Chapter 26

The Current Status and Future Expectations in Industrial Production of Lactic Acid by Lactic Acid Bacteria

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1. Introduction

Conversion of carbohydrates to lactic acid is one of the most employed fermentation processes in food industry. Applications of lactic acid fermentation are found in dairy industry, production of wine and cider, production of fermented vegetable products and meat industry.

The main markets for lactic acid have been in food, pharmaceutical and cosmetics industry, but presently the main growing application of lactic acid is in the production of biodegradable and renewable raw material based poly lactic acid (PLA) polymers. Production of lactate esters (*e.g.* butyl lactate) is another growing application as environmentally friendly solvents [1]. Lactic acid has two optical isomers, L-(+)-lactic acid and D-(−)-lactic acid. Lactic acid is classified as GRAS (generally recognized as safe) for use as a food additive, although D(-)-lactic acid can be harmful to human metabolism and result in *e.g.* acidosis [2]. The optical purity of lactic acid is required for the production of PLA. The properties of PLA may however be adjusted by the ratio of the L- and D-PLA in a copolymer D-form increasing the melting point of the copolymer [3]. Optically pure L- or D-lactic acid can be obtained by microbial fermentation and presently more than 95 % of industrial production of lactic acid is based on fermentation.

Production figure of 260,000 t as 100 % lactic acid for conventional (excluding PLA) markets in 2008 and forecast over 1 million ton annual production of lactic acid for conventional markets and PLA by 2020 has been presented in 2010 [4]. DuPont patented PLA already in 1954 but it took almost 50 years before first large-scale production was started. The US-based NatureWorks is the largest producer of PLA having lactic acid production capacity of 180,000 t/a. The sustainability of the PLA product Ingeo® from NatureWorks has been

evaluated [5]. Greenhouse gas emissions and nonrenewable energy consumption for Ingeo from cradle to factory-gate are 1.3 kg CO_2 eq./kg polymer and 42 MJ/kg polymer. These compare favorably with *e.g.* fossil-based PET (polyethylene terephtalate) with 3.2 kg CO_2 eq./kg polymer and 80 MJ/kg polymer, respectively. There is a huge potential for biodegradable and renewable raw materials based polymers if and when the economics for these become competitive. It is estimated that altogether 140 million tons of petroleum-based synthetic polymers are produced annually [6]. It should be emphasized that also many petroleum-based synthetic polymers (*e.g.* polyesters) are biodegradable. However at the moment there are only three commercial synthetic polymers replacing petroleum-based ones and produced on renewable raw materials: PLA, PTT (polytetramethylene terephtalate which is partly renewable) and PHA (polyhydroxyalkanoates). Natural polymers such as starches and celluloses are biodegradable and based on renewable raw materials, but their applications are limited by their properties. Reliance Life Sciences is producing copolymers of PLA and glycolic acid mainly for high-value medical applications. Lactic acid in this case is produced by bacterial fermentation.

The price of PLA is ca. 2.2 $/kg, the target being half of that [7]. This means that the price of lactic acid in captive use should be less than 0.8 $/kg. A major cost factor is the raw material used in fermentation medium. This is especially the case with fastidious lactic acid bacteria. Processes based on cheap polymeric waste and side stream materials are indeed widely studied. So far research on alternative fermentation modes and reactor systems has been mainly academic. PLA production requires both optically and chemically pure lactic acid. Optical purity can be guaranteed with several microbial strains under optimized fermentation conditions. Chemical purity is mainly dependent on the constituents in the fermentation medium especially when cheap materials are being used. Contrary to many other fermentation products lactic acid yield on monosaccharides is usually very high (> 90 %) the main impurity being the cell mass itself, which is easily separated from the product. The key economic drivers in the fermentative production of lactic acid are optimization of the production medium, high product yields, productivity, and the concentration of products formed, which influences the down-stream processing costs [8].

Lactic acid bacteria (LAB) are a group of Gram-positive bacteria belonging to genera Aerococcus, Alloiococcus, Atopobium, Bifidobacterium, Carnobacterium, Enterococcus, Lactobacillus (Lb.), Lactococcus (L.), Leuconostoc (Leuc.), Oenococcus, Pediococcus, Streptococcus (S.), Tetragenococcus, Vagococcus and Weissella (W.). LAB are non-sporulating rods or cocci which produce lactic acid as the main fermentation product under suitable substrates. LAB are oxidase and benzidine negative, lack cytochromes, and do not reduce nitrates to nitrite [9]. Most of the LAB are anaerobic, but some of them can shift to oxygen-dependent metabolism in aerobic conditions [10,11]. Lactic acid bacteria have complex nutrient requirements, including specific minerals, B vitamins, several amino acids, and purine and pyrimidine bases.

LAB ferment sugars via homo-, hetero-, or mixed acid fermentation. Homofermentative LAB produce lactic acid as main product from sugars, while hetero- or mixed acid fermentations produce also ethanol and/or acetic acid, formic acid and carbon dioxide.

Although it is a common practice to divide LAB into homo- and heterofermentative strains, the division is not that straightforward as the actual metabolism is dependent on both the nature of the C/energy substrate (*e.g.* hexose vs. pentose sugars) and fermentation conditions (*e.g.* growth rate and availability of the C/energy source). LAB used for lactic acid production are used to be classified as homofermentative (*Lactococcus*, *Enterococcus*, *Streptococcus* and some lactobacilli) as their hexose metabolism under non-limiting conditions is entirely via Embden-Meyerhof pathway to pyruvate which is then used to regenerate the reducing power (NADH) in the lactate dehydrogenase (LDH) catalyzed reaction to lactic acid. However at slow growth rate and low glycolytic flux mixed acid fermentation may take place and acetic acid, formic acid and ethanol are formed in addition to lactic acid [12]. The key enzyme in this metabolic shift *e.g.* in *L. lactis* is claimed to be pyruvate-formate lyase (PFL) [13]. There are two types of LDH for both enantiomers D-LDH and L-LDH. In addition some species have a racemase enzyme catalyzing the reaction between the two enantiomers. Thus enantiomerically pure lactic acid is produced by species with only one type of LDH and no racemase. A comprehensive list of different LAB strains used in lactic acid production is available elsewhere [1].

Biotechnical production of lactic acid may be based on several alternative micro-organisms. In addition to lactic acid bacteria filamentous fungi (*e.g. Rhizopus* spp.), other gram-positive bacteria (*e.g. Bacillus coagulans*) and metabolically engineered yeasts have been used also in industrial scale. The advantage of fungi is that they are active at and tolerate low medium pH. Low pH reduces significantly the consumption of neutralizing agent ($Ca(OH)_2$) in the fermentation stage and subsequent formation of gypsum ($CaSO_4$) in the product recovery stage. The advantage of filamentous fungi, *Bacillus* spp. and yeasts compared to lactic acid bacteria is their simple nutrient requirement in the fermentation medium. Filamentous fungi and *Bacillus* spp. are better suited to lignocellulosic fermentation raw materials as they are in general able to utilize pentose sugars in addition to hexoses. Anaerobic fermentation is generally speaking more feasible and this favors yeasts and lactic acid bacteria. When optimized the technical parameters such as product yield, R_P and final product concentration are quite similar for each of these production organisms.

In the wide literature on lactic acid production two examples based on other than lactic acid bacteria should be taken up. The first of them presents results with a thermotolerant *B. coagulans* strain [14]. High lactic acid $Y_{P/S}$ on both glucose and xylose (96 % and 88 %, respectively) were achieved at reasonable R_P (2.5 g/lh) and product concentration (100 g/l). Exceptionally high levels of lactic acid (200 g/l) were produced in fed-batch fermentation. Yeasts have been metabolically engineered aiming at lactic acid production since 1990's [15]. A recent article reported on metabolic engineering of *Candida utilis* having pyruvate decarboxylase deleted and a bovine L-lactate dehydrogenase expressed under the *pdc* promoter resulting in the production of lactic acid with high yield from glucose (95 %) and reasonable R_P (4.9 g/lh) ending up with lactic acid concentration of 103.3 g/l and more than 99.9 % enantiomeric purity [16].

Heterofermentative LAB *(Leuconostoc, Weissella* and some lactobacilli such as *Lb. brevis*) utilize both hexose and pentose sugars via phosphoketolase pathway (PKP). Several LAB

possess the genes for PPP as well. The different pathways are presented in Fig. 1. Heterofermentative LAB may be applied for the production of side products such as polyols (mannitol, erythritol) and ethanol or acetic acid. This is only feasible if the markets for the side products are comparable to those of lactic acid and the production more than covers the added down-stream processing costs.

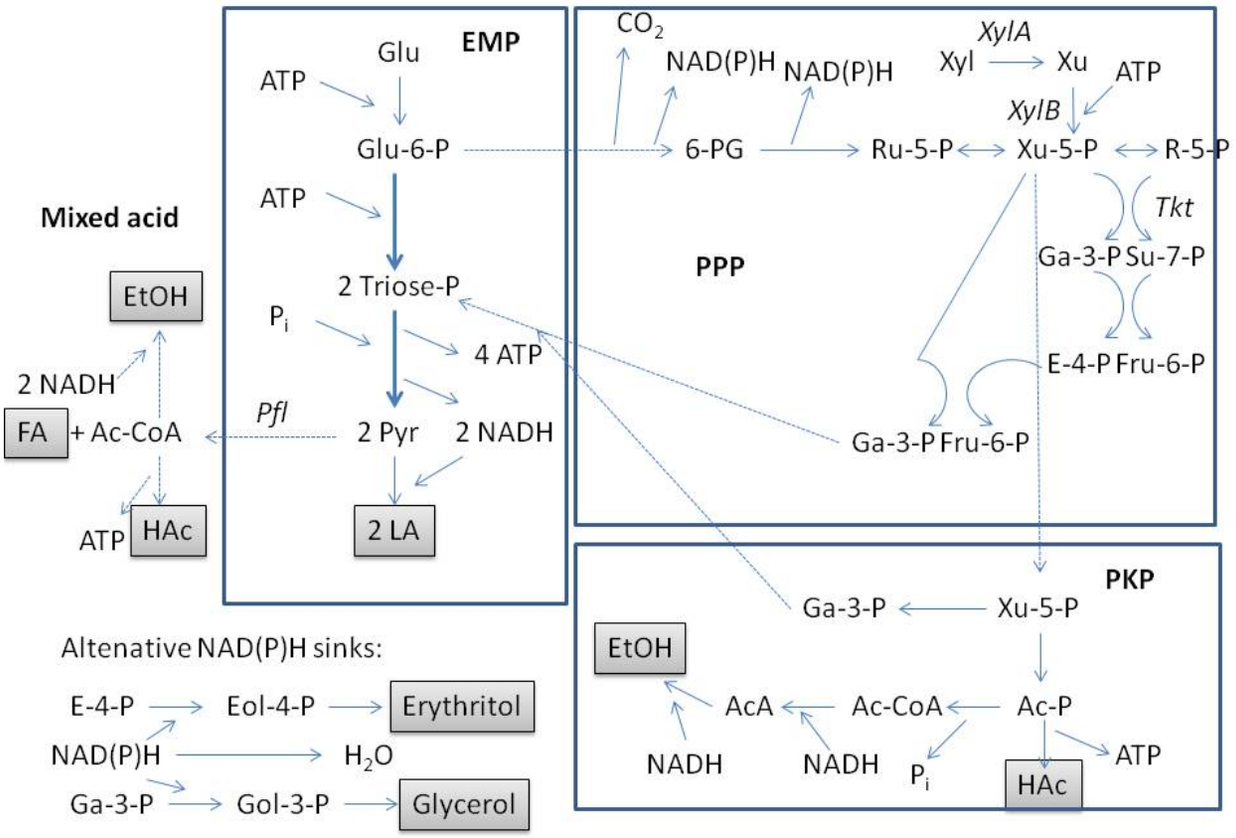

Figure 1. The main metabolic pathways in LAB. EMP: Embden-Meyerhof-Parnas pathway. PPP: pentose phosphate pathway. PKP: phosphoketolase pathway. Glu: glucose. LA: lactic acid. HAc: acetic acid. FA: formic acid. EtOH: ethanol. -P: energy-rich phosphate group. Pi: inorganic phosphate. Xyl: xylose. Xu: xylulose. 6-PG: 6-phosphogluconate. Ru: ribulose. R: ribose. Ga: glyceraldehyde. E: erythrose. Su: seduheptulose. Fru: fructose. Ac-CoA: acetyl-coenzymeA. Pyr: pyruvate. AcA: acetaldehyde. Ac-P: acetyl-phosphate. Pfl: pyruvate-formate lyase. XylA: xylose isomerase. XylB: xylulokinase. All carbohydrates are in D-form. Various metabolic end-products are presented with the dark background.

2. Future raw materials for production of lactic acid by LAB

The carboxylate platform is comprised of biological and chemical pathways that can be used in order to convert waste to bioproducts, such as lactic acid [17]. Lactic acid is a relatively cheap product, and one of the major challenges in its large-scale fermentative production is the cost of the raw material. This is the situation even in case of so called low-cost substrates [18]. Therefore, development of processes that utilize cheap raw materials at minimal costs have been under extensive studies. These substrates can be roughly classified as starch-

based non-processed biomasses, lignocellulosic non-processed biomasses, and waste or side stream feedstocks. The former are nowadays generally considered as non-ideal feedstocks due to ethical reasons, and therefore they are not discussed in this review. Extensive reviews including starch-based feedstocks are available elsewhere [19]. With respect to future applications, the most likely raw materials for the lactic acid production are industrial side-streams and lignocellulosic biomasses. Recent advances in case of both raw material groups are discussed in the following.

As in other bioconversion processes, also in lactic acid production the focus of research has turned towards the use of lignocellulosic feedstocks. The major driving forces are fossil fuel deprivation and general paradigm change to bioeconomy, and the abundancy of lignocellulose materials. Generally, the effective utilization of lignocellulosic biomass for biochemical processes is limited due to seasonal availability, scattered distributions and high logistics cost [20]. The fermentation of lignocellulosic biomasses can also be hampered by inefficient pretreatment, high enzyme costs and end-product inhibition, formation of unwanted by-products under metabolism of pentoses, and carbon catabolite repression caused by the heterogeneous substrates. These challenges are further discussed in a recent review [8].

Paper industry residues and recycled paper products include various possible feedstocks for lactic acid production, which are together with agroindustrial residues discussed in a recent review [21]. Due to economical and ecological reasons, an intensive research interest is currently devoted to complex industrial by-products. In this field the advances presented during the past five years include the utilization of cellulosic biosludges from a Kraft pulp mill [22,23], and recycled paper sludge [24]. In both cases a nutrient supplement has increased the lactic acid productivity. LAB could be used for the bioconversion of hemicellulose fractions, *e.g.* from alfafa processing [25] to lactic acid. Direct conversion of xylan to lactic acid by LAB is already possible by use of genetically modified strains [26].

Food industry residues comprise a large variety of different biomasses and sludges that can be roughly categorized to agricultural wastes and food production wastes. Since the use of agricultural residues for lactic acid production is summarized in a recent review [21], it is not futher discussed here. Food production residues have been tested for bioconversion applications for ages, and the variety of used materials is large. Whey and other dairy industry residues are the prominent raw materials with respect to lactic acid production, reviewed in *e.g.* [27,28]. Whey retains about 55% of total milk nutrients, from which approximately 70% consists of lactose [29]. Availability of the lactose carbohydrate reservoir and the presence of other essential nutrients, such as proteins and phosphates, for the growth of microorganisms make whey and other cheese-making residues potent raw materials for the production of biochemicals.

Other quite often referred raw materials include brewery residues, especially spent grain [30], and winery wastes [31-35]. Additionally, there are various other proposed food industry residues that could fit to the lactic acid fermentation. The recently proposed include *e.g.* apple pomace [36], canned pineapple syrup [37], cashew apple juice [38], Jerusalem artichoke tubers [39], macaroni milk and rice-green pea-salad refectory wastes

[40], rice residues [41], sap from palmyra and oil palms [42], and spent coffee grounds [43]. Despite of the large variety of the raw materials, the main conclusions of these studies are that the optimization and control of pH and temperature is critical for the process, and that the supplementation of low-cost substrate with *e.g.* inorganic salts and yeast extract is necessary or at least improves the productivity remarkably. In a recent study the use of mixed cultures of *Lb. casei*, *Lb. helveticus*, and *S. thermophilus* was observed to reduce the demand of supplements compared to single strain cultures [44].

The required supplements and their concentrations depend on the low-cost substrate. Drawbacks of complex supplements are their cost and extensive down-stream processing required for the purification of lactic acid from fermentation broth, especially in applications requiring high purity. Therefore, the optimization of supplement concentration is essential. Although yeast extract is often considered superior to other supplements in terms of efficiency, its major drawback is the relatively high cost, and therefore substitutive supplements have been suggested. Equal productivities may be achieved via use of cheaper alternatives, such as inorganic phosphates [45], and microbial lysates [46-47]. It is notable that the use of lysates in combination to *e.g.* whey proteins could cause unwanted proteolytic activity. Other options for the increased productivity include *e.g.* the addition of manganese, which is a constituent of lactate hydrogenase [48], whey protein hydrolyzate [49], malt combing nuts [49], corn steep liquor [50], fish hydrolyzates and other fishery by-products [51-53], hydrolyzed spent cells [54] or red lentil flour [55]. It is likely that this is one of the future trends in lactic acid production, *i.e.* fermentation media are optimized from mixtures of different low-cost raw materials in order to avoid the use of expensive complex supplements.

The modern biorefineries are looking into oceans in order to find new abundant and less land- or water-using biomasses for the production of commodities. Among the plenty marine biomasses, brown seaweed and especially species *Laminaria japonica*, a common food in Japan, has been recognized as a potential raw material for the production of platform chemicals. *L. japonica* is interesting due to its high carbohydrate content and fast growth. Production of lactic acid from *L. japonica* hydrolyzates was reported in a recent study [56]. Another potential raw material for bioconversion is shrimp shell waste, which is produced in vast amounts as a by-product of food industry. It has been reported that the production of lactic acid can be combined to the recovery of biopolymer chitin, a precursor for largely applied chitosan [57-58]. Since the recovery of chitin is traditionally done via chemical processing, the integrated process offers both economical and ecological advantage. Similar to the previous examples of other food industry residues, also the marine food processing industry generates various different side streams, such as fish waste and shells that could perhaps be combined in biochemical production.

3. Novel LAB strains

Metabolic engineering in general is applied when *e.g.* $Y_{P/S}$, R_P, substrate flux through a desired pathway in growth phases or resting cells are aimed at. Metabolic engineering studies aiming at increased flux in glycolysis to lactic acid in LAB are fairly scarce. That may

be explained by the fact that the metabolism of LAB is already tuned for efficient lactic acid production.

Some of these studies are listed in a review on metabolic engineering for lactic acid production [59]. The overexpression of L-LDH in *Lb. plantarum* can result 13-fold increase in LDH activity, and still show no effect on lactic acid production [60]. It has also been shown by overexpression that glyceraldehyde-3-P dehydrogenase (GAPDH) is not limiting the glycolytic flux either in growing or resting cells of a *L. lactis* strain [61]. Metabolic flux and control analysis (MFA and MCA) combined with the estimation of the kinetic parameters of the enzymes of a pathway are indeed needed in systematic and systemic approach to study and optimize also such seemingly simple - there is always growth and maintenance functions involved as well - metabolic pathway as that from glucose to lactic acid in LAB. An excellent view on topic is available in a review [62], which includes several references also for LAB (*e.g.* [63-66]).

More straightforward work on lactic acid production has been performed to achieve high enantiomeric purity by expressing and deleting respective genes for LDH. There are several examples of these as discussed in the recent review [59], such as construction of two different strains of *Lb. helveticus* for optically pure L-lactic acid production [67]. These strains differed from each other at the level of L-LDH activity (53 and 93 % higher than the wild type strain). Lactic acid production in a fermentation batch was equal to that of the wild type strain. However, at low pH when the growth and production are uncoupled, the strain with higher activity produced 20 % more lactic acid compared to construct with the lower activity.

Another straightforward target for the construction of genetically modified strains is widening of the raw materials for the production of lactic acid especially to lignocellulosic biomass-based materials. There are no reports on work to produce cellulolytic enzymes in LAB. Instead several groups have tried to produce xylanase in LAB [26]. This is however focused on heterofermentative LAB as they are naturally able to utilize pentoses and especially *Lb. brevis* as it has been shown to have endogenous beta-xylosidase activity [68]. Another approach is based on *L. lactis* IO-1 strain being able to metabolize xylose both via PKP and PPP [69]. PPP provides a homolactic fermentation route for pentoses. As the molecular biology tools or protocols for this strain were not available, another strain of *L. lactis* was used as the host. *XylRAB* genes from IO-1 strain were expressed in the host. *XylA* and *XylB* encode genes for xylose isomerase and xylulokinase, respectively. *XylR* is a putative transcriptional activator of the *XylAB* operon. In addition the gene for phosphoketolase was disrupted. Such a strain construct had homolactic fermentation for xylose. The rate of xylose fermentation was further improved by overexpressing the gene for transketolase, one of the enzymes in PPP. Almost theoretical $Y_{P/S}$ of lactic acid (1.58 vs. 1.67 mol/mol xylose) was achieved with lactic acid concentration of 50,1 g/l. Acetic acid concentration was as low as 0.3 g/l. Enantiomeric purity was very high (99.6 %). Similar approach has been applied for the production of D-lactic acid from xylose and L-arabinose [70,71].

Typical LAB fermentations are run at minimum pH of 5 – 5.5, which is much higher than the pKa-value of lactic acid (*i.e.* 3.8). Thus more than 90 % of the product exists as lactate. This is

a major cost factor in the product recovery stage as well as the cause of high salt burden and/or gypsum formation. The tolerance to acid and low pH is difficult to explain at genetic level and thus hardly be affected by metabolic engineering methods on specific genes. A successful approach to engineer LAB strains for lower fermentation pH has been genome shuffling. *E.g.* populations from nitrosoguanidine (NTG) mutations and low pH acclimatization in chemostat cultivation have been used for the shuffling [72]. The resultant population grew at pH 3.8 and lowered pH by lactic acid formation down to 3.5. This is a promising result even though the population was not used with realistic sugar concentrations. Similar approach has been reported aiming at improving acid tolerance as well as R_P and glucose tolerance, respectively, with *Lb. rhamnosus* [73,74]. NTG and UV irradiation were used for mutagenesis and lethal mutants were fused from protoplasts. The best strain of [73] lowered pH down to 3.25 and increased average R_P by 60 % compared to the wild type strain. However, average R_P was still moderately low (ca. 1 g/lh). Final lactic acid concentration and $Y_{P/S}$ from glucose were 84 g/l and 82 %, respectively. In [74] higher $Y_{P/S}$ (> 95 %) and R_P (ca. 3.6 g/lh) were reached with the best strains on industrially relevant fermentation medium with 150 g/l glucose. The $Y_{P/S}$ from 200 g/l glucose was still 90 %, but the average R_P decreased to 2 g/lh. In a recent study *Lb. casei* mutants induced by NTG were screened in high glucose concentration (360 g/l) [75]. A mutant strain with highest osmotic tolerance produced 198.2 g/l lactic acid from 210 g/l glucose with increased R_P (5.5 g/lh).

4. Novel process technologies

From fermentor design point of view lactic acid production by LAB is quite simple and conventional as the process requires no gassing, gas exchange or gas mass transfer. When the production strain and fermentation conditions are optimized for lactic acid production there is no or little formation of side products (metabolites, cellular mass, exopolysaccharides). Thus *e.g.* the rheology of the fermentation broth is Newtonian and very close to that of water. Power consumption is mainly for the sake of homogeneity and reduction of gradients of pH-controlling agents. The biggest challenges for process technology are to minimize osmotic effects by substrates and the product, to reach high R_P and to minimize the costs and waste formation in the product recovery stage.

Typical fermentation approaches other than simple batch include repeated batch and fed-batch fermentation and continuous fermentation with cell-recycle as solutions with free cells and the use of immobilized cells in different reactor types (fixed or fluidized bed). A novel fed-batch strategy was developed recently by combining pH-control and substrate feeding [76]. The rationale behind the strategy was the linear relationship between the consumption amounts of alkali and that of substrate. Thus these two components were mixed together in the feeding liquid. This resulted in higher efficiency compared to batch fermentation, but the efficiency parameters were not especially high if compared with data from several other reports. By far the most studied method to increase the R_P and/or separate cell growth from product formation is based on the immobilization of the cells. These have also been reviewed [27]. Several immobilization methods have been applied including entrapment within gels such as alginate [77,78], modified alginate [79,80], or pectate [81], adsorption on granulated DEAE-

cellulose [82] or porous glass [83], and biofilm formation on solid supports [84-85]. Solid incompressible supports and carrier materials such as granulated cellulose and porous glass may be applied in any scale and in various reactor designs while gels as compressible materials suit less well for larger scale especially in fixed-bed column reactors.

Immobilized cells may be utilized in various fermentation modes and reactor designs such as repeated batch or fed-batch, continuous fermentation with cell retention or recycle, in continuous stirred tank reactors (CSTR), fixed-bed or fluidized-bed reactors. High R_P (19-22 g/lh) have been achieved in a two-stage process with immobilized cells [86]. A special arrangement consisting of a CSTR for pH-control and substrate feeding and a fixed-bed reactor with immobilized cells was used in a concept with intermittent refreshing of the cells in a patent [87]. Short residence time within the column was possible because of the incompressible nature of the carrier material. Chemically pure product was achieved by using a production medium with few nutrients. Once the productivity decreased below a threshold value based on the consumption of alkali the cells were refreshed with nutrients. Incompressible carriers for cell adsorption have obvious advantages. However, new solutions to secure cell adherence on the carrier are required. This would facilitate efficient use of fluidized-bed reactors with minimal pressure losses in the reactor. Biological means for cell adherence may be one solution which could offer a further advantage to selectively keep the productive cells in the reactor.

Another approach to increase R_P is high cell-density fermentation with free cells recycled by membrane separation technique. This has been in use in industrial scale for lactic acid production already in 1980's in Finland. Several academic reports on this approach have been since published demonstrating very high R_P of 26 g/lh [88], 31.5 g/lh [89] and up to 57 g/lh [90].

It should be kept in mind that R_P is affected by the concentration of lactic acid. Thus not all published figures are comparable. Product inhibition may be diminished by in-situ recovery of the product. Electrodialysis [91-92], nanofiltration [93] and ion-exchange [94-95] have thus been coupled with the fermentation system.

Conventional lactic acid recovery from fermentation broth consists of cell and other solids separation, lactic acid precipitation as calcium lactate and precipitate recovery, acidification of the precipitate by sulfuric acid and the separation of the gypsum precipitate formed. The amount of gypsum is usually higher than the amount of lactic acid produced. Lately NatureWorks has reported to have reduced the formation of gypsum significantly. Probably this has been achieved by performing the fermentation at lower pH *e.g.* by using metabolically engineered yeast for the production of lactic acid. The amount of gypsum can be avoided by using electrodialysis for the acidification and separation of the acid and alkali formed with bipolar membranes [96]. The alkali formed may be recycled back to the fermentation. Electrodialysis has been considered too expensive technology for lactic acid recovery [97]. However, specific energy consumption of only 0.25 kWh/ kg lactic acid is presented [96]. Nanofiltration has been used as a pretreatment method to remove Mg- and Ca- and sulfate-ions and color before electrodialysis increasing significantly the capacity in electrodialysis [98]. Alternative techniques for lactic acid recovery are extraction [99] and use of ion-exchange [100-101], neither of which is a proper solution to the salt burden.

5. Conclusions

Lactic acid production in LAB has both cell mass and growth dependent portions. Typically LAB require several nutrient components for their growth increasing the fermentation and down-stream processing costs. Down-stream processing is especially important in the production of lactic acid for PLA. As R_P is the a major investment factor affecting costs, the minimization of medium and product purification costs should be accompanied by methods increasing cell mass concentration without excess growth. For this several different strategies have been applied so far mainly in academia (cell immobilization, cell-recycling and cell-retention). As history shows some of these could be applicable in industrial production as well, however pilot and demonstration plant studies and some risk-taking are required.

The main C/energy source spectrum available for LAB has been widened significantly. Reports of new possible substrates are frequently published, and the utilization of industrial side streams is a growing trend. Into this direction major successes have also been achieved with metabolic engineering providing strains for efficient production of lactic acid from pentoses as well, which is to promote sustainable use of renewables.

In an ideal fermentation process product inhibition should be minimized so that high R_P would be achieved even at high lactic acid concentrations resulting in feasible average productivities. For this purpose both acclimatization and mutagenesis has been applied successfully. However, it has to be considered how far can we go in respect to fermentation pH and lactic acid concentration. There are already remarkable alternatives to LAB with naturally better properties in this sense. Some success has been achieved with in-situ product recovery, but also these procedures lack experiences in any larger scale.

Conventional lactic acid production process with LAB is accompanied with the formation of large amounts of gypsum in the product recovery stage. Fermentation at lower pH diminishes this amount, but does not prevent its formation. Electrodialysis has been considered too expensive technique for the recovery of such cheap, bulk products as lactic acid. However, recent reports claim promising results with this technology. Forecasted figures for lactic acid market show up to one million tons per year. The growth would come mainly from the growth of PLA as a biodegradable polymer based on renewable raw materials. Economies of scale should decrease the production costs, but new technical approaches are also needed to reach these figures.

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Abbreviations

η % - Efficiency, i.e. the ratio of YP/S to the maximum theoretical value
D-LDH - D-lactate dehydrogenase
LAB – lactic acid bacteria
L-LDH - L-lactate dehydrogenase
NTG – Nitrosoguanidine
R_P - Volumetric productivity g/l*h
SSF – Simultaneous saccharification and fermentation
PLA – poly lactic acid
PPP - Pentose phosphate pathway
PKP - Phosphoketolase pathway
$Y_{P/S}$ – Yield of lactic acid per substrate consumed g/g
$Y_{P/X}$ – Yield of lactic acid per cell mass g/g

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