Recent Trends in the Production, Purification and Application of Lactic Acid

J. Vijayakumar,[a] R. Aravindan,[b,*] and T. Viruthagiri[c]
[a] M. Tech Industrial Biotechnology,
[b] Lecturer in Chemical Engineering,
[c] Professor and Head, Department of Chemical Engineering,
Biochemical Engineering Laboratory, Annamalai University,
Annamalai nagar – 608002, Tamil nadu, India

Review
Received: March 12, 2007
Accepted: September 15, 2007

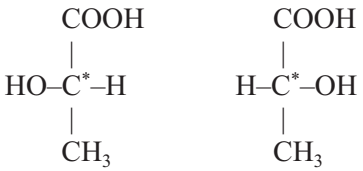
Lactic acid, a naturally occurring multifunctional organic acid, is a valuable industrial chemical used as an acidulant, preservative in the food industry, pharmaceutical, leather, and textile industries, as well as a chemical feedstock. One of the most promising applications of lactic acid is its use for biodegradable and biocompatible lactate polymers, such as polylactic acid. Lactic acid can be produced either by fermentation or by chemical synthesis but the biotechnological fermentation process has received significant importance due to environmental concerns, use of renewable resources instead of petrochemicals, low production temperature, low energy requirements and high purity. There are numerous investigations on the development of biotechnological methods for lactic acid production, with an ultimate objective to enable the process to be more efficient and economical. This review discusses the various recent fermentation technologies to produce lactic acid, different microorganisms involved in the production of lactic acid, purification and wide industrial applications of lactic acid.

Key words:
Lactic acid, lactic acid bacteria, fermentation, purification, applications

Introduction

Lactic acid has a long history of uses for fermentation and preservation of human foodstuffs. 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. In 1839, *Fremy*, produced lactic acid by fermentation of carbohydrates such as sucrose, lactose, mannitol, starch and dextrin. The first commercial production of lactic acid started in the United States by a microbial process in 1881.

Lactic acid is produced by humans, animals, plants and microorganisms. Lactic acid is the simplest hydroxyl carboxylic acid with an asymmetrical carbon atom. Lactic acid occurs naturally in two optical isomers, D(–) and L(+)-lactic acids. Since elevated levels of the D-isomer are harmful to humans, L(+)-lactic acid is the preferred isomer in food and pharmaceutical industries.[1,2]



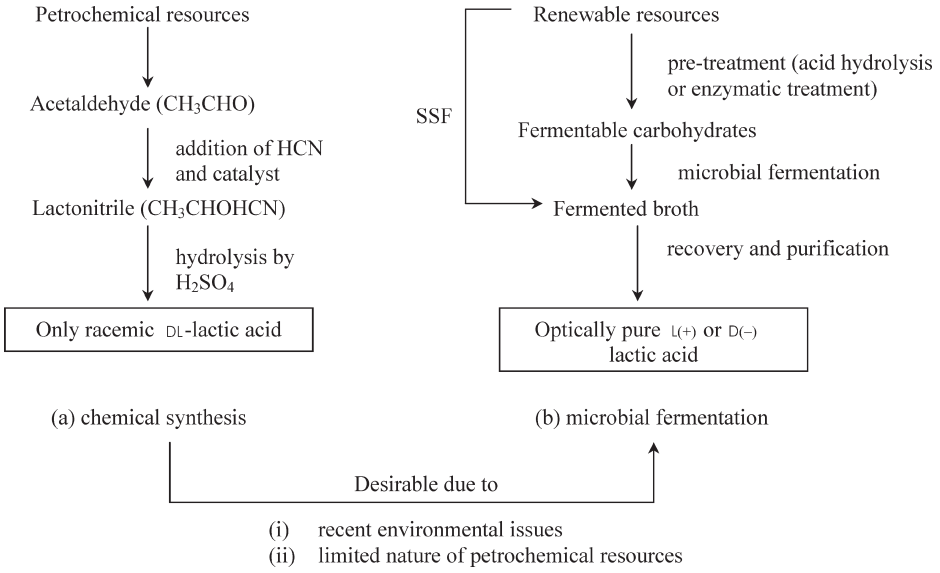
L(+) lactic acid D(–) lactic acid

C* – asymmetric carbon atom

The L(+) form of lactic acid is used for food and drug industry, because the human body is only adapted to assimilate this form. Lactic acid is a valuable industrial chemical used in the food industry and in numerous other applications in the pharmaceutical, leather and textile industries, and also for the production of biodegradable and biocompatible polylactate polymers, such as polylactic acid (PLA), an environmentally friendly alternative to biodegradable plastics.[3–5] Polylactide is used for the preparation of scaffolds for biocompatible artificial organs, self-dissolving sutures and as a means for sustained drug release.[3] Owning to the unique property of PLA, lactic acid has the potential to be a substitute for biodegradable plastics manufacture and becomes a very large-volume commodity chemical intermediate.[3–5]

Lactic acid can be produced commercially by either chemical or biochemical methods as shown in Fig. 1. The most commonly used synthetic

* Correspondence: E-mail: donaravind@yahoo.com
Phone: 91-4144-239737



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

method for chemical production of lactic acid is based on the hydrolysis of lactonitrile, derived from acetaldehyde and hydrogen cyanide.[1–5] The biotechnological process may yield either form [D(+) or L(+)] alone, or a mixture in different proportions of two isomers, depending on the microorganism, substrate and growth conditions used, whereas the chemical production only results in a mixture of the two isomers.[1,6,7] Another significant advantage over the chemical synthesis is that biological production can use cheap raw materials, such as whey, molasses, starch waste, beet and cane-sugar and other carbohydrate-rich materials.[7–11] In commercial processes, sugars and starches have been widely used as substrates for biological production of lactic acid. The most effective way for L-lactic acid synthesis is through biosynthesis rather than chemical process.

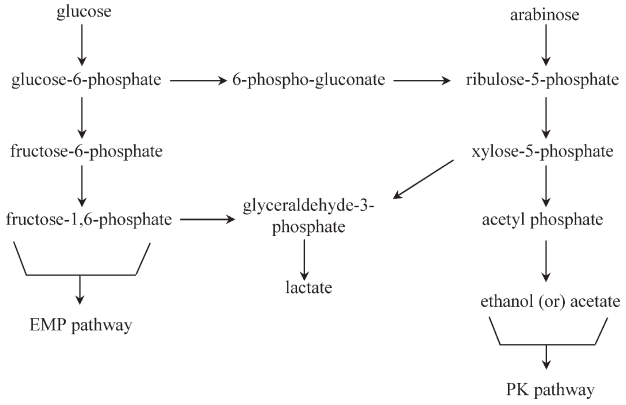
Lactic acid has been classified by the US FDA (Food and Drug Administration) as GRAS (Generally Recognized as Safe)[3] for use as a food additive, and it has been utilized in a broad range of applications in the food and pharmaceutical industries.[3,12,13] At present, 90 % of the world production of lactic acid is by bacterial fermentation and the rest is produced synthetically. The worldwide market growth is increasing every year and the production in 2006 was about 68,000 tons per year. The worldwide market growth is expected to be between 10 % and 15 % per year[14] The Dutch Company CCA, together with its holding in Spain and Brazil produces 20,000 – 25,000 tons per year. Croda, United Kingdom produces about 25,000 tons per year. Synthetic lactic acid is produced by sterling in United States (5,000 tons per year) and Musashino in Japan (7,000 tons per year).[15] Cargill Dow LLC, the primary US manufacturer of PLA, has reported that the global PLA market might expand to 5,000,000 tons per year by 2010. Therefore, considerable increase in the worldwide demand for lactic acid is definitely expected in the coming years.

Characteristics of lactic acid bacteria

Lactic acid bacteria are usually gram-positive, non-motile, non-spore-forming rods and cocci. They lack the ability to synthesize cytochromes and porphyrins (components of respiratory chains) and therefore cannot generate ATP by creation of a proton gradient. Since they do not use oxygen in their energy production, lactic acid bacteria grow under anaerobic conditions, but they can also grow in the presence of oxygen. They are protected from oxygen byproducts (e.g. H_2O_2) because they have peroxidases and these organisms are aero tolerant anaerobes. They are differentiated from other organisms by their ability to ferment hexoses to lactic acid. Lactic acid bacteria can be divided into homo fermentative and hetero fermentative based upon the products produced from the fermentation of glucose. Homo fermentative organisms ferment glucose to two moles of lactic acid, generating a net of 2 ATP per mole of glucose metabolized. Lactic acid is the major product of this fermentation. Hetero fermentative lactic acid bacteria ferment 1 mole of glucose to 1 mole of lactic acid, 1 mole of ethanol, and 1 mole of CO_2. One mole of ATP is generated per mole of glucose, resulting in less growth per

mole of glucose metabolized. Because of the low energy yields, lactic acid bacteria often grow more slowly than microbes capable of respiration, and produce smaller colonies of 2 – 3 mm. Table 1 shows the list of homo and hetero fermentative lactic acid bacteria and configuration of lactic acid.[16]

It is easy to determine whether a lactic acid bacterium has a homo or heterofermentative metabolism by the hot-loop test. A major end-product of heterofermentation is CO_2. In a medium containing glucose this gas is highly soluble at high pH and will stay in solution. If the temperature of the solution is increased; CO_2 will become insoluble and will be released in the gaseous form. The hot-loop test consists of growing a test isolates to saturation in a medium containing glucose. After incubation, a wire loop (inoculating loop) is heated to redness and plunged into the broth culture. This causes the liquid around the loop to heat up. If a test microorganism is heterofermentative, CO_2 bubbles will evolve close to the loop. The homofermentative lactic acid bacteria usually metabolize glucose via the Embden-Meyerhof pathway (i.e., glycolysis). The two major pathways for better assimilation of glucose and xylose in lactic acid are the Embden-Mayerhof-Parnas (EMP) pathway and the pentose phosphoketolase (PK) pathway shown in Fig. 2. The lactic acid bacteria have limited biosynthetic ability, requiring amino acids, B vitamins, purines, pyrimidines and typically a sugar as carbon and energy source.



F i g . 2 – *Simplified illustration of EMP pathway and PK pathway in the left and right side respectively*[23]

Microorganisms for the lactic acid production

There are large number of species of bacteria and some species of molds that possess the ability to form relatively significant quantities of lactic acid from carbohydrates. Lactic acid bacteria are important not only for the desirable reactions which they catalyze but also for the undesirable activities which they promote.

Bacteria and fungi are the two groups of microorganisms that can produce lactic acid.[12] 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.[17–19] *Rhizopus* species such as *R. oryzae* and *R. arrhizus* have amylolytic enzyme activity, which enables them to convert starch directly to L(+)-lactic acid, but it also requires vigorous aeration because *R. oryzae* is an obligate aerobe.[18] In fungal fermentation, the low production rate, below $P = 3$ g L^{-1} h^{-1} is probably due to the low reaction rate caused by mass transfer limitation.[20] The lower product yield from fungal fermentation is attributed partially to the formation of by-products, such as fumaric acid and ethanol.[18]

Several attempts have been made to achieve higher cell density, lactic acid yield, and productivity in fungal fermentation. Haung *et al*.[21] produced lactic acid from potato starch wastewater using *R. oryzae* and *R. arrhizus*. Park *et al.*[22] produced lactic acid from waste paper by using *R. oryzae*. Tay and Yang[18] immobilized *R. oryzae* cells in a fibrous bed to produce lactic acid from glucose and starch. Kosakai *et al.*[19] cultured *R. oryzae* cells with the use of mycelial floc formed by the addition of mineral support and poly ethylene oxide. The microorganisms selected for recent investigations of the biotechnological production of lactic acid are listed in Table 2.

Garde *et al.*[23] obtained lactic acid from wheat straw hemicellulose by using mixed culture of *Lactobacillus pentosus* and *Lactobacillus brevis.* Yun *et al.*[24] investigated the production of lactic acid from single and mixed sugars using *Enterococcus faecalis RKY1*. The volumetric productivity, cell growth and concentration of lactic acid were highest in glucose/fructose (mixed sugar) than single sugar. Rivas *et al*.[25] produced lactic acid from corn cobs by simultaneous saccharification and fermentation using *Lactobacillus rhamnosus*.

Wee *et al.*[26] reported the economical L(+)-lactic acid production from sugar molasses by batch fermentation of *Enterococcus faecalis*. Kourkoutas *et al*.[27] used immobilized *Lactobacillus casei* cell on fruit pieces to produce lactic acid. Narita *et al.*[28] reported the efficient production of L(+)-lactic acid from raw starch by *Streptococcus bovis 148.* Chauhan *et al*.[29] used the statistical screening of medium components by Placket-Burman design for lactic acid production by *Lactobacillus sp. KCP01* using date juice. Patil *et al.*[30] produced lactic acid from cane sugar using mutant of *Lactobacillus delbrueckii NCIM 2365*. John *et al.*[31] reported the

Table 1 – *Homo and heterofermentative lactic acid bacteria and configuration of lactic acid*

Genera and species	Homo-fermentative	Hetero-fermentative	Configuration of lactic acid
Lactobacillus			
L. delbrueckii	+	–	D(–)
L. lactis	+	–	D(–)
L. bulgaricus	+	–	D(–)
L. casei	+	–	L(+)
L. plantarum	+	–	DL
L. curvatus	+	–	DL
L. brevis	–	+	DL
L. fermentum	–	+	DL
Sporolactobacillus			
S. inulinus	+	–	D(–)
Streptococcus			
S. faecalis	+	–	L(+)
S. cremoris	+	–	L(+)
S. lactis	+	–	L(+)
Leuconostoc			
L. mesenteroides	–	+	D(–)
L. dextranicum	–	+	D(–)
Pediococcus			
P. damnosus	+	–	DL
Bifidobacterium			
B. bifidum	–	+	L(+)

solid state fermentation for L-lactic acid production from agro wastes using *Lactobacillus delbrueckii.* Amrane and Prigent[32] designed a two-stage continuous reactor to produce lactic acid from lactose by using *Lactobacillus helveticus* and obtained high product concentration of lactic acid at very low dilution rate. Senthuran *et al*.[33] explained lactic acid production by immobilized *Lactobacillus casei* in recycle batch reactor. Fu and Mathews[34] reported the lactic acid production from lactose by *Lactobacillus plantarum*. Nolasco-Hipolito *et al.*[35] explained the continuous production of L(+)-lactic acid from hydrolyzed sago starch using *Lactobacillus lactis*. Amrane[36] reported the unstructured models for biomass formation, substrate consumption and lactic acid production from whey using *Lactobacillus helveticus*.

Nancib *et al*.[37] explained the joint effect of nitrogen sources and B-vitamin supplementation of date juice on lactic acid production by *Lactobacillus casei sub sp. rhamnosus.* Oh *et al*.[38] used agricultural resources for the production of lactic acid by *Enterococcus faecalis*.

Schepers *et al.*[39] reported the continuous lactic acid production in whey permeate with immobilized *Lactobacillus helveticus*. Ohkouchi and Inoue[40] studied the direct production of L(+)-lactic acid from starch and food wastes using *Lactobacillus manihotivorans LMG 18011*. Vasala *et al*.[41] used high salt containing dairy products to produce lactic acid by using *Lactobacillus Salivarius ssp*. *Salicinius*.

Xu *et al*.[42] reported the development of a continuous cell-recycle fermentation system for production of lactic acid by *Lactobacillus paracasei*. Xu *et al.*[43] used mixed culture of *Lactobacillus sake* and *Lactobacillus casei* for the production of lactic acid from soybean stalk hydrolysate. Sakai *et al*.[44] reported the production of lactic acid in pH-swing open fermentation of kitchen refuse by selective proliferation of *Lactobacillus plantarum*.

Berry *et al.*[55] produced lactic acid by batch culture of *Lactobacillus rhamnosus* in a defined medium. Burgos-Rubio *et al*.[53] reported the kinetic investigation of the conversion of different substrates into lactic acid with the use of *Lactobacillus bulgaricus.* Hujanen and Linko[52] investigated the effects of culture temperature and nitrogen sources on lactic acid production by *Lactobacillus casei*. Bustos *et al.*[51] used *Lactobacillus pentosus* for the production of lactic acid from vine-trimming wastes. The strains of amylase-producing *Lactobacillus amylophilus* were used often for the direct conversion of starch into lactic acid.[13,50,61] However, among the genus *Lactobacillus*, *Lactobacillus delbrueckii* has appeared commonly in many investigation for the production of lactic acid, Kutzanmanidis *et al.*[45] used *Lactobacillus delbrueckii NC1MB 8130* for lactic acid production from beet molasses. Monteagudo *et al.*[62] and Goksungur *et al.*[46] also attempted to produce lactic acid from beet molasses with *Lactobacillus delbrueckii*. Several amylolytic lactic acid bacteria, such as *Lactobacillus amylophilus,*[63,64] *Lactobacillus amylovorus*[65] and *Lactobacillus plantarum A6*[66] can convert starch directly to lactic acid. The most common bacterium for the industrial production of lactic acid is *Lactobacillus delbrueckii,* which is employed in fermentations utilizing corn dextrose media. Other bacteria of industrial importance include *Lactobacillus bulgaricus*, which utilizes lactose as a carbon source and finds use in lactic acid production from whey media, and *Lactobacillus pentosus*, which is able to utilize the pentoses of sulfite waste liquor

Table 2 – *Microorganisms used in recent investigations of the biotechnological production of lactic acid*

Microorganism	Lactic acid γ (g L^{-1})	Yield Y (g g^{-1})	Productivity P (g L^{-1} h^{-1})	Ref.
Enterococcus faecalis	95.7	0.94	4.0	26
Lactobacillus delbrueckii NC1MB8130	90.0	0.97	3.8	45
Lactobacillus delbrueckii IFO 3202	60.3	0.95	3.4	46
Streptococcus bovis 148		0.88	14.7[a]	28
Rhizopus oryzae	93.8	0.77	1.38	47
Latobacillus paracasei	88 – 106	0.91 – 0.95	3.31 – 3.67	48
Lactobacillus lactis	109	0.93	1.09	49
Enterococcus faecalis RKY1	102	0.97	4.87	38
Lactobacillus amylophilus GV6	76.2	0.7	0.8	50
L. pentosus ATCC 8041	21.8	0.77	0.8	51
L. plantarum ATCC 21028	41.0	0.97	1.0	34
L. casei NRRL B-441	82.0	0.91	5.6	52
L. bulgaricus NRRL B-548	38.7	0.9	3.5	53
L. helveticus ATTC 15009	65.5	0.66	2.7	54
L. rhamnosus ATCC 10863	67.0	0.84	2.5	55
R. oryzae NRRL 395	104.6	0.87	1.8	20
R. oryzae ATCC 52311	83.0	0.88	2.6	17
L. salivarius sp. *salivarius ATCC 11742*	28.0	0.92	11	56
L. amylovorus ATCC 33622	93.0	0.52	2.0	57
L. plantarum ATCC14917			2.0	58
L. acidophilus R	8.60	0.17		59
S. thermophilus	18.0	0.50	5.9	60

a – 14.7 g L^{-1} of lactic acid from 20 g L^{-1} of raw starch

for lactic acid production. Other homofermentative species of potential industrial importance are *Lactobacillus casei*, *Lactobacillus leichmanii*, and *Streptococcus lactis*. All of these bacteria are considered anaerobes, although they can withstand some oxygen. However, *Streptococcus lactis* is less sensitive to oxygen and therefore may also be considered a facultative aerobe.

The nutritive requirements of the lactic acid bacteria, specifically members of the genera *Lactobacillus*, *Leuconostoc* and *Streptococcus*, are rather complex. Various vitamins of the B-complex and certain amino acids are required for the growth of these microbes in addition to the usual elements. Yeast extract and malt sprouts may be used as sources of vitamins of the B-complex in media used for the isolation, growth and maintenance of lactic acid bacteria. Occasionally the addition of extra thiamin may be necessary or desirable for the growth of some species. They require some elements for growth, such as carbon and nitrogen sources, in the form of carbohydrates, amino acids, vitamins, and minerals.[67] Fatty acids also influence lactic acid bacteria 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.

Traditionally, the most common nutrients for the preparation of fermentative media are yeast extract and peptone, which turn out to be very expensive being able to account for almost 30 % of the total cost of the process. It is desired to find some new nutrients suitable for an industrial process and

to replace yeast extract. Generally, the proteins in nutrients are hydrolyzed into peptides and amino acids before used for lactic acid production. Some nutrients, such as casaminoacids,[68] soybean hydrolysate[69,70] and ram horn protein hydrolyzate,[71] have been used for lactic acid production after hydrolyzed with acids. Hydrolyzed whey protein has been shown to constitute a rich nutrient source for the lactic acid bacteria.[72–75] As lactic acid bacteria have a limited capacity to synthesize B-vitamins and amino acids,[2] yeast extract is often used to supply all of these factors in bacterial cultures.

For the feasibility of biotechnological production of lactic acid, 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 byproduct formation, ability to be fermented with little or no pretreated and year round availability.[76] There have been many attempts to screen for cheap raw materials for the economical production of lactic acid. Cheap raw materials, such as starchy and cellulose materials, whey, and molasses have been used for lactic acid production.[2] Among these, starchy and cellulose materials are currently receiving a great deal of attention, because they are cheap, abundant, and renewable.[8,77,78] The starchy materials used for lactic acid production include sweet sorghum,[8,48] wheat straw,[23] corn,[25] cassava,[79] potato,[21] rice[80,81] and barley.[82] These materials have to be hydrolyzed into fermentable sugars before fermentation, because they consist mainly of $\alpha(1,4)$ and $\alpha(1,6)$ linked glucose. This hydrolysis can be carried out simultaneously with fermentation.[82] Although a number of different substrates have been used for the biotechnological production of lactic acid, most studies for lactic acid production have been focused on the pure substrates, such as glucose,[83,84] or lactose,[32,85] and the natural polysaccharides such as starch,[79,82] or cellulose.[78,86] Patil *et al.*[30] reported the production of lactic acid from cane sugar. Wee *et al*.[26] used sugar cane molasses for the production of lactic acid by batch fermentation of *Enterococcus faecalis*.

Whey is a major byproduct of the dairy industry, and it contains lactose, protein, fat, and mineral salts. Amrane *et al*.[87] reported the production of lactic acid from whey and explained the influence of peptidic nitrogen deficiency. There have been several attempts to produce lactic acid from whey by batch culture of *Lactobacillus casei.*[88,89] Schepers *et al.*[54] reported the lactic acid production during pH controlled batch cultures in whey permeate/yeast extract medium. Oh *et al.*[38] reported the production of lactic acid from agricultural resources. Ohkouchi *et al.*[40] reported the direct production of lactic acid from starch and food wastes.

Xu *et al.*[43] used soybean stalk to produce lactic acid production and Ohkouchi *et al.*[90] explained the production of lactic acid from organic wastes. 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.[54] Nancib *et al.*[37] explained the supplementation of vitamin-B and effect of nitrogen sources during the production of lactic acid. Commonly, lactic acid is prepared by using refined sugars and starch materials.[18,91] In recent years for the sake of decreasing environmental pollution and expense of lactic acid production, various wastes like kitchen waste,[92–94] wastewater sludge,[95,96] food processing waste,[97] crop residue including corn cobs, wheat stalk and bran have been used for lactic acid production.[23,25,98]

Lactic acid fermentation

Batch, fed-batch, repeated batch, and continuous fermentations were used for lactic acid production. Higher lactic acid concentration was obtained in batch and fed-batch cultures than in continuous cultures, whereas higher productivity was achieved by the use of continuous cultures.[2] Another advantage of the continuous culture compared to the batch culture, is the possibility to continue the process for a longer period of time. Kwon *et al.*[84] attempted to produce lactic acid by a two-stage cell-recycle culture of *L. rhamnosus*. They connected the membrane cell-recycle bioreactors in a series, and obtained $\gamma = 92$ g $L^{-1}$of lactic acid with a productivity of $P = 57$ g L^{-1} h^{-1}. 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.[99,100] Senthuran *et al.*[33] reported the production of lactic acid by continuous culture of *Lactobacillus 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.

Amrane *et al.*[32] designed a novel reactor, specialized function two-stage reactor (SFTS) to produce lactic acid. For such systems, volumetric productivity was improved with the help of a novel two-stage reactor equipped with a separate feeding line in the second stage. Ninetyseven percent of the total amount of lactic acid was produced in the second stage. The volumetric productivity obtained from this novel configuration was close to that of

batch in similar conditions. An efficient bioreactor, termed a 'synchronized fresh cell bioreactor', was developed to produce lactic acid from hydrolyzed sago starch using *Lactococcus lactis IO-1*.[35] Volumetric lactic acid productivities of P = 8.2 g L^{-1}, 19.3 g L^{-1} and 33 g L^{-1} were obtained at dilution rates of D = 0.21 h^{-1}, 0.50 $h^{-1}$and 1.1 h^{-1} respectively. Lactic acid is produced from corncobs by solid-state fermentation.[25] Oh *et al.*[38] produced lactic acid from agricultural resources by SSF using *Enterococcus faecalis RKY1*. Schepers *et al.*[39] obtained high lactic acid productivity (P = 19–22 g L^{-1} h^{-1}) and low residual sugar mass concentration (γ < 1 g L^{-1}) during continuous fermentation of whey permeate/yeast extract medium with immobilized *Lactobacillus helveticus* in a two stage process. In this two-stage immobilized cell/free-cell process, an overall lactic acid productivity of P = 13.5 g L^{-1} h^{-1} was reached with γ = 1 g L^{-1} residual sugar at an overall dilution rate of D = 0.27 h^{-1}. Extrapolation of experimental results in this study suggested that a high lactic acid productivity of up to P = 23 g L^{-1} h^{-1} with low residual sugar could be attainable in a two-stage immobilized cell process. The effects of different culture parameters and operating strategies were tested on lactic acid production from whey permeate/yeast extract medium by immobilized *Lactobacillus helveticus* in a continuous two-stage process. High lactic acid productivities of P = 19–22 g L^{-1} h^{-1} and low residual sugar was achieved with an overall dilution rate of D = 0.5 h^{-1} and γ = 10 g L^{-1} yeast extract. Lowering the yeast extract mass concentration from γ = 10 to 1 g L^{-1} resulted in a gradual loss of activity with time in both reactors, leading to an overall lactic acid productivity of P = 10.5 g L^{-1} h^{-1} with γ = 24 g L^{-1} residual sugar after t = 47 h. Inversion of the first and second reactor in the two-stage process led to an important drop in productivity which was only partly restored in the next 3 days of culture.

Xu *et al.*[42] developed a novel reactor called membrane cell-recycle bioreactor (MCRB) to produce lactic acid by *Lactobacillus paracasei*. Using a MCRB system with a diaphragm pump and tangential flow-rate controlling, a maximum value of OD_{620} of 98.7 was obtained which was six times greater than that of the fed-batch fermentation. Maximum productivity of P = 31.5 g L^{-1} h^{-1} was recorded which was 10 times greater than the counter part of fed-batch fermentation. Lactic acid production from sugar molasses by batch fermentation of *Enterococcus faecalis RKY1* was investigated in order to reduce the manufacturing cost of lactic acid.[26] The maximum lactic acid mass concentration of γ = 134.9 g L^{-1} and the maximum productivity of P = 4.3 g L^{-1} h^{-1} were obtained. Huang *et al.*[21] studied the biochemical kinetics of simultaneous saccharification and fermentation (SSF) for lactic acid production by fungal species of *Rhizopus arrhizus 36017* and *Rhizopus oryzae 2062* resulting in lactic acid yield of Y = 0.85–0.92 g g^{-1} associated with γ = 1.5–3.5 g L^{-1} fungal biomass produced in t = 36–48 h fermentation. John *et al.*[31] produced lactic acid from two agro industrial wastes, cassava baggasse and sugarcane baggasse, as a raw material and inert solid support using solid-state fermentation (SSF). A maximum yield of Y = 0.249 g g^{-1} L(+)-lactic acid was obtained after 5 days of fermentation under the optimized conditions with a conversion efficiency of about 99 % of the initial reducing sugars. Ding *et al.*[101] studied different fed batch feeding strategies to produce lactic acid. The pulse fed-batch, constant feed rate fed-batch and constant glucose concentration fed-batch methods were not satisfactory for lactic acid production. The exponential fed-batch method had better results in L-lactic acid concentration, while the exponential feeding glucose and yeast extract had the best L-lactic acid production. Compared with the batch culture, the exponential feeding glucose and yeast culture showed 56.5 % improvement in L-lactic acid production, 68.6 % improvement in dry cell mass and 59.7 % improvement in productivity, respectively. The maximum lactic acid mass concentration (γ = 210 g L^{-1}) and L-lactic acid mass concentration (γ = 180 g L^{-1}) in exponential feeding glucose solution (γ = 850 g L^{-1}) and yeast extract (1 %) was obtained, respectively. The yield, the maximal dry cell mass and productivity of lactic acid were up to Y = 90.3 %, γ = 4.30 g L^{-1}, and P = 2.14 g L^{-1} h^{-1} respectively.

Ohashi *et al.*[102] studied a perfusion culture system used for continuous production of lactic acid by retaining cells at a high density of *Lactococcus lactis* in a stirred ceramic membrane reactor (SCMR). After the cell mass concentration increased to γ = 248 g L^{-1}, half of the culture broth volume was replaced with the fermentation medium. Subsequently, a substrate solution containing glucose or molasses was continuously supplied to the cells retained in the SCMR. Simultaneously, the culture supernatant was extracted using a ceramic filter with a pore diameter of d_p = 0.2 μm. The mass concentration and productivity of lactic acid reached γ = 40 g L^{-1} and P = 10.6 g $L^{-1}h^{-1}$, respectively, by continuously replenishing the culture medium at a dilution rate of D = 0.26 h^{-1}. These results demonstrated that the filtration capacity of the SCMR was sufficient for a continuous and rapid replenishment of molasses solution from the dense cell culture and, therefore, the perfusion culture system was considered to provide a low-cost process for continuous production of lactic acid from cheap resources. Kamoshita *et al.*[103] developed the

improved SCMR system. Using the improved SCMR system, a cell mass concentration of $\gamma = 178$ g L^{-1} and viability of 98 % were obtained after $t =$ 198 h of culture, while it took $t = 238$ h to obtain a cell mass concentration of $\gamma = 141$ g L^{-1} and 94 % viability without the use of the membrane cleaning system. The perfusion culture system was applied to the rapid batch fermentation of lactic acid by retaining cells at a high density in the SCMR. When the cell mass concentration reached $\gamma = 80$ g L^{-1}, the culture supernatant was extracted and replaced with the fermentation medium. Batch fermentation using the retained cells was repeated six times. The mass concentration of lactic acid increased to more than $\gamma = 30$ g L^{-1} within $t = 2$ h in each fermentation, while $t = 1.2$ h was necessary for replacing the culture supernatant to repeat the batch fermentation. The production rate of lactic acid was increased in proportion to the cell concentration, and a high fermentation activity of the retained cells was maintained via the repeated batch fermentation. These results demonstrate 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.

Moueddeb *et al.*[104] developed a new type of membrane bioreactor for the transformation of lactose into lactic acid by *Lactobacillus rhamnosus.* However, as for the majority of membrane systems, the decrease of permeate flow by membrane fouling is observed. From an industrial point of view the problem of sterilization (always present in reactors with supported microorganisms) and membrane fouling was partially solved using the fact that inorganic membranes were easily cleaned and sterilized. It was observed that large contact time and high microorganism concentration were necessary in order to have good substrate conversions. This model was a useful tool to estimate the bacteria concentration profiles into a porous media and to study biological transformations in a confined media like in soils.

Mostafa *et al.*[105] produced lactic acid from deproteinized whey using the efficient strain immobilized in an agar gel and fermented in a continuous fixed bed reactor. The optimal processing temperature and dilution rate were found to be 40 °C and $D = 0.33$ h^{-1}. Maximum volumetric productivities of lactic acid from whey were $P = 7.28$ and 9.36 g L^{-1} h^{-1} without and with recycle, respectively. *Silva et al.*[106] investigated the kinetics and long-term stability of the fibrous-bed bioreactor for continuous lactic acid production from un-supplemented acid whey containing $w = 3.7$ % lactose and $w = 0.8$ % lactic acid, using immobilized cells of *Lactobacillus helveticus* at pH 5.5 and 42 °C. Depending on dilution rate and lactate mass concentration, reactor volumetric productivities ranged from $P = 2.6$ g L^{-1} h^{-1} to 7 g L^{-1} h^{-1}. The fibrous bed bioreactor greatly improved in its packing design to allow for more uniform structure and to minimize diffusion limitations, thereby improving cell efficiency and reactor productivity. A much higher specific cell productivity was attained in the fibrous bed when diffusion limitations were eliminated, such as in the cell-recycle membrane bioreactor systems.

Factors affecting the lactic acid production

Effect of temperature

The effect of temperature on the production of lactic acid was studied for various microorganisms (Table 3). The temperature giving the highest productivity was in some cases lower than the temperature resulting in highest lactic acid mass concentration and yield,[52,64] whereas in others the same temperature gave the best results in all categories.[1,52] For *Lactobacillus amylophilus*, which is known to grow at 15 °C but not at 45 °C,[107] the optimal temperatures were 25 °C and 35 °C for maximum productivity and yield, respectively.[64] For *Lactobacillus casei* and *Lactobacillus paracasei* the optimal temperature was reported to be between 37 °C and 44 °C,[48,52,82] which is contradictory to the information that the strains grow at 15 °C but not at 45 °C.[107] In agreement with previous observations,[107,108] *Lactobacillus lactis* and *Lactobacillus rhamnosus* exhibited the highest yields and productivities at 33 to 35 °C[1] and 41 to 45 °C,[52] respectively. Huang *et al.*[21] investigated the cultivation temperature on the solid-state fermentation of lactic acid production by controlling the growth temperatures at 22, 30, 35, and 40 °C. The results from measuring the residual starch and reducing sugar in 4 h and 8 h indicated that there was an increase in starch hydrolysis and reducing sugar accumulation as the temperature increased from 22 – 30 °C, and a further increase from 30 – 40 °C resulted in a slight improvement for the saccharification in both *Rhizopus oryzae 2062* and *Rhizopus oryzae 36017* cultures. The lactic acid production and biomass growth were affected by the temperature. The fermentation performance by the *Rhizopus arrhizus 36017* appeared to be relatively less sensitive than by the *Rhizopus oryzae 2062* with respect to the temperature. Consequently, 30 °C appeared to be an optimum cultivation temperature for both saccharification and fermentation by the *Rhizopus* species in the SSF. Narita *et al.*[28] examined the effect of temperature on the production of lactic acid from $\gamma = 20$ g L^{-1} raw starch by *Streptococcus bovis 148*.

Table 3 – *Effect of temperature on lactic acid production*

Microorganism	Substrate	Temp. θ/°C	Lactic acid mass concentration γ/g L^{-1}	yield Y/g g^{-1}	Productivity P/g L^{-1} h^{-1}	Ref.
L. amylophilus ATCC 49845	starch	25	26	0.52	0.54	64
	starch	28	29	0.58	0.44	
	starch	35	30	0.60	0.33	
L. casei NRRL B-441	glucose	30	80	0.89	3.2	52
	glucose	37	80	0.89	5.6	
	glucose	41	82	0.91	5.6	
	glucose	45	42	0.47	1.2	
L. paracasei No.8	sweet sorghum	30			1.5	48
	sweet sorghum	36			1.9	
	sweet sorghum	44			2.2	
L. rhamnosus ATCC 10863	glucose	30	67	0.74	3.3	48
	glucose	37	70	0.78	3.3	
	glucose	41	68	0.78	3.5	
	glucose	45	75	0.83	3.3	
S. bovis 148	raw starch	30	10.6			28
	raw starch	37	14.73			
	raw starch	45	10.77			
L. lactis sp. lactis ATCC 19435	glucose	30	60	1.3	2.2	1
	glucose	34	65	1.5	2.8	
	glucose	37	60	1.5	2.3	
	glucose	40	50	1.2	1.5	
L. delbrueckii	pineapple waste	37	28.73	0.917		109

The maximum lactic acid mass concentrations at 30, 37, and 45 °C were γ = 10.60, 14.73 and 10.77 g L^{-1}, respectively. The highest yield (Y = 0.88 g g^{-1}) and lactic acid mass concentration (γ = 14.73 g L^{-1}) was obtained at 37 °C.

Effect of pH

The fermentation pH is either set at the beginning and then left to decrease due to acid production or it is controlled by base titration, or by extraction, adsorption, or electrodialysis of lactic acid. The optimal pH for lactic acid production varies between 5.0 and 7.0. A pH below 5.7 was optimal for *Lactobacillus* strains, which are known to tolerate lower pH than *lactococci*. Wee *et al.*[26] investigated the influence of culture pH on lactic acid fermentation from molasses where lactic acid fermentations were performed on a jar fermentor at 38 °C and pH 5.0–9.0 using γ = 200 g L^{-1}of molasses. Although the optimum pH for cell growth of *Enterococcus faecalis RKY1* was seen to be 8.0, the lactic acid fermentation at pH 7.0 was completed faster than that at pH 8.0. The cell growth at pH 5.0 almost ceased even after 10 h of fermentation. The highest lactic acid mass concentration (γ = 4.0 g L^{-1}) was obtained at pH 7.0 with a comparable yield with pH 6.0. Huang *et al.*[21] determined the impact of pH on the starch saccharification and fermentation of lactic acid by the *Rhizopus arrhizus* 3*6017* and *Rhizopus oryzae 2062,* the pH was controlled at 4.0, 5.0, 6.0 and 7.0 by adding c = 4 mol L^{-1} NaOH solution at t = 4 h intervals during the course of cultivation. It was interesting to note that the volumetric concentration of lactic acid and biomass in the *Rhizopus arrhizus 36017* cultures increased with the increase in pH. A growth condition with starch mass concen-

tration approximately $\gamma = 20$ g L^{-1} at pH 6.0 and was favorable for both starch saccharification and lactic acid fermentation, resulting in lactic acid yield of $Y =$ 0.85–0.92 g g^{-1} associated with $\gamma = 1.5$–3.5 g L^{-1} fungal biomass produced in $t = 36$–48 h fermentation. Senthuran *et al.*[33] investigated the lactic acid production by *Lactobacillus casei* at different pH values showed that reactor productivity was highest at pH 6.0 with free cells and at pH 6.5 with immobilized cells. Productivity as well as free cell density decreased at both lower and higher pH values. The productivity was seen to decrease for successive batches in immobilized cell reactor at pH 5.5. Fu *et al.*[34] produced lactic acid in batch fermentations with synthetic lactose media by using *Lactobacillus plantarum* at various pH values ranging from 4 to 7. The optimal pH range of 5 – 6 yielded the highest values of biomass ($\gamma = 11.0$ g L^{-1}) and lactic acid mass concentration ($\gamma = 41$ g L^{-1}). Ohkouchi *et al.*[40] produced lactic acid economically by direct bioconversion from starchy substrates by using *Lactobacillus manihotivorans LMG18011*. The optimum initial pH was found to occur between 5.0 and 5.5. Above pH 6.0 or below pH 4.5, this strain could not convert all of the starch to lactic acid.

John *et al.*[31] studied the influence of initial pH of the fermented medium for lactic acid production using *Lactobacillus delbrueckii*. The effect of pH was tested at various pH values from 4–10, with and without buffering. The pH of the moistening medium was adjusted with $c = 1$ mol L^{-1} $Ca(OH)_2$ and $c = 1$ mol L^{-1} HCl. In the absence of buffering, the pH decreased to less than 3.5 within three days of fermentation and at low pH resulted in low lactic acid production. The pH 6.5 was proved the optimum for the lactic acid production ($Y = 0.237$ g g^{-1}). From pH 7–9 the yield was quite stable between $Y = 0.1923$ and 0.1992 g g^{-1}. Idris *et al.*[109] reported the effect of various initial pH on the lactic acid production of the immobilized *Lactobacillus delbrueckii* during the batch fermentation of liquid pineapple waste. At initial pH 6.5, cell started to utilize glucose earlier and at a faster rate than at other initial pH. Maximum lactic acid concentration was attained at initial pH 6.5 with a yield of $\gamma =$ 29.02 g L^{-1} or $Y = 92.7$ %. Further increase in initial pH beyond 6.5 does not improve the lactic acid production. It is possible that the higher initial pH brought too much stress on the microorganism metabolic abilities.[46]

Effect of carbon sources

A number of different substrates have been used for the fermentative production of lactic acid by LAB. The purest product is obtained when a pure sugar is fermented, resulting in lower purification costs. However, this is economically unfavorable, because pure sugars are expensive and lactic acid is a cheap product. Instead, waste products from agriculture and forestry were utilized as shown in Table 4. The study on lactic acid production by Senthuran *et al.*[33] with free cell cultivations in a medium containing different sugars revealed that *Lactobacillus casei* preferred lactose as a carbon source for its growth and lactic acid production, followed by glucose and maltose, while sucrose was poorly utilized. This was in contrast to the report by Ohleyer *et al.*[110] where glucose was the preferred substrate by *Lactobacillus delbrueckii*. Lactose used at the mass concentration of $\gamma = 50$ g L^{-1} in synthetic medium was completely utilized by the cells giving a productivity of $P = 2.0$ g L^{-1} h^{-1} and final cell mass of $\gamma = 5.1$ g L^{-1}, while delayed growth and incomplete substrate utilization was observed in the medium containing only glucose at the same concentration. The preliminary experiment of Yun *et al.*[24] with vial cultivation in a medium containing the different sugars revealed that *Enterococcus faecalis RKY1* utilized glucose, fructose, and maltose as carbon sources for growth and lactic acid production, while galactose and sucrose were metabolized to formic acid and acetic acid as main products, and xylose, glycerol, whey, and starch were poorly utilized. When *Enterococcus faecalis RKY1* was cultivated on these three carbon sources, cell growth and lactic acid formation patterns were similar. The highest volumetric productivity was found to be with cells grown in a medium containing fructose, which was completely utilized within $t = 35$ h. The average volumetric productivity and yield of lactic acid was $P = 4.12$ g L^{-1} h^{-1} and $Y = 0.96$ g-lactic acid (g-fructose)$^{-1}$ respectively. In many lactic acid bacteria (LAB), fructose metabolism generally differs from glucose metabolism in that fructose acts both as a growth substrate and electron acceptor. When *Enterococcus faecalis RKY1* was grown on a mixture of glucose ($\gamma = 75$ g L^{-1}) and fructose ($\gamma = 75$ g L^{-1}) the cell growth and volumetric productivity were higher than growth on each sugar alone. Furthermore, for the lactic acid fermentations with glucose/fructose, glucose/maltose, and fructose/maltose mixtures as carbon sources, *Enterococcus faecalis RKY1* grown on a mixture of glucose/fructose simultaneously consumed these sugars, and the cell growth and average volumetric productivity were higher than when grown on the individual sugars. Ohkouchi *et al.*[40] selected glucose, soluble starch, and starch from rice or potato as carbon sources. At initial pH 6.5 the saccharification of starch was inhibited. Therefore, *Lactobacillus manihotivorans LMG18011* was unable to take up carbohydrate or produce lactic acid under this condition.

Table 4 – *Effect of carbon sources on lactic acid production*

Microorganism	Substrate	Lactic acid mass concentration γ/g L^{-1}	Yield Y/g g^{-1}	Productivity P/g L^{-1} h^{-1}	Ref.
L. amylophilus ATCC 49845	glucose	21	0.95	1.6	111
	corn starch	33	0.73	0.88	
L. amylovorus	cassava starch	4.8	0.48	0.69	79
	corn starch	10	1.0	1.2	
	potato starch	4.2	0.42	0.14	
	rice starch	7.9	0.79	0.86	
	wheat starch	7.8	0.78	1.2	
L. delbrueckii sp. bulgaricus CBS 743.84	glucose	35	0.85		112
	lactose	37	0.82		
L. delbrueckii sp. bulgaricus CNRZ359	glucose	56	2.8		113
	cellobiose	32	1.6		
L. paracasei No. 8	glucose	95	0.95	5.6	48
	sweet sorghum	91	0.91	10	
L. pentosus	glucose	46	0.92	2.4	114
	xylose	27	0.54	0.59	
	glucose+xylose	90	1.8	4.0	
L. rhamnosus ATCC 10863	glucose	17	0.86		115
	fructose	14	0.71		
	glucose + fructose	16	0.81		
	sucrose	15	0.73		
L. plantarum	hydrolyzed soluble starch	15	0.30		116
	hydrolyzed tapioca starch	15	0.30		
	hydrolyzed tapioca flour	17	0.35		
L. plantarum NRRL B-531	glucose	5.4	0.54		117
	galactose	3.7	0.37		
	mannose	5.7	0.57		

However, at initial pH 5.5, lactic acid production occurred with all carbon sources. Kadam *et al.*[118] used different carbohydrate sources to produce lactic acid. When the medium containing glucose, fructose, lactose or galactose was used as carbon source, mutant Uc-3 could produce lactic acid more efficiently than the parent strain. The maltose, xylose and sucrose were utilized very poorly for growth by both the mutant and parent strains resulting in no lactic acid production. The optimal carbon sources for lactic acid production were found to be glucose, fructose, lactose or galactose.

Effect of nitrogen sources

The medium composition has been investigated from many aspects, including the addition of various mass concentrations of nutrients in the form of yeast extract, peptone or corn steep liquor. The addition of nutrients and higher nutrient mass concentrations generally had a positive effect on the lactic acid production. MRS medium, which contains yeast extract, peptone and meat extract, was superior to yeast extract, which in turn was better than malt extract. Senthuran *et al.*[33] used a synthetic medium which contains γ = 10 g L^{-1} yeast extract as a

nitrogen source. Higher concentration or a better nitrogen source may improve the reactor performance. Yeast extract is considered an essential nutrient for *lactobacilli* for an efficient lactic acid production.[119] The performance of lactic acid fermentation with hydrolyzed whey protein and yeast extract was compared. Whey can be directly subjected to enzymatic treatment.[75] Replacing the yeast extract containing synthetic medium with whey protein based medium (maintaining the same level of elemental nitrogen) containing lactose and glucose at the same mass ratio (ζ = 1:19) resulted in a higher lactate production rate by the free cells giving a productivity of 2.8 and P = 1.7 g L^{-1} h^{-1}. A variety of nitrogen sources have been tested for lactic acid production, but they did not give the product concentrations as high as those obtained with yeast extract.[120] Ohkouchi *et al.*[90] reported that lactic acid productivities were improved by the supplementation of nitrogen sources with tryptone, beef extract, and MRS-complex. In particular, supplementation with tryptone or MRS-complex, the total nitrogen contents were improved to 0.58 % and 0.72 %, respectively, and both the acceleration of the bioconversion rate and a doubling of lactic acid production, from γ = 35 g L^{-1} up to γ = 70 g L^{-1}, were observed. There was a little improvement of the bioconversion only by nitrogen supplementation with beef extract (the total nitrogen content; w = 0.48 %). Zhou *et al*.[121] attempted nitrogen supplements for the bioconversion of municipal solid waste to lactic acid by *Lactobacillus pentosus B-227*.

Nancib *et al.*[37] reported the effects of supplementing date juice with different nitrogen sources such as yeast extract, ammonium sulfate, tryptic soy, urea, peptone and casein hydrolysate on the lactic acid product performance of *Lactobacillus casei subsp. rhamnosus*. None of the non-yeast-extract nitrogen sources gave lactic acid concentrations as high as that of yeast extract. On the other hand, ammonium sulfate seems to be a good alternative to yeast extract. Among the nitrogen sources tested, urea gave the lowest mass concentrations of lactic acid (γ = 14.1 g L^{-1}). Traditionally, the most common nutrients for the preparation of fermentative media are yeast extract and peptone, which turn out to be very expensive being able to account for almost 30 % of the total cost of the process. Because of this, the search for alternative, financially competitive nutrient sources is particularly interesting.

Effect of mineral salts

Mineral salts play a vital role in the lactic acid fermentation. Chauhan *et al.*[122] screened various medium components by Plackett-Burman design at the confidence level of 95 % on the basis of their effects. The components KH_2PO_4, $MgSO_4 \cdot 7\ H_2O$, NaCl, tri-sodium citrate and sodium succinate were found to be less significant on lactic acid products. The components, peptone, beef extract, yeast extract, K_2HPO_4, sodium acetate, sodium sulfate, $FeSO_4 \cdot 7\ H_2O$, and $MnSO_4 \cdot 4\ H_2O$ were found to be significant. Nitrogen source was found to be significant at γ = 1.0 g L^{-1} concentration during solid-state fermentation using wheat bran and during selection of media components for lactic acid production by *Lactobacillus plantarum NCIM 2084* at 1.0 g L^{-1}.[98,123] Sodium acetate was also found significant at 99.43 % confidence level. It enhanced the cell growth and thus influenced the production indirectly.[124] Tween-80 has also been reported to be significant component for lactic acid production using wheat bran under solid-state fermentation[98] and for production of enzymes. Among the phosphate sources used, only K_2HPO_4 was significant and there was considerable difference in lactic acid production among the two phosphate sources.

Vasala *et al.*[41] produced lactic acid from *Lactobacillus ssp. salicinius.* It grew slowly in the absence of peptides even in the presence of high amount of whey proteins. In order to reach a high concentration of lactic acid, reasonable high number of bacteria should be achieved before product inhibition stops the growth.[125] Fast growth and correspondingly, significantly higher lactic acid accumulation was achieved by supplementing the medium with either yeast extract or by treating the medium with proteolytic enzymes.

Purification of lactic acid

Lactic acid is sold in various commercial grades, and the better grades require that well-purified substrates be utilized in the fermentation medium in order to reduce the levels of impurities present during recovery which, without great difficulty, cannot be separated from the lactic acid. Also, in this regard, the sugar should be depleted from the medium by harvest of the fermentation. One of the commercial grades of lactic acid, "crude" or "technical" grade is a colored product prepared for commercial usage at mass fraction in water of w = 22, 44, 50, 66 and 80 %. It is prepared by employing sulfuric acid to remove the calcium from the calcium lactate derived from the heated and filtered fermentation broth, followed by filtration, concentration, and refiltration to remove additional calcium sulfate. Thus, this grade of lactic acid contains many of the impurities from the fermentation medium, and it finds many industrial uses where purity of the product is not essential as, for

example, in the deliming of hides in the leather industry. The 'edible' grade of lactic acid is snow colored and is marketed 50–80 % strengths. Thus, it receives additional refining over that of technical lactic acid. Colorless, high purity lactic acids are the plastic grade, marketed at 50–80 % strength, and "U.S.P". Lactic acid marketed at 50–80 % strengths. Other commercial preparations of lactic acid are calcium lactate, sodium lactate, and copper lactate (a salt used in electroplating). The final recovered yields of technical and edible-grade lactic acids, based on the original carbohydrate of the medium, are approximately 85–90 % and 80 %, respectively. Plastic and U.S.P grades are prepared by further refining of technical grade lactic acid and therefore, slight to moderate yield losses are incurred during this refining. Lactic acid is commercially available at different grades (qualities). They are technical grade lactic acid (20–80 %), food grade lactic acid (80 %), pharmacopoeia grade lactic acid (90 %), and plastic grade lactic acid. Pharmaceutical and food grade lactic acids are considerable to be of most important.

For the recovery of lactic acid, additional calcium carbonate is added to the medium, the pH is adjusted to approximately 10, and the fermentation broth is heated and then filtered. This procedure converts all of the lactic acid to calcium lactate, kills bacteria, coagulates protein of the medium, removes excess calcium carbonate and helps to decompose any residual sugar in the medium. 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 sulfuric acid to remove the calcium as calcium sulfate. The lactic acid is then re-crystallized as calcium lactate, and activated carbon is used to remove colored 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. This is a counter current continuous extraction, and the lactic acid is recovered from the isopropyl ether by further counter-current washing of the solvent with water. In a third procedure, the methyl ester of the free lactic acid is prepared, and this is separated from the fermentation broth by distillation followed by hydrolysis of the ester by boiling in dilute water solution (the methyl ester decomposes in water). The lactic acid is then obtained from the aqueous solution by evaporation of the water, and the methanol is recovered by distillation. In a fourth procedure, secondary or tertiary alkyl amine salts of lactic acid are formed and then extracted from aqueous solution with organic solvents; the solvent is removed by evaporation, and the salt then is decomposed to yield the free acid. An older procedure, not utilized commercially to any extent today, involves direct high-vacuum steam distillation of the lactic acid from the fermentation broth, but decomposition of some of the lactic acid occurs.

The fermentation broth is generally heated to 70 °C to kill the bacteria and then acidified with sulfuric acid to pH 1.8. The clarified lactic liquor is then ion exchanged and concentrated to 80 %. Smell and taste can be improved further by oxidative treatment with hydrogen peroxide. The lactic acid obtained at this stage is suitable for some food industries. The lactic acid produced from biological fermentation requires extensive purification operations. It is of particular importance that the recovery processing equipment be resistant to the corrosive action of the high concentrations of lactic acid that accumulate. Therefore, special stainless steel equipment is most often employed for this purpose.

Sun *et al.*[126] used two reactors with a rectifying column carried out recovery of lactic acid from the fermentation broth. Ammonium lactate obtained by fermentation was used directly to produce butyl lactate by reacting with butanol for 6 h, and the esterification yield of ammonium lactate was Y = 87.7 %. In this procedure, a cation exchange resin which was modified by $SnCl_2$ replaced sulphuric acid as a catalyst, and neutral ammonium lactate replaced former lactic acid as a starting material, which not only eliminated corrosion of a reactor, but also avoid generating calcium salts as a byproduct. Then butyl lactate was rectified, and the purified butyl lactate was sequentially hydrolyzed into lactic acid in presence of the cation exchange resin in the H^+ form as a catalyst for 4 h, and the hydrolysis yield was 89.7 % and the purity of recovered lactic acid was 90 %.

Bouchoux *et al.*[127] investigated nanofiltration for usability in a specific lactic acid production process based on conventional and bipolar electrodialysis operations. Industrial fluids, corresponding to two potential integration levels and coming from an existing installation, were investigated. Nanofiltration was able to efficiently remove magnesium and calcium ions from a sodium lactate fermentation broth before its concentration and conversion by electrodialysis (first potential integration level). Maximum impurities rejections and lactate recovery were obtained at maximum transmembrane pressures. Mg^{2+} and Ca^{2+} rejections were 64 ± 7 and 72 ± 7 %, respectively and lactate recovery flux reached 25 ± 2 mol m^{-2} h^{-1} for pressure p = 20 bar. Sulfate and phosphate ions were also partially removed from the broth (40 % rejection). At the in-

vert, chloride ions were negatively retained by the membrane and were consequently more concentrated in the permeate. Nanofiltration also led to a nearly total decolouration of the fermentation broth. On the other hand, sulfate and phosphate rejections obtained from the filtration of a converted broth containing the lactic acid under its neutral form (second potential integration level) were also satisfactory, i.e. 47 ± 5 and 51 ± 5 %, respectively. High recovery fluxes were observed in that case, i.e. J = 48 ± 2 mol m^{-2} h^{-1} at p = 20 bar.

Tong *et al.*[128] reported the purification results of lactic acid from the fermentation broth with paper sludge as a cellulosic feedstock using weak anion exchanger Amberlite IRA-92. Some factors such as flow rate, sample volume loaded, pH, and column were systematically examined to improve the purity, yield and productivity in lactic acid purification. Adsorption isotherm of standard lactic acid and lactic acid in the fermentation broth by anion exchanger IRA-92 were also investigated. Results indicate that in purification process the increase of pH of the fermentation broth ranging from 5.0 to 6.0 can significantly enhance the recovery yield, purity and productivity. The decrease of flow rate and sample volume loaded can also improve the recovery yield and purity but apparently reduce the productivity. In addition, the scale-up of purification process in laboratory size had little influence on the recovery yield and purity. After optimization, the yield, purity and productivity were found to be about 82.6 %, 96.2 % and 1.16 g LA / (g-resin day), respectively.

Madzingaidzo *et al.*[129] studied purification of cell free sodium lactate solutions by mono- and bi-polar electro dialysis. Lactate was concentrated by mono-polar electrodialysis to a maximum of γ = 150 g L^{-1}. At high feed mass lactate flux reached G = 300 g m^{-2} h^{-1} with correspondingly high current efficiency in the 90 % range. Relatively low water transport rates were observed during processing with mono-polar electrodialysis. A low incidence of impurities was observed in the concentrate solutions with less than γ = 2 g L^{-1} glucose and γ = 1.5 g L^{-1} acetate detected respectively. Subsequent purification with bi-polar electrodialysis yielded good performance parameters with water transport rates as low as m = 70 g H_2O per mol L^{-1} lactate and lactate flux reaching a high of G = 300 g m^{-2} h^{-1}. Current efficiency for bi-polar electrodialysis was in excess of η = 90 %. Free lactic acid mass concentration reached a moderate of 160 g L^{-1} while colour and other chemical impurities were significantly reduced. Additional bleaching and de-ionisation process steps should however be integrated to polish the free lactic acid for high-grade applications in the biodegradable thermoplastic and pharmaceutical industries. Acetic acid impurity remained at around γ = 1 g L^{-1}. Significant reduction in colour and minerals in the product streams was observed during electrodialysis purification.

The physico-chemical and operating effects of lactic acid, sodium lactate and ammonium lactate on the RO process have been investigated using a polyamide composite membrane by Liew *et al.*[130] This particular type of membrane was found to swell at pH 2.2 but had no detectable solute-membrane affinity. The flux and permeate concentration were found to be an inverse function, whereas the solute reduction factor was a direct function of the pH value of feed. This was attributed to the greater concentration of ions dissociated into the solution as the pH was increased, and the fact that hydrated ions possess larger sizes than molecules by merit of their charge densities is believed to help enhance rejection by their lower rates of diffusion. At this stage, ammonium hydroxide is considered to be the optimum pH-controlling agent for lactic acid production by virtue of its reasonably high flux and solute rejection as well as its capability to augment cell growth by acting as a nitrogen source. In terms of operating conditions, an increase in pressure from p = 1 MPa to 7 MPa has contributed to a higher flux, which outweighs the effect of the increasing total solute loss and consequently leads to an increase in solute rejection. On the contrary, an increase in feed mass fraction, especially to $w \geq$ 3.80 %, has incurred pronounced effects on concentration polarization, leading to a reduced solute rejection and concentration efficiency.

Applications of lactic acid

Food industry

Lactic acid is widely used in almost every segment of the food industry, where it serves in a wide range of functions. The major use of lactic acid is in food and food-related applications, which, in the U.S., accounts for approximately 85 % of the demand. The rest (~ 15 %) of the uses are for non-food industrial applications. Lactic acid occurs naturally in many food products. It has been in use as an acidulant, preservative and pH regulator for quite some time. There are many properties of lactic acid which make it a very versatile ingredient in the food industry. It has a pronounced preservative action, and it regulates the microflora. It has been found to be very effective against certain type of microorganisms. Some times a combination of lactic acid and acetic acid is used as it has a greater bactericidal activity. The calcium salt of lactic acid, calcium lactate, has greater solubility than the corresponding salt of citric acid. In such products

where turbidity caused by calcium salts is a problem, the use of lactic acid gives products that are clear. L(+)-lactic acid is the natural acid found in biological systems and hence its use as an acidulant and does not introduce a foreign element into the body. Moreover, lactic acid is used commercially in the processed meat and poultry industries, to provide products with an increased shelf life, enhanced flavor, and better control of food-born pathogens. Another potential application of lactic acid in the food industry is the mineral fortification of food products.

Confectionery

Lactic acid finds use as an acidulant in the confectionery industry. It is a better acidulant than citric acid since the sugar inversion is less when used for hardboiled candies. It does not have the initial burst of flavor and tanginess of citric acid. Lactic acid imparts a mellower and lasting sourness and enhances the flavor much more. The use of buffered lactic acid in continuous production lines for high boiled sweets is a more recent application. Liquid buffered lactic acid may be converted easily to the molten syrups, even at the high temperatures used in depositing lines. In sugar confectionery it is used in continuous production lines for high boiled sweets (like bonbons) to make perfectly clear sweets, with minimum sugar inversion and with no air trapped. Lactic acid is used in confectionery, not only for flavor, but also to bring the pH of the cooked mix to the correct point for setting.

Beer and wine

Lactic acid is a natural beer acid and hence it is used for pH adjustments during the mashing process and in wort cooking. Lactic acid improves the microbial stability and also enhances the flavor of beer during the manufacturing process.

Beverages

Lactic acid is used as an acidulant in delicately flavored soft drinks and fruit juices. It does not mask or over power the natural flavor. Its flavor enhancing property makes the beverage more palatable and leaves a lingering taste. Lactic acid is preferred over citric acid for these reasons. Use of buffered lactic acid improves the taste and flavor of many beverages, such as soft drinks, mineral water, carbonated fruit juices etc.

Olives, pickles, cabbage, gherkins

Green olives, gherkins and others are often packed in a solution of salt, lactic acid and water. The lactic acid acts as a preservative and improves the clarity of the brine and flavor. A mixture of acetic acid and lactic acid in pickled products such as gherkins, silver skin onion etc. imparts a milder taste and flavor, and improves microbial stability. Calcium lactate is reported to be used as firming salt, which have been used for canned fruits and vegetables.

Dairy products

Direct acidification with lactic acid, in dairy products such as cottage cheese, is preferred to fermentation as the risks of failure and contamination can be avoided. The processing time also can be reduced. Lactic acid and calcium lactate are used extensively in the production of Channa and Panneer by direct acidification. Lactic acid is also used as an acidulant in dairy products like cheese, margarine and yogurt powder. In dairy products such as cottage cheese, addition of lactic acid is preferred to fermentation.

Bakery products

For direct acidification of certain breads, lactic acid is the natural sour dough acid. The general appearance of a loaf of bread is greatly improved by the use of bacterial lactic acid, a larger loaf results per weight of bread with improved bloom, and color of crust. Lactic acid is directly added to certain types of fermented dough crispy biscuits. Lactic acid added to dough increases the shelf life due to its retarding action on molds and rope. The sodium and calcium stearoyl lactylates find use as emulsifiers in the baking industry as they provide substantial quality improvement of baked products besides reducing shortening levels. In bakery products it is used for direct acidification of rye or rye-wheat breads. It increases butter stability and volume. Part of the egg albumen can be replaced by less expensive calcium lactate. A large fraction ($w >$ 50 %) of the lactic acid for food-related uses goes to produce emulsifying agents used in foods, particularly for bakery goods. These emulsifying agents are esters of lactate salts with longer chain fatty acids, and the four important products are calcium and sodium, stearoyl-2-1actylate, glyceryl lactostearate, and glyceryl lactopalmirate. Of the stearoyl lactylates, the calcium salt is a very good dough conditioner, and the sodium salt is both a conditioner and an emulsifier for yeast leavened bakery products. The glycerates and palmitates are used in prepared cake mixes and other bakery products and in liquid shortenings.

Meat and meat products

Lactic acid is widely used in meat products as an antimicrobial agent. Decontamination of beef, poultry and pork carcasses in slaughterhouse opera-

tions is practiced to reduce *Salmonella* infection. In sausages, sodium lactate is used to reduce water activity and achieve higher shelf life. Recent research publication indicates the use of hot lactic acid spray on carcasses where reduction of over 99 % of *E. coli* has been observed. Lactic acid is also used in the improvement of shelf-life of buffalo meat.[131] An emerging new use for lactic acid or its salts is in the disinfection and packaging of carcasses, particularly those of poultry and fish, where the addition of aqueous solutions of lactic acid and its salts during the processing increased shelf life and reduced the growth of anaerobic spoilage organisms such as *Clostridium botulinum*.

Cosmetic industry

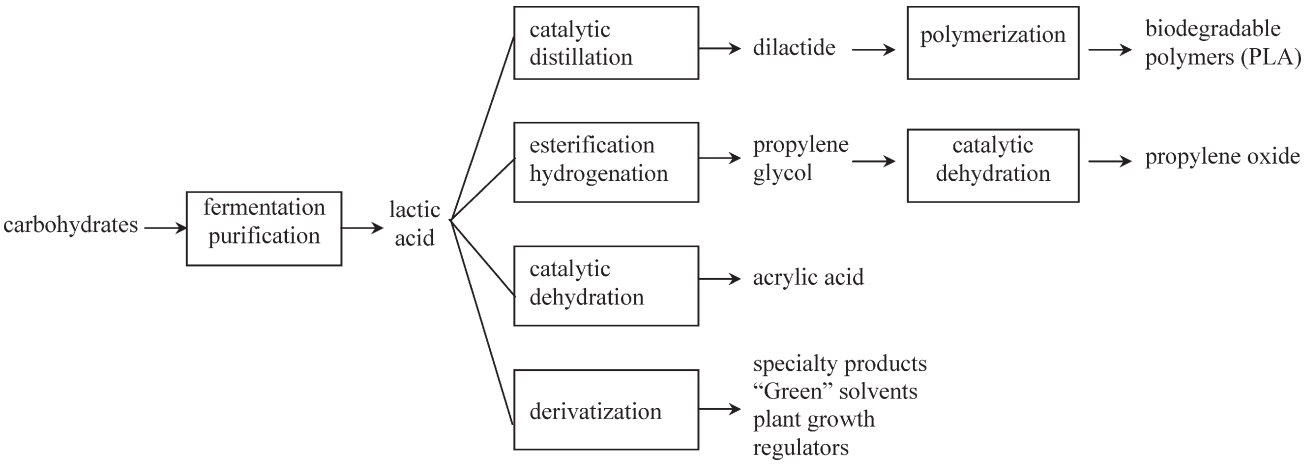
Lactic acid offers natural ingredients for cosmetic applications. Although primarily used as moisturizers and pH regulators, they possess multiple other properties such as antimicrobial activity, skin lightening, and skin hydration. The moisturizing effect is related directly to lactate's water retaining 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. Lactic acid is popularly known as an alpha hydroxy acid (AHA) in the cosmetics industry. It is widely used as a milder alternative to glycolic acid. It is primarily used as an anti-aging chemical claimed to soften lines, reduce photo damage from the sun, improve skin texture and tone and improve overall appearance. Precautions should be taken when using lactic acid as a cosmetic agent because it can increase sensitivity to the sun's UV radiation.

Chemical industry

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) (Fig. 3). In the chemical industries, lactic acid is used in the dyeing of silks and other textile goods, as a mordant in the printing of woolens, in the bating and plumping of leathers, in the deliming of hides, in vegetable tanning, and as a flux for soft solders. The water-white grade is used in plastic industry. 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.

Pharmaceutical industry

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 includes tablets, prostheses, surgical sutures,



F i g . 3 – *Lactic acid potential products and technologies*[3]

and controlled drug delivery systems. Lactic acid has many pharmaceutical formulations, particularly in topical ointments, lotions, anti acne solutions, humectants, parental solutions and dialysis applications, for anti carries agent. Its biodegradable polymer has medical applications as sutures, orthopedic implants, controlled drug release etc. Poly L-lactic acid is used in many medical products such as stitches and screws used to repair broken bones. The calcium salt is widely used for calcium-deficiency therapy and as an effective anti-caries agent.

They provide the energy and volume for blood besides regulation of pH. Calcium, sodium, ferrous and other salts of lactic acid are used in the pharmaceutical industry in various formulations. Lactate salts have better absorption, solubility and are easily metabolized resulting in administration of some very important drugs like ciprofloxacin as a lactate salt. Lactic acid based formulations find use for their antitumor activity. The antimicrobial action of lactic acid is taken advantage for use as sanitizers. It is reported that lactic acid finds use in the treatment of dermatological problems like warts.

Polymer industry

Lactic acid has recently received a great deal of attention as a feedstock monomer for the production of polylactic acid (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. Table 5 shows the uses of various potential polymer products of lactic acid. 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.

Table 5 – *Potential products from lactic acid*

Product	Uses
degradable plastics	packaging, films
oxychemicals:	
propylene glycol	polymers, food deicers, humectants
acrylates	polymers, plastic films, coatings
propylene oxide	polymers, plastics
“green” chemicals/solvents:	plasticizers, food processing
esters	packaging
ester derivatives	same as above
plant growth regulators:	
poly-L-lactates	mulch film for vegetable and fruit crops

Other applications

Technical grade lactic acid is used as an acidulant in vegetable and leather tanning industries. Lactic acid is being used in many small scale applications like pH adjustment hardening baths for cellophanes used in food packaging, terminating agent for phenol formaldehyde resins, alkyl resin modifier, solder flux, lithographic and textile printing developers, adhesive formulations, electroplating and electro-polishing baths, detergent builders. It is also used for the extraction of fish skin gelatin.[132] In recent days it is used in the field of soft tissue augmentation and also used as adhesive in lamination industries.[133]

Lactic acid has better descaling properties than conventional organic descalers due to which reason it is used in many decalcification applications such as cleaners for toilets, bathrooms etc. Lactate esters like ethyl, methyl lactate etc. are used for degreasing since they have excellent action for oils, oligomeric and polymeric stains. Lactic acid is used in Ni – plating process because of its unique complexing constant for Ni. Lactic acid is used as a pH regulator and complexing agent in various binder systems for water–based coatings such as electro-deposition coatings. Lactates find use as neutralizers in the production of certain types of surfactants, used in special detergents and personal care products.

Conclusions

Lactic acid is one of the most important chemical that can be derived from renewable resources like refined sugars, molasses, whey, raw starchy materials and lignocellulose which is used to make a wide variety of useful products. The current major markets for lactic acid are food related industries, but the emerging markets for polylactic acid polymer would cause a significant increase in growth of lactic acid consumption. Many investigations explained the various factors such as plant size, raw material cost, and various microorganisms involved in the production of lactic acid, and capital investment. However, there are still several researches that need to be addressed in order to produce lactic acid biotechnologically within the targeted cost, development of high-performance lactic acid producing microorganisms and lowering the cost of the raw materials.

List of symbols

c – concentration, mol L^{-1}
D – dilution rate, h^{-1}
G – mass flux, g m^{-2} h^{-1}
J – molar flux, mol m^{-2} h^{-1}
m – mass, g
P – productivity, g L^{-1} h^{-1}
p – pressure, bar, MPa
t – time, h
w – mass fraction, %
Y – yield, %
γ – mass concentration, g L^{-1}
θ – temperature, °C
η – efficiency, %
ζ – mass ratio

References

1. *Akerberg, C., Hofvendahl, K., Zacchi, G., Hahn-Hagerdal, B.,* Appl. Microbiol. Biotechnol. **49** (1998) 682.
2. *Hofvendahl, K., Hahn-Hagerdal, B.,* Enzyme Microb. Technol. **26** (2000) 87.
3. *Datta, R., Tsai, S. P., Bonsignore, P., Moon, S. H., Frank, J. R.,* FEMS Microbiol. Rev. **16** (1995) 221.
4. *Hofvendahl, K., Akerberg, C., Zacchi, G.,* Appl. Microbiol. Biotechnol. **52** (1999) 163.
5. *Khalaf, S. A.,* Egypt. J. Microbiol. **36** (1) (2001) 89.
6. *Yin, P., Nishiya, N., Kosakai, Y., Yahira, K., Park, Y. S., Okabe, M.,* J. Ferment. Bioeng. **84** (1997) 249.
7. *Tsao, G. T., Cao, N. J., Du, J., Gong, C. S.,* Adv. Biochem. Eng. Biotechnol. **65** (1999) 245.
8. *Richter, K., Berthold, C.*, J. Agric. Eng. Res. **71** (1998) 181.
9. *Anuradha, R., Suresh, A. K., Venkatesh, K. V.,* Process Biochem. **35** (1999) 367.
10. *Aristidous, A., Penttila, M.,* Curr. Opin. Biotechnol. **11** (2000) 187.
11. *Huang, L. P., Jin, B., Lant, P., Zhou, J. T.,* J. Chem. Technol. Biotechnol. **78** (2003) 889.
12. *Litchfield, J. H.,* Adv. Appl. Microbiol. **42** (1996) 45.
13. *Naveena, B. J., Altaf, M., Bhadrayya, K., Reddy, G.,* Food Technol. Biotechnol. **42** (2004) 147.
14. *Wassewar, K. L.,* Chem. Biochem. Eng. Q. **19** (2005) 159.
15. *Gross, R. A., Kalra, B.,* Science **2** (2002) 803.
16. *Gottschalk, G.,* Bacterial metabolism, second ed., Springer-Verlag, New York 1986.
17. *Zhou, Y., Dominguez, J. M., Cao, N., Du, J., Tsao, G. T.,* Appl. Biochem. Biotechnol. **78** (1999) 401.
18. *Tay, A., Yang, S. T.,* Biotechnol. Bioeng. **80** (2002) 1.
19. *Kosakai, Y., Park, Y. S., Okabe, M.,* Biotechnol. Bioeng. **55** (1999) 461.
20. *Park, E. Y., Kosakai, Y., Okabe, M.,* Biotechnol. Progr. **14** (1998) 699.
21. *Huang, L. P., Jin, B., Lant, P., Zhou, J.,* Biochem. Eng. J. **23** (2005) 265.
22. *Park, E. Y., Anh, P. N., Okuda, N.,* Bioresour. Technol. **93** (2004) 773.
23. *Garde, A., Jonsson, G., Schmidt, A. S., Ahring, B. K.,* Bioresour. Technol. **81** (2002) 217.
24. *Yun, J. S., Ryu, H. W.,* Process Biochem. **37** (2001) 235.
25. *Rivas, B., Moldes, A. B., Dominguez, J. M., Parajo, J. C.,* Enzyme Microb. Technol. **34** (2004) 627.
26. *Wee, Y. J., Kim, J. N., Yun, J. S., Ryu, H. W.,* Enzyme Microb. Technol. **35** (2004) 568.
27. *Kourkoutas, Y., Xolias, V., Kallis, M., Bezirtzoglou, E., Kanellaki, M.,* Process Biochem. **40** (2005) 411.
28. *Narita, J., Nakahara, S., Fukuda, H., Kondo, A.,* J. Biosci. Bioeng. **97** (2004) 423.
29. *Chauhan, K., Trivedi, U., Patel, K. C.,* Bioresour. Technol. **98** (2007) 98.
30. *Patil, S. S., Kadam, S. R., Patil, S. S., Bastawde, K. B., Khire, J. M., Gokhale, D. V.,* Lett. Appl. Microb. **43** (2006) 53.
31. *John, R. P., Nampoothiri, K. M., Pandey, A.,* Process Biochem. **41** (2006) 759.
32. *Amrane, A., Prigent, Y.,* J. Biotechnol. **45** (1996) 195.
33. *Senthuran, A., Senthuran, V., Hatti-Kaul, R., Mattiasson, B.,* J. Biotechnol. **73** (1999) 61.
34. *Fu, W., Mathews, A. P.,* Biochem. Eng. J. **3** (1999) 163.
35. *Nolasco-Hipolito, C., Matsunaka, T., Kobayashi, G., Sonomoto, K., Ishizaki, A.,* J. Biosci. Bioeng. **93** (2002) 281.
36. *Amrane, A.,* Enzyme Microb. Technol. **28** (2001) 827.
37. *Nancib, A., Nancib, N., Meziane-Cherif, D., Boubendir, A., Fick, M., Boudrant, J.,* Bioresour. Technol. **96** (2005) 63.
38. *Oh, H., Wee, Y. J., Yun, J. S., Han, S. H., Jung, S., Ryu, H. W.,* Bioresour. Technol. **96** (2005) 1492.
39. *Schepers, A. E., Thibault, J., Lacroix, C.,* Enzyme Microb. Technol. **38** (2006) 324.
40. *Ohkouchi, Y., Inoue, Y.,* Bioresour. Technol. **97** (2006) 1554.
41. *Vasala, A., Panula, J., Neubauer, P.,* J. Biotechnol. **117** (2005) 421.
42. *Xu, G. Q., Chu, J., Wang, Y. H., Zhuang, Y. P., Zhang, S. L., Peng, H. Q.,* Process Biochem. **41** (2006) 2458.
43. *Xu, Z., Wang, O., Wang, P., Cheng, G., Ji, Y., Jiang, Z.,* Process Biochem. **42** (2007) 89.
44. *Sakai, K., Fujii, N., Chukeatirote, E.,* J. Biosci. Bioeng. **102** (2006) 227.
45. *Kutzanmanidis, C., Roukas, T., Skaracis, G.,* World J. Microbiol. Biotechnol. **18** (2002) 442.
46. *Goksungur, Y., Guvenc, U.,* J. Chem. Eng. Biotechnol. **69** (1997) 399.
47. *Soccol, C. R., Marin, B., Lebeault, J. M., Raimbault, M.,* Appl. Microbiol. Biotechnol. **41** (1994) 286.
48. *Richter, K., Trager, A.,* Acta Biotechnol. **14** (1994) 367.
49. *Hofvendahl, K., Hahn-Hagerdal, B.,* Enzyme Microb. Technol. **20** (1997) 301.
50. *Vishnu, C., Seenayya, G., Reddy, G.,* Bioprocess Eng. **23** (2000) 155.
51. *Bustos, G., Moldes, A. B., Cruz, J. M., Dominguez, J. M.,* J. Sci. Food Agric. **84** (2004) 2105.
52. *Hujanen, M., Linko, Y. Y.,* Appl. Microbiol. Biotechnol. **45** (1996) 307.
53. *Burgos-Rubio, C. N., Okos, M. R., Wankat, P. C.,* Biotechnol. Prog. **16** (2000) 305.
54. *Schepers, A. W., Thibault, J., Lacroix, C.,* Enzyme Microb. Technol. **30** (2002) 176.
55. *Berry, A. R., Franco, C. M. M., Zhang, W., Middelberg, A. P. J.,* Biotechnol. Lett. **21** (1999) 163.
56. *Siebold, M., von Frieling, P., Joppien, R., Rindfleisch, D., Schugerl, K., Roper, H.,* Process Biochem. **30** (1995) 81.
57. *Javanainen, P., Linko, Y.-Y.,* Biotechnol. Tech. **9** (1995) 543.

58. *Samuel, W. A., Lee, Y. Y., Anthony, W. B.,* Biotechnol. Bioeng. **22** (1980) 757.
59. *Gupta, R., Gandhi, D. N.,* Indian J. Dairy Sci. **48** (1995) 636.
60. *Audet, P., Paquin, C., Lacroix, C.,* Appl. Microbiol. Biotechnol. **29** (1988) 11.
61. *Altaf, M., Naveena, B. J., Reddy, G.,* Food Technol. Biotechnol. **43** (2005) 235.
62. *Monteagudo, J. M., Rodriguez, L., Rincon, J., Fuertes, J.,* J. Chem. Technol. Biotechnol. **68** (1997) 271.
63. *Nakamura, L. K., Crowell, C. D.,* Dev. Ind. Microbiol. **20** (1979) 531.
64. *Yumoto, I., Ikeda, K.,* Biotechnol. Lett. **17** (1995) 543.
65. *Zhang, D. X., Cheryan, M.,* Biotechnol. Lett. **13** (1991) 733.
66. *Giraud, E., Champailler, A., Raimbault, M.,* Appl. Environ. Microbiol. **60** (1994) 4319.
67. *Amrane, A*., World J. Microbiol. Biotechnol. **16** (2000) 207.
68. *Yoo, I.-K., Chang, H. N., Lee, E. G., Chang, Y. K., Moon, S. H.,* J. Ferment. Bioeng. **84** (1997) 172.
69. *Hsieh, C. M., Yang, F.-C., Iannotti, E. L.,* Process Biochem. **34** (1999) 173.
70. *Kwon, S., Lee, P.-C., Lee, E. G., Chang, Y.-K., Chang, N.,* Enzyme Microb. Technol. **26** (2000) 209.
71. *Kurbanoglu, E. B., Kurbanoglu, N. I.,* FEMS Microbiol. Lett. **225** (2003) 29.
72. *Leh, M. B., Charles, M.,* J. Ind. Microbiol. **4** (1989) 77.
73. *Krischke, W., Schroder, M., Trosch, W.,* Appl. Microbiol. Biotechnol. **34** (1991) 573.
74. *Lund, B., Norddahl, B., Ahring, B.,* Biotechnol. Lett. **14** (1992) 851.
75. *Senthuran, A., Senthuran, V., Mattiasson, B., Kaul, R.,* Biotechnol. Bioeng. **55** (1997) 841.
76. *Vickroy, T. B.,* Lactic acid. In: Comprehensive Biotechnology, *Moo-Young, M.* (Ed.), Vol. 3, Pergamon Press., New York, USA, 1985, pp 761–776.
77. *Akerberg, C., Zacchi, G.,* Bioresour. Technol. **75** (2000) 119.
78. *Venkatesh, K. V.,* Bioresour. Technol. **62** (1997) 91.
79. *Xiaodong, W., Xuan, G., Rakshit, S. K*., Biotechnol. Lett. **19** (1997) 841.
80. *Nguyen, T. T. T., Loiseau, G., Icard-Verniere, C., Rochette, I., Treche, S., Guyot, J. P.,* Food Chem. **100** (2007) 623.
81. *Yun, J. S., Wee, Y. J., Kim, J. N., Ryu, H. W.,* Biotechnol. Lett. **26** (2004) 1613.
82. *Linko, Y. Y., Javanainen, P.,* Enzyme Microb. Technol. **19** (1996) 118.
83. *Olmos-Dichara, A., Ampe, F., Uribelarrea, J. L., Pareilleux, A., Goma, G.,* Biotechnol. Lett. **19** *(*1997) 709.
84. *Kwon, S., Yoo, I. K., Lee, W. G., Chang, H. N., Chang, Y. K.,* Biotechnol. Bioeng. **73** (2001) 25.
85. *Venkatesh, K. V., Okos, M. R., Wankat, P. C.,* Process Biochem. **28** (1993) 231.
86. *Chen, R., Lee, Y. Y.,* Appl. Biochem. Biotechnol. **63** (1997) 435.
87. *Amrane, A., Prigent, Y.,* J. Biotechnol. **55** (1997) 1.
88. *Buyukkilci, A. O., Harsa, S.,* J. Chem. Technol. Biotechnol. **79** (2004) 1036.
89. *Fitzpatrick, J. J., Ahrens, M., Smith, S.,* Process Biochem. **36** (2001) 671.
90. *Ohkouchi, Y., Inoue, Y.,* Bioresour. Technol. **98** (2007) 546.
91. *Gao, M. T., Hirata, M., Koide, M., Takanashi, H., Hano, T.,* Process Biochem. **39** (2004) 1903.
92. *Wang, Q. H., Narita, J. Y., Xie, W. M., Ohsumi, Y., Kusano, K., Shirai, Y., Ogawa, H. I.,* Bioresour. Technol. **84** (2002) 13.
93. *Wang, Q. H., Narita, J. Y., Ren, N. Q., Ohsumi, Y., Kusano, K., Shirai, Y., Ogawa, H. I.,* Water Air Soil Pollut. **144** (2003) 405.
94. *Tong, W. Y., Fu, X. Y., Lee, S. M., Yu, J., Liu, J. Y., Wei, D. Z., Koo, Y. M.,* Biochem. Eng. J. **18** (2004) 89.
95. *Zakasaki, K., Akakura, N., Adachi, T., Akiyama, T.,* Environ. Sci. Technol. **33** (1999) 198.
96. *Oda, Y. J., Park, B. S., Moon, K. H.,* Bioresour. Technol. **60** (1997) 101.
97. *Pintado, J., Guyot, J. P., Raimbault, M.,* Enzyme Microb. Technol. **24** (1999) 590.
98. *Naveena, B. J., Altaf, M. D., Bhadriah, K., Reddy, G.,* Bioresour. Technol. **96** (2005) 485.
99. *Goksungur, Y., Guvenç, U.,* J. Chem. Technol. Biotechnol. **74** (1999) 131.
100. *Cotton, J. C., Pometto, A. L., Gvozdenovic-Jeremic, J*., Appl. Microbiol. Biotechnol. **57** (2001) 626.
101. *Ding, S., Tan, T.,* Process Biochem. **41** (2006) 1451.
102. *Ohashi, R., Yamamoto, T., Suzuki, T.,* J. Biosci. Bioeng. **87** (1999) 647.
103. *Kamoshita, Y., Ohashi, R., Suzuki, T.,* J. Ferment. Bioeng. **85** (1998) 422.
104. *Moueddeb, H., Sanchez, J., Bardot, C., Fick, M.,* J. Memb. Sci. **114** (1996) 59.
105. *Mostafa, N. A.,* Energ. Convers. Manage. **37** (1996) 253.
106. *Silva, E. M., Yang, S. T.,* J. Biotechnol. **41** (1995) 59.
107. *Hammes, W. P., Vogel, R. F.,* The genus Lactobacillus, In: Wood BJB, *Holzapfel, W. H,* (Ed.) The Genera of lactic acid Bacteria. Blackie Academic and Professional, Glasgow, UK, 1995, pp 19–54.
108. *Teuber, M.,* The genus *Lactococcus*. In: Wood BJB, *Holzapfel, W. H.,* (Ed.), The Genera of lactic acid Bacteria. Blackie Academic and Professional, Glasgow, UK 1995, pp. 173–234.
109. *Idris, A., Suzana, W*., Process Biochem. **41** (2006) 1117.
110. *Ohleyer, E., Wilke, C. R., Blanch, H. W.,* Appl. Biochem. Biotechnol. **11** (1985b) 457.
111. *Mercier, P., Yerushalmi, L., Rouleau, D., Dochain, D.,* J. Chem. Technol. Biotechnol. **55** (1992) 111.
112. *Veringa, H. A.,* US Patent **5** (1994) 781.
113. *El Sabaeny, A. H.,* Microbiologia **12** (1996) 411.
114. *Linko, P., Stenroos, S.-L., Linko, Y.-Y., Koistinen, T., Harju, M., Heikonen, M.,* Ann NY Acad Sci. **434** (1984) 406.
115. *Aksu, Z., Kutsal, T.,* Biotechnol. Lett. **8** (1986) 157.
116. *Shamala, T. R.,* J. Ind. Microbiol. **3** (1988) 175.
117. *McCaskey, T. A., Zhou, S. D., Britt, S. N., Strickland, R.,* Appl. Biochem. Biotechnol. **45** (1994) 555.
118. *Kadam, S. R., Patil, S. S., Bastawde, K. B., Khire, J. M., Gokhale, D. V.,* Process Biochem. **41** (2006) 120.
119. *Aeschlimann, A., von Stockar, U.,* Appl. Microbiol. Biotechnol. **32** (1990) 398.
120. *Nancib, N., Nancib, A., Boudjelal, A., Benslimane, C., Blanchard, F., Boudrant, J.,* Bioresour. Technol. **78** (2001) 149.
121. *Zhou, S. D., MaCaskey, T. A., Broder, J.,* Appl. Biochem. Biotechnol. **57** (1996) 517.
122. *Chauhan, K., Trivedi, U., Patel, K. C.,* Bioresour. Technol. **98** (2007) 98.
123. *Krishnan, S., Prapulla, S. G., Rajalakshmi, D., Misra, M. C., Karanth, N. G.,* Bioprocess Eng. **19** (1998) 61.

124. *Peters, V. J., Snell, E. E.*, J. Bacteriol. **67** (1954) 69.
125. *Concalves, L. M. D., Xavier, A. M. R. B., Almeida, J. S., Carrondo, M. J. T.,* Enzyme Microb. Technol. **13** (1991) 314.
126. *Sun, X., Wang, Q., Zhao, W., Ma, H., Sakata, K.,* Sep. Purif. Technol. **49** (2006) 43.
127. *Bouchoux, A., Balmann, H. R., Lutin, F.,* Sep. Purif. Technol. **52** (2006) 266.
128. *Tong, W. Y., Fu, X. Y., Lee, S. M., Jie Yu., Liu, J. W., Wei, D. Z., Koo, Y. M.,* Biochem. Eng. J. **18** (2004) 89.
129. *Madzingaidzo, L., Danner, H., Braun, R.,* J. Biotechnol. **96** (2002) 223.
130. *Liew, M. K. H., Tanaka, S., Morita, M.,* Desal. **101** (1995) 269.
131. *Naveena, B. M., Muthukumaran, M., Sen, A. R., Babiji, Y., Murthy, T. R. K.,* Meat sci. **74** (2006) 409.
132. *Gimenez, B., Turnay, J., Lizarbe, M. A., Montero, P. V., Gomez-Guillen, M. C.,* Food hydrocolloids **19** (2005) 941.
133. *Viljanmaa, M., Sodergard, A., Tormala, P.,* Int. J. Adhes. **23** (2003) 151.