett. 26* (2004) 1613–1616.

52. Y.Y. Linko, P. Javanainen, Simultaneous liquefaction, saccharification, and lactic acid fermentation on barley starch, *Enzyme Microb. Technol. 19* (1996) 118–123.

53. C. Vishnu, G. Seenayya, G. Reddy, Direct fermentation of various pure and crude starchy substrates to L(+)-lactic acid using *Lactobacillus amylophilus* GV6, *World J. Microbiol. Biotechnol. 18* (2002) 429–433.

54. R. Yáñez, A.B. Moldes, J.L. Alonso, J.C. Parajó, Production of D(–)-lactic acid from cellulose by simultaneous saccharification and fermentation using *Lactobacillus coryniformis* subsp. *torquens*, *Biotechnol. Lett. 25* (2003) 1161–1164.

55. S. Miura, T. Arimura, N. Itoda, L. Dwiarti, J.B. Feng, C.H. Bin, M. Okabe, Production of L-lactic acid from corncob, *J. Biosci. Bioeng. 97* (2004) 153–157.

56. R. Yáñez, J.L. Alonso, J.C. Parajó, D-lactic acid production from waste cardboard, *J. Chem. Technol. Biotechnol. 80* (2005) 76–84.

57. E.Y. Park, P.N. Anh, N. Okuda, Bioconversion of waste office paper to L(+)-lactic acid by the filamentous fungus *Rhizopus oryzae*, *Bioresour. Technol. 93* (2004) 77–83.

58. A.B. Moldes, J.L. Alonso, J.C. Parajó, Strategies to improve the bioconversion of processed wood into lactic acid by simultaneous saccharification and fermentation, *J. Chem. Technol. Biotechnol. 76* (2001) 279–284.

59. Y.J. Wee, J.S. Yun, D.H. Park, H.W. Ryu, Biotechnological production of L(+)-lactic acid from wood hydrolyzate by batch fermentation of *Enterococcus faecalis*, *Biotechnol. Lett. 26* (2004) 71–74.

60. A.W. Schepers, J. Thibault, C. Lacroix, *Lactobacillus heveticus* growth and lactic acid production during pH-controlled batch cultures in whey permeate/yeast extract medium. Part II: Kinetic modeling and model validation, *Enzyme Microb. Technol. 30* (2002) 187–194.

61. A.O. Büyükkilci, S. Harsa, Batch production of L(+)-lactic acid from whey by *Lactobacillus casei* (NRRL B-441), *J. Chem. Technol. Biotechnol. 79* (2004) 1036–1040.

62. K.V. Venkatesh, Simultaneous saccharification and fermentation of cellulose to lactic acid, *Bioresour. Technol. 62* (1997) 91–98.

63. M. Chatterjee, S.L. Chakrabarty, B.D. Chattopadhyay, R.K. Mandal, Production of lactic acid by direct fermentation of

starchy wastes by an amylase-producing *Lactobacillus*, *Biotechnol. Lett. 19* (1997) 873–874.

64. C. Ruengruglikit, Y.D. Hang, L(+)-lactic acid production from corncobs by *Rhizopus oryzae* NRRL-395, *Lebensm. Wiss. Technol. – Food Sci. Technol. 36* (2003) 573–575.

65. H.K. Sreenath, A.B. Moldes, R.K. Koegel, R.J. Straub, Lactic acid production from agricultural residues, *Biotechnol. Lett. 23* (2001) 179–184.

66. A. Garde, G. Jonsson, A.S. Schmidt, B.K. Ahring, Lactic acid production from wheat straw hemicellulose hydrolyzate by *Lactobacillus pentosus* and *Lactobacillus brevis*, *Bioresour. Technol. 81* (2002) 217–223.

67. E. Palmqvist, B. Hahn-Hägerdal, Fermentation of lignocellulosic hydrolyzates. II: Inhibitors and mechanisms of inhibition, *Bioresour. Technol. 74* (2000) 25–33.

68. E. Palmqvist, B. Hahn-Hägerdal, Fermentation of lignocellulosic hydrolyzates. I: Inhibition and detoxification, *Bioresour. Technol. 74* (2000) 17–24.

69. A. Amrane, Y. Prigent, Lactic acid production rates during the different growth phases of *Lactobacillus helveticus* cultivated on whey supplemented with yeast extract, *Biotechnol. Lett. 20* (1998) 379–383.

70. U. Kulozik, J. Wilde, Rapid lactic acid production at high cell concentrations in whey ultrafiltrate by *Lactobacillus helveticus*, *Enzyme Microb. Technol. 24* (1999) 297–302.

71. J.J. Fitzpatrick, U. O'Keeffe, Influence of whey protein hydrolyzate addition to whey permeate batch fermentations for producing lactic acid, *Process Biochem. 37* (2001) 183–186.

72. J.J. Fitzpatrick, M. Ahrens, S. Smith, Effect of manganese on *Lactobacillus casei* fermentation to produce lactic acid from whey permeate, *Process Biochem. 36* (2001) 671–675.

73. T. Pauli, J.J. Fitzpatrick, Malt combing nuts as a nutrient supplement to whey permeate for producing lactic acid by fermentation with *Lactobacillus casei*, *Process Biochem. 38* (2002) 1–6.

74. V.B. Shukla, S. Zhou, L.P. Yomano, K.T. Shanmugam, J.F. Preston, L.O. Ingram, Production of D(–)-lactic acid from sucrose and molasses, *Biotechnol. Lett. 26* (2004) 689–693.

75. O.D. Dailey Jr., M.K. Dowd, J.C. Mayorga, Influence of lactic acid on the solubilization of protein during corn steeping, *J. Agric. Food Chem. 48* (2000) 1352–1357.

76. E.B. Kurbanoglu, N.I. Kurbanoglu, Utilization of lactic acid production with a new acid hydrolysis of ram horn waste, *FEMS Microbiol. Lett. 225* (2003) 29–34.

77. G. Bustos, A.B. Moldes, J.M. Cruz, J.M. Domínguez, Formulation of low-cost fermentative media for lactic acid production with *Lactobacillus rhamnosus* using vinification lees as nutrients, *J. Agric. Food Chem. 52* (2004) 801–808.

78. Y.J. Wee, J.S. Yun, Y.Y. Lee, A.P. Zeng, H.W. Ryu, Recovery of lactic acid by repeated batch electrodialysis and lactic acid production using electrodialysis wastewater, *J. Biosci. Bioeng. 99* (2005) 104–108.

79. H. Oh, Y.J. Wee, J.S. Yun, H.W. Ryu, Lactic acid production through cell-recycle repeated-batch bioreactor, *Appl. Biochem. Biotechnol. 107* (2003) 603–613.

80. S. Kwon, I.K. Yoo, W.G. Lee, H.N. Chang, Y.K. Chang, High-rate continuous production of lactic acid by *Lactobacillus rhamnosus* in a two-stage membrane cell-recycle bioreactor, *Biotechnol. Bioeng. 73* (2001) 25–34.

81. J.C. Cotton, A.L. Pometto III, J. Gvozdenovic-Jeremic, Continuous lactic acid fermentation using plastic composite support biofilm reactor, *Appl. Microbiol. Biotechnol. 57* (2001) 626–630.

82. P.V. Iyer, Y.Y. Lee, Simultaneous saccharification and extractive fermentation of lignocellulosic materials into lactic acid in a two-zone fermentor-extractor system, *Appl. Biochem. Biotechnol. 78* (1999) 409–419.

83. Y. Nomura, M. Iwahara, J.E. Hallsworth, T. Tanaka, A. Ishizaki, High-speed conversion of xylose to L-lactate by electrodialysis bioprocess, *J. Biotechnol. 60* (1998) 131–135.

84. G. Min-Tian, M. Koide, R. Gotou, H. Takanashi, M. Hirata, T. Hano, Development of a continuous fermentation system for production of lactic acid by *Lactobacillus rhamnosus*, *Process Biochem. 40* (2005) 1033–1036.

85. R. Jeantet, J.L. Maubois, P. Boyaval, Semicontinuous production of lactic acid in a bioreactor coupled with nanofiltration membranes, *Enzyme Microb. Technol. 19* (1996) 614–619.

86. J.M. Monteagudo, M. Aldavero, Production of L-lactic acid by *Lactobacillus delbrueckii* in chemostat culture using an ion exchange resins system, *J. Chem. Technol. Biotechnol. 74* (1999) 627–634.

87. A. Senthuran, V. Senthuran, R. Hatti-Kaul, B. Mattiasson, Lactic acid production by immobilized *Lactobacillus casei* in recycle batch reactor: A step towards optimization, *J. Biotechnol. 73* (1999) 61–70.

88. L.M.D. Gonçalves, A. Ramos, J.S. Almeida, A.M.R.B. Xavier, M.J.T. Carrondo, Elucidation of the mechanism of lactic acid growth inhibition and production in batch cultures of *Lactobacillus rhamnosus*, *Appl. Microbiol. Biotechnol. 48* (1997) 346–350.

89. T. Hano, M. Matsumoto, S. Uenoyama, T. Ohtake, Y. Kawano, S. Miura, Separation of lactic acid from fermented broth by solvent extraction, *Bioseparation*, *3* (1993) 321–326.

90. Y.H. Kim, S.H. Moon, Lactic acid recovery from fermentation broth using one-stage electrodialysis, *J. Chem. Technol. Biotechnol. 76* (2001) 169–178.

91. Bulletin of the Purac (*http://www.purac.com/*).

92. Bulletin of the Galactic (*http://www.lactic.com/*).

93. R.E. Drumright, P.R. Gruber, D.E. Henton, Polylactic acid technology, *Adv. Mater. 12* (2000) 1841–1846.

94. E.T.H. Vink, K.R. Rábago, D.A. Glassner, P.R. Gruber, Applications of life cycle assessment to NatureWorks™ polylactide (PLA) production, *Polym. Degrad. Stabil. 80* (2003) 403–419.

95. Prices & people, *Chemical Market Reporter*, *267* (2005) 24–26.

96. L. Jarvis, Prospects for lactic acid are healthy as demand for all end uses grows, *Chemical Market Reporter*, *263* (2003) 12.

97. Bulletin of the NatureWorks LLC (*http://www.natureworksllc.com/*).

98. C. Boswell, Bioplastics aren't the stretch they once seemed, *Chemical Market Reporter*, *260* (2001) FR15–FR17.