LACTIC ACID PRODUCTION FROM POLYSACCHARIDES-CONTAINING RAW MATERIALS

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Abstract

To date, more than 90% of global lactic acid production is achieved by microbial fermentation of sugars. Despite the significant advantages of microbial production process over the chemical means, the final production cost is relatively high and still limits product application. In commercial scale, it is considered that a significant part of production cost is determined by the substrate cost, so the use of sugars is not so economically feasible. A promising alternative to overcome this problem is the development of new strategies for utilizing cheaper and abundant in nature materials, such as biomass or polysaccharides containing industrial residues.

In this review are summarized the current problems and limitations of biological production process like the last achievements in lactic acid production from renewable raw resources, such as lignocelluloses, starchy materials and inulin-containing substrates. Although all these attempts are still on laboratory scale, they receive extensive attention because of their potential possibilities to replace sugars utilization in microbial lactic acid production and to provide an economically competitive and eco-friendly production process.

Keywords: *lactic acid production, renewable materials, lignocelluloses, starch, inulin*

1. INTRODUCTION

Lactic acid (LA, 2-hydroxypropanic acid, $CH_3CH(OH)COOH$) is firstly discovered and purified from yoghurt by the Swedish scientist Carl Willhelm Scheele in 1780 [1]. LA is non-volatile odorless acid, colourless as liquid or soluble in water white crystal in solid phase. LA can be found in two optically active stereoisomeric forms – L(+)-LA and D-(-)-LA. Lactic acid is classified by FDA in USA and other regulatory agencies as GRAS compound (Generally Recognized As Safe) and harmless as a food additive [2].

LA has a wide application in food industry as natural oxidative component, emulsifier, flavouring, conserving and antibacterial agent. It is used in pharmaceutical, cosmetic and other chemical industries [3]. A new important application LA finds as a building block for polylactate synthesis – biodegradable polymer, applicable in medicine or as a substitute of synthetic plastics, produced from processing petroleum products [4].

In nature, LA is found in its both isomeric forms, but the L-(+)- form is considered more valuable for food and pharmaceutical production. The reason is that in human organism only one lactate-dehydrogenase is presented [5]. In last years many scientists refute this fact, claiming that the human organism is able to assimilate both isomeric forms [6].

When co-polymerized, the two isomeric forms give amorphic material, while the homopolymers constitute a crystal structure. Because of this, for polylactate formation, an optically pure LA is needed (no matter if the isomeric form is L-(+)- or D-(-)-LA) [7].

2. BIOLOGICAL PRODUCTION

LA can be produced by chemical means or by microbial fermentation. Its chemical synthesis occurs by lactonitrile hydrolysis [8]. The main disadvantage of chemical production is that the product is obtained in a racemic mixture of the two isomers, which limits its application. For LA microbial production, depending on the choice of the microbial producer, either racemic mixture or optically pure isomer (L-(+)- or D-(-)-LA) can be obtained [9]. Another significant advantage of LA biological synthesis is the possibility of using cheap raw materials like molasses, whey, starch residues, beet, cane-sugar, inulin-containing substrates, etc. [10]. The price of the used raw material is a basic economic indicator for the process efficiency. Lower production temperature and the lower energy cost are other important priorities of microbial LA synthesis. For these reasons more than 90 % of the total LA production is conducted by biological means [11].

Although the LA synthesis for polylactate production grows constantly, in the last decade the annual polylactate production (450 000 tons) is still negligible, compared to plastics production (200 millions tons per year) [12]. High production price of LA is what limits the polylactate synthesis, as at least 34 % of its cost (no matter the type of the used substrate) represents the price of the raw substrate [13]. Because of this, experiments are focused mainly on discovering new cheaper substrates to be fermented - rice bran [14], paper production wastes [15], green algae [16].

To date, as the most perspective for LA production are considered renewable materials like lignocellulose, starch from agricultural residues, wood and other wide spread natural polysaccharides. A basal problem for the utilization of these materials is the necessity of high cost pretreatment, as raw materials in general are not in suitable for fermentation form. Another limitation is LA derivation and purification from the fermentation mixture, which is impeded by the presence of different admixtures in the raw materials [17].

The natural LA producers are the lactic acid bacteria (LAB) - facultative anaerobes or microaerofilic organisms containing the following characteristics: (i) they grow at temperatures between 5 and 45 ^{o}C; (ii) they grow at pH 4.0 ÷ 4.5, some of them even at 3.2 ÷ 9.6; (iii) for cultivation and LA production LAB need mainly organic nitrogen, vitamins and minerals [18]. 90 % of the published studies for microbial LA synthesis refer to LAB. They possess the ability to produce LA as a main metabolic product with high yield and productivity. They have been used for food fermentations for more than 4000 years, as their growth significantly diminishes the carbon content and pH of the product (because of LA accumulation). This way, the LAB inhibit the growth of most microorganisms in fermented foods, including most of the human pathogens (the so called “probiotic effect”).

The group of the LAB includes the representatives of 20 genera; the most important of them are *Lactobacillus, Lactococcus, Streptococcus, Leuconostoc, Pediococcus, Aerococcus, Carnobacterium, Enterococcus, Oenococcus, Tetragenococcus, Vagococcus and Weisella.* Among them, an optically pure L-(+)-LA produce several strains like *Enterococcus mundtii and Lactococcus lactis* [19] while an optically pure D-(-)-LA can be synthesized by *Lactobacillus delbrueckii* [20].

Although mainly LAB are used for LA production, it can be synthesized by another organisms, too, like the bacteria from *Bacillus* genus and some fungi strains. *Bacillus* sp. 17C5 and 36D1 are isolated from soils and produce L-(+)-LA from sugar cane with maximal productivity of 6,7 mM/Lh in a process of simultaneous saccharification and fermentation (SSF) [21]. Another study describes the thermophilic strain *Bacillus coagulans* P4-102B, which is capable of converting paper residues to LA [15].

The fungi *Rhizopus oryzae* are a well known LA producer. They don’t require specific media additives but LA synthesis is strongly affected by the micelium morphology and media oxygen content. The first report for LA production by *Rhizopus oryzae* dates back to 1936 [22].At present, as the most perspective fungi strain for LA production is considered the type strain *Rhizopus oryzae* NRRL 395 [23]. There was reported a case of simultaneous utilization of cellulose and hemicellulose from corncobs by *Rhizopus oryzae* [24].

3. LACTIC ACID PRODUCTION FROM PURIFIED SUGARS

As LAB are not capable of synthesizing various elements for their own growth, they need a media, containing organic nitrogen source, vitamins and minerals. It is considered that carbon/nitrogen ratio (C/N) is of great importance for LA secretion [25]. The most important for LA production are the organic nitrogen sources of animal origin: yeast extract (YE), peptone, tryptone and meat extract. According to the major part of reports, the nitrogen source with greatest impact is YE. But as it raises the process cost too much, the experiments are focused on finding cheaper alternatives. Numerous materials of plant and animal origin are studied - hydrolysed fish residues [26], corn hydrolysate, wheat bran extract [28], silkworm larvae [29], etc. The utilization of *Lactobacillus* sp. MKT-LC878 results in promoted LA production at the expense of decreased C/N ratio from 37:1 to 19:1. However, further decrease of the ratio to 12:1 leads to no positive effect [30].

The presence of some vitamins and minerals in LAB cultivation media markedly increases LA production. For example, the addition of pyridoxine (vitamin B6) stimulates the growth of some LAB, but when other vitamins are added (2,4,5-trimethyl-3-hydroxypyridine or 2,4-dimethyl-3-hydroxy-5-hydroxymethil pyridine), such effect is not observed [31]. A study of the mineral content of media for *Lactobacillus* sp. KCP01 shows that the addition of K_2HPO_4, $MnSO_4.4H_2O$, $FeSO_4.7H_2O$, sodium sulfate and sodium acetate in the media has a significant positive effect on LA production while the presence of $MgSO_4.7H_2O$, KH_2PO_4, NaCl, sodium citrate and sodium succinate has a negligible influence [32].

The carbon sources which can be assimilated by LAB are divided in two groups: purified sugars and renewable sugar containing materials.

Different purified sugars like glucose, xylose, lactose and cellobiose are used as a carbon source for LA synthesis [33, 34]. However, for the vast majority of microbial producers, glucose remains the most appropriate sugar when it is used as a sole carbon source [35]. On the other hand, in media consisting of various carbon sources, glucose limits the utilization of other sugars - a phenomenon called catabolite repression. This phenomenon strongly restricts the exploitation of natural substrates consisting of a mixture of sugars [36].

For overcoming the catabolite repression and effective LA synthesis from mixed sugar media, numerous attempts for natural producers modification are conducted. For example, in *Lactobacillus plantarum* NCIMB 8826 genome, genes for xylose isomerase (*xylA*), xylulokinase (*xylB*) are inserted and the genes for L-(+)-lactate dehydrogenase (*ldhL1*) and phosphoketolase (*xpk2*) are deleted. Modified that way, *Lactobacillus plantarum* strain is able to utilize xylose and arabinose in the presence of glucose. It produces optically pure D-(-)-LA. This is the first report for pure isomer obtaining from hexose and pentoses without catabolite repression [35]. Sometimes this is a natural ability of the wild isolates. *Enterococcus mundtii* QU 25 is able to produce only L-(+)-LA from glucose, cellobiose and xylose. It metabolizes glucose and cellobiose simultaneously with final LA yield of 0.9 g/g without any co-products accumulation [17].

Yun and Ryu (2001) study LA production with *Enterococcus faecalis* RKY 1 in co-fermentation of different sugar mixtures – glucose/fructose, glucose/maltose and maltose/fructose. When cultivated in media containing glucose/fructose, this strain consumes the sugars without catabolite repression, while in the other two mixtures, maltose utilization is repressed by the presence of both glucose and fructose [36].

Taniguchi et al. (2004) report for pure L-(+)-LA secretion from lignocellulosic hydrolysate which contains glucose and xylose with an yield of 0.63 g/g. For achieving this yield the authors apply co-cultivation of *Enterococcus casseliflavus* IFO 12256 and *Lactobacillus casei* 2218. In this case the catabolite repression is not overcame completely but still both of the carbon sources (50 g/L xylose and 100 g/L glucose) are fully consumed [33].

4. FERMENTATION CONDITIONS

Process parameters as pH of the media, cultivation temperature and the ratio between the volumes of the inoculum and working media are of key importance for cellular growth, productivity, yield and maximal product concentration.

Depending on the used LAB, the optimal pH varies between 5.0 and 7.0. In a process without controlling pH, it strongly decreases because of LA accumulation, which leads to cellular metabolism inhibition. The initial pH of the process is also an important parameter. Idris and Suzana (2006) study the influence of initial pH in the spectrum between 4.5 and 8.5 on LA production with *Lactobacillus delbrueckii* ATCC 9646 and conclude that at initial pH 6.5 fastest sugar assimilation (i.e. shortest lag-phase) and highest maximal LA concentration are observed [37]. It is estimated that maximal LA concentration is achieved at pH 6.0 – 12.6 g/L with *Lactobacillus manihotivorans* LMG 18010^T. In a similar process without controlling the pH, after twice longer fermentation process, just 7.6 g/L are achieved. The difference between the optical densities (OD^{600}) is also significant – 5.10 *vs* 1.65 [38].

As LAB are mesophilic bacteria, they can grow and synthesize LA at temperatures up to 50°C. Optimal growth temperature depends on genus; for most LAB, it is between 33 and 37 ^{o}C. But for *Lactobacillus delbrueckii* subsp. *bulgaricus*, the optimal temperature is higher: 42-44 ^{o}C. On the other hand, the temperature optimiums for cellular growth and LA secretion can be different for the same organism. In this case, the maximal LA concentration (g/L), LA yield (g/g) and productivity (g/Lh) also would be reached at different temperatures. For example, for *Lactobacillus amylophylus* ATCC 49845, it is estimated that the optimal temperature for maximal productivity is 25 ^{o}C and for a maximal yield – 35 ^{o}C [39].

For reaching the logarithmic phase of growth faster, the inoculation volume usually is 5-10 % (v/v) of the cultural media volume [40]. However, inoculation volume over 5 % raises the process cost too much. Aiming to examine the effect of inoculation volume on LA production, different ratios of inoculation (1 – 5 %) with *L. casei* NBIMCC 103 are varied. Maximal LA concentration (33.72 g/L) is reached, applying inoculation volume of 2 – 4 % (v/v) [41]. Similar experiment conduct Qi and Yao (2007), fermenting rice straw with *L. casei* GIM 1.159. As the authors vary the inoculation volume in the ranges 3 – 8 % (v/v), they observe the highest LA concentration at 6 % (v/v). The lowest inoculation volume results in insufficient cellular growth and the highest inoculation volume leads to too quick depletion of the growth compounds [42].

5. PROBLEMS AND LIMITATIONS FOR LA FERMENTATION

At present, more than 90 % of the world LA production is carried out by microbial fermentation and although the biotechnological production is more profitable than the chemical synthesis, for the improvement of this method still have to be got over number of limitations. One of the existing obstacles is the catabolite repression which limits the complete utilization of the cheaper natural substrates, containing various carbon sources. Up to now there are several isolates and some modified organisms capable of simultaneous assimilation of more than one sugar compound – glucose/cellobiose [43], xylose/arabinose/glucose [44] and cellobiose/glucose/xylose [45]. For solving this problem, co-cultivation of organisms with different substrate spectrum is applied [33].

Another significant limitation is producers tolerance to LA. To address this problem, different methods for selective LA recovery (*in situ*) from the fermentation broth during the process are developed. Another approach is the selection of high LA tolerant producers [46].

When heterofermentative producers are used, the presence of co-products like ethanol or acetate significantly decreases LA yield (theoretical yield 0.6 g/g) [47]. Decreasing the yield results in higher cost for product extraction and purification. With reference to this, in *Lactobacillus plantarum* are inserted genes for xylose assimilation. The modified strain *Lactobacillus plantarum ΔldhL1-xpk1::tkt* is able to assimilate xylose and to homofermentatively secrete LA [48].

Purified sugars exploitation is economically unprofitable and for the future development of fermentation technologies, it is necessary to be replaced by proper alternatives of the group of renewable sugar-containing materials. The use of purified sugars is preferred in industry. The reasons are the following: (i) necessity of high cost pretreatment of the polysaccharide-containing materials; (ii) unavailability of producers metabolizing the polysaccharides to LA in economically favorable yields and productivity; (iii) inability of the natural producers to simultaneously assimilate the sugars from the mixed substrates (catabolite repression); (iv) accessibility to renewable sugar-containing substrates.

6. LACTIC ACID PRODUCTION FROM RENEWABLE MATERIALS

As the most appropriate alternatives of the purified sugars for LA synthesis are considered the following materials: (i) lignocellulosic materials (wood, agricultural wastes); (ii) starchy materials (starch from potato, corn, wheat, sago palm); (iii) inulin-containing substrates (Jerusalem artichoke, dahlia, chicory roots, etc.). All of these materials contain polymers of the most utilized sugars for LA synthesis.

6.1. Lignocelluloses

Lignocellulose consists of cellulose, hemicelluloses and lignin [49]. Lignocellulosic materials cannot be fermented directly. They require pretreatment for separating the three fractions – cellulose, hemicelluloses and lignin. The methods, used for primary treatment can be physical (mechanical treatment, grinding), physico-chemical (hydrothermal treatment, vapour treatment, oxidation, etc.), chemical (treatment with concentrated or diluted acids, bases, ionic solutions) and biological (microbial or enzymatic treatment) [50]. The goal of pretreatment is separating the fractions, increasing the available surface of the material and partial depolymerization of cellulose. When treated with strong acids (H_2SO_4, H_3PO_4) at low pH, cellulose usually hydrolyses to monomers (xylose, arabinose) which decreases the necessity of additional treatment with hemicelluloses [51]. However, in most cases, the employment of a single pretreatment step is not sufficiently, therefore a combined pretreatment has to be conducted. Sasaki et al. (2012) process sugar cane production waste by steam explosion at 20 atm. After that, they apply enzymatic saccharification. After the second step, the authors observe liberation of 73-95 % of glucose in the raw material [52]. Kurakake and co-workers (2007) conduct combined treatment with microorganisms and enzymes for paper waste hydrolysis. They use two strains - *Sphingomonas paucimobilis* and *Bacillus circulans* and then they perform enzymatic hydrolysis with the waste. The authors reach up to 94 % liberation of sugars [53].

For complete enzymatic hydrolysis of cellulose macromolecules to glucose, the action of four groups of cellulases is required: (i) cellulases (endo-1,4-β-D-glucosidases), which diminish the stage of polymerization of the cellulose macromolecule by attacking the available surfaces with low crystallization; (ii) glucan-1,4- β-glucosidases – they attack the polymeric chains ends, liberating glucose; (iii) cellulose-1,4-β-cellobiosidases – they attack the ends of the cellulose chains, liberating cellobiose; (iv) β-glucosidases – they hydrolyse soluble cellodextrines and cellobiose to glucose [54].

By contrast with cellulose, hemicellulosic macromolecules are not homogenous. The highest content in the cellulose fraction are the xylanes – heteropolymers containing xylose, arabinose, glucuronic acid and some its esters, acetic, ferulic and coumaric acids. There are presented numerous enzymes, attacking the hemicellulosic macromolecules – glucuronidases, xylanases, β-xylosidases, galactomananases and glucomananases [8].

Apart from LAB (Table 1), for obtaining LA from cellulosic materials, *Rhizopus oryzae* (fungi) and *Bacillus coagulans* (G+ bacteria) which assimilate xylose and hemicellulosic hydrolysate are used [55]. For obtaining LA from hemicelluloses, Ilmen et. al. (2007) modify the yeast strain *Pichia stipitis*, which also possess the ability to metabolize xyloze [56].

In last years, for easier hydrolysis of lignocellulosic materials, a new approach is applied – genetic modification of lignocellulosic biomass. For example, in cultivating rice cultures, the overexpression of genes encoding exoglucanase [57] and arabinofuranosidase [58] leads to development of a culture

which straw is being saccharified much more easier. In spite of this, at present the use of lignocellulose for industrial LA production is still just a challenge, far from commercialization.

Table 1. The highest lactic acid titers obtained to date from renewable materials. CM – corncob molasses; WH – wood hydrolysate; WBH – wheat bran hydrolyzate; CW – cassava waste; IJT – inulin from Jerusalems artichoke tubers; SH – soybean meal hydrolyzate; CbCt – cellobiose and cellotriose; CR – corncob residue; CF – chicory flour; AF – alfalfa fibers; HAS – hydrolyzed acorn starch; WS – wheat starch; RWB – rice and wheat bran; FM – fermentation mode; FB – fed-batch; C – continuous process; SSF – simultaneous saccharification and fermentation; SHF – separate hydrolysis and fermentation.

Organism	Substrate	FM	Titer (g/L)	Yield (g/g)	Productivity (g/Lh)	Source
Bacillus sp. strain XZL9	CM	FB	74.7	0.50	0.38	[44]
Enterococcus faecium RKY1	WH	Batch	93.0	0.93	1.70	[88]
Lactobacillus bifermentans DSM 20003^T	WBH	Batch	62.8	0.83	1.20	[89]
Lactobacillus casei NCIMB 3254	CW	SSF	83.8	0.96	1.40	[90]
Lactobacillus casei G-02	IJT	FB	120.5	0.95	3.35	[85]
Lactobacillus casei G-02	IJT	FB	141.5	0.94	4.70	[86]
Lactobacillus casei LA-04-1	SH	FB	180.0	0.90	2.14	[91]
Lactobacillus delbrueckii mutant Uc-3	CbCt	Batch	90.0	0.90	2.3	[92]
Lactobacillus delbrueckii mutant Uc-3	Molasses	Batch	166.0	0.87	4.20	[93]
Lactobacillus delbrueckii mutant ZU-S2	CR	Batch	48.7	0.95	1.01	[94]
Lactobacillus paracasei B41	CF	SSF	123.7	0.91	1.08	[95]
Lactobacillus paracasei B41	CF	SSF	151.0	0.83	1.09	[87]
Lactobacillus plantarum 14431	LF	SSF	46.4	0.46	0.64	[96]
Lactobacillus rhamnosus HG 09	HAS	Batch	57.6	0.46	1.60	[25]
Lactobacillus sp. MKT 878	WS	SHF	118.0	0.94	1.71	[97]
Lactobacillus sp. RKY2	RWB	Batch	129.0	0.95	3.10	[98]

6.2. Starchy materials

Unlike lignocelluloses, starchy materials require lighter pretreatment. In order to be in adequate for fermentation form, starch-containing materials need preliminary gelatinization and liquification. This is a two-step process. At first the material is preheated to 90-130 ^oC for 15 minutes and then follows an enzymatic hydrolysis [59]. The essential enzymes, necessary for starch polymer decomposition are: α-amylases, β-amylases and glucoamylases [60].

Purified enzymes treatment however is a high cost process. On the other hand, the process including two consistent stages of enzymatic hydrolysis and fermentation is economically unfavorable. The process of bioconversion can be effective only when the steps of hydrolysis and fermentation occur in a single process of simultaneous saccharification and fermentation. This process is successfully applied for LA production with the amylolytic strains *Lactobacillus* and *Lactococcus* (Fig. 1) [61], [62], [63], [64], [65], [66].

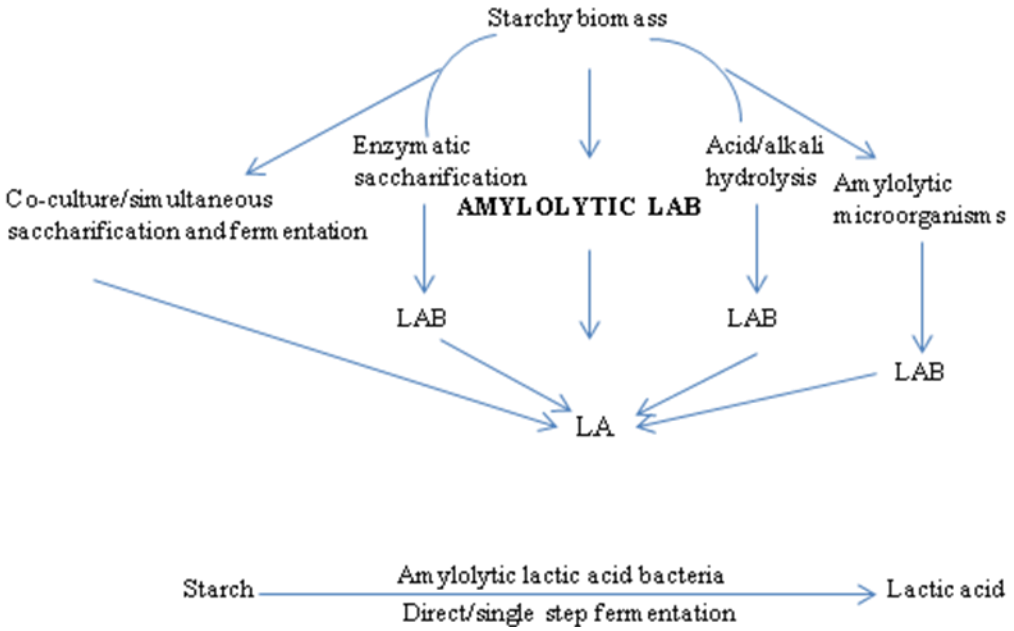

Figure 1. LA production from starch [67]

Till now, there are reports for several amylolytic strains capable of direct starch conversion to LA without preliminary hydrolysis. On laboratory scale, LA is directly obtained with *Lactobacillus manihotivorans* OND32^T [68]; *Lactobacillus manihotivorans* LMG18010^T [38]; *Lactobacillus amylophilus* GV6 [69]; *Lactobacillus amylophilus* B4437 [70]; *Lactobacillus amylovorus* [71]; *Lactobacillus paracasei* B41 [64]; *Lactobacillus plantarum* A6; *Lactobacillus plantarum* LMG18053 [72]; *Lactobacillus plantarum* BOM 816 [65]; *Lactobacillus pentosus* N3 [65]; *Streptococcus bovis* 148 [73]; *Lactococcus lactis* [62].

Streptococcus bovis 148 possess α-amylase (with enzymatic activity up to 1.41 U/ml) and produces 14.2 g/L LA from starch [73]. Quantities close to these (14.25 g/L LA) produces the strain *Lactobacillus plantarum* from 20 g/L mixed substrate [74]. In a process of simultaneous saccharification and fermentation, *Lactobacillus manihotivorans* LMG18011 secretes 19.5 g/L LA from soluble starch. *Lactobacillus plantarum* produces LA from starch with an yield of 0.81 g/g [75]. Assimilating 100 g/L soluble starch, the strain *Lactobacillus amylophilus* JCM 1125 produces 53.4 g/L LA [39]. *Lactobacillus amylophilus* B4437 converts 45 g/L corn starch to 29 g/L LA and *Lactobacillus amylovorus* converts 120 g/L soluble starch to 96.2 g/L LA [76]. Another strain *Lactobacillus amylovorus* assimilates crude corn, rice and wheat starch, producing 10.1 g/L, 7.9 g/L and 7.8 g/L LA, respectively. The reached concentrations are lower when cassava and potato starch are used as substrates – 4.8 and 4.2 g/L, respectively. The addition of 1 % peptone increases the conversion of cassava starch from 43 to 70 % and 7.7 g/L LA is produced [77].

Genetic manipulations for producers development are applied, as well. A gene encoding α-amylase from *Streptococcus bovis* 148 is cloned in *Lactobacillus casei*. The hybrid organism demonstrates increased amylolytic activity to soluble starch. 21.8 g/L LA are produced from 50 g/L soluble starch for 24 hours [73].

Lactobacillus cellobiosus produces LA directly from potato mess wastes. In media containing 5 % (w/v) potato mess and 3 % (w/v) calcium carbonate (pH neutralizer), 50 % of starch is converted to LA for 48 hours [79].

From the examples given above, it is visible that the starch solubility and the source of the starchy material are of great importance for LA yield even for the strains possessing the highest amylolytic activity.

6.3. Inulin-containing substrates

Inulin is of plant origin and is firstly isolated from *Inula helenium* roots [80]. It is a polyfructane with linear carbon chain where the fructose residues are connected by β-2, bonds. Usually, one of the chains ends with glucose residue, connected through α-1,2 bond. Inulin is the second most spread reserve carbon source in nature after starch. The highest inulin content is presented in chicory roots (*Chicorium intybus*), Jerusalem artichoke tubers (*Helianthus tuberosus*) and dahlia (*Dahlia pinnata*) – up to 20 % of the whole weight and above 50 % of the weight after dehydration [81]. Depending on the plant source, the polysaccharide has different molecule weight – for chicory the polymerization degree is from DP8 to DP15, for Jerusalem artichoke – from DP15 to DP20, for dahlia – up to DP36. The polymerization degree of inulin molecules depends on climate conditions, the duration of plant growth and the continuation of crop storage [82].

Inulin degrading enzymes are called inulinases; they are isolated for the first time from plant tissues. Despite that, their content in these tissues is not sufficient for an industrial application. The most appropriate source of inulinases for commercial applications is the wide spectrum of microorganisms producing them. Microbial inulinases are an important class of industrial enzymes as their producers are easy to cultivate, they accumulate protein quickly and in a high final yield. Multiple fungi, yeasts and bacteria possess inulinase activity. Among fungi, the best inulinases producers are from *Aspergillus* and *Penicillium* genera, among yeasts – from *Kluyveromyces* and among bacteria – *Bacillus, Pseudomonas* and *Streptomyces* [83]. Inulinases are produced by *Lactobacillus, Arthrobacter, Paenibacillus, Xanthomonas, Klebsiella, Yarrowia, Chaetomium, Chrysosporium, Rhizopus, Trichoderma*, too [84].

Inulinases can be endo- or exoenzymes, depending on the position of the β-2,1 bond which they attack. An exoinulinase activity possess the enzymes fructane-β-fructosidase and β-fructofuranosidase, which attack the last β-2,1 bond in inuline and consecutively separate the end fructose residues of the non-reducing end. In contrast by the invertase, fructane-β-fructosidase acts on β-2,6 bonds in polyfructanes (if available). The endoinulinase enzyme (inulinase) acts casually on the inner β-1,2 bonds in the molecule. As a result, di-, tri-, tetra- or oligosaccharides are breaking off, instead of fructose. Unlike exoinulinases, the endoinulinase does not possess invertase activity therefore isn't capable of degrading sucrose to fructose and glucose. Hence, as they convert inulin to monosaccharides (fructose, glucose) only, the exoinulinases producers are preferred for industrial application. Except for exo- and endo-, depending on the place of their secretion, the inulinases can be divided into extracellular (the enzyme is secreted from the cellular wall), connected to the cell and intracellular.

At present, the newest and the most perspective substrates for LA synthesis are considered the inulin-containing substrates. The chicory and Jerusalem artichoke are a very appropriate choice as they grow on rocky and non-cultivable lands and don't require special care.

Although the process of simultaneous saccharification and fermentation are reputed to be the most industrially profitable approach for LA production, the application of polysaccharides containing substrates is raising problems. For the inulin-containing substrates, the problems concern the lack of strains able to hydrolyze inulin and to simultaneously convert the sugars to LA [11]. Till now, there is only one report for LA over-producer with a strong inulinase activity, too. The strain *Lactobacillus paracasei* B41 possess en effective exoinulinase – Inu B41.In SSF process it converts completely 136 g/L chicory flour to 123.7 g/L LA. But such strains are rare. For that reason, for LA production is usually applied co-cultivating of a strain which possess a strong exoinulinase and invertase activity, and LA over-producer strain. For example, in a process of co-cultivation of *A. niger* SL-09 and *Lactobacillus* sp. G-02 in SSF process of inulin from Jerusalem artichoke, for 36 hours, 120.5 g/L LA are obtained [85]. In cultivation of *Lactobacillus* sp. G-02 in an optimized media, containing a solution of the inulinase of *A. niger* SL-09, 141.5 g/L LA are reached [86].

If there is no strain containing both of the enzymatic activities, another approach is applied – enzymatic hydrolysis of inulin and fermentation of the hydrolysate. That way, the strain *Bacillus*

coagulans XZL4 produces 134 g/L LA [11] from inuline hydrolysate of Jerusalem artichoke, while the strain *Pediococcus lactic* DQ2 – 111.5 g/L [82].

In all of the cases givven above, the media content is of key importance for reaching maximal inulinase activity. The experiments show that the inulinases secretion is strongly dependent on the type of the nitrogen source and the presence of different metal ions. Experiments with purified enzymes from *L. paracasei* B41 demonstrate that the addition of definite microelements like Mn^{2+}, Ca^{2+} or Co^{2+} can increase the inulinase activity. The strongest impact is that of Mn^{2+}. The addition of 2.5 mM Mn^{2+} increases the enzymatic activity of InuB41 by 54 % [87]. Even higher is the impact of Mn^{2+} on LA synthesis from inulin. In chicory flour fermentation with *L. paracasei* B41, the addition of 15 mM Mn^{2+} to the media results in augmented LA concentration from 107.9 to 151 g/L (40 % increase). The yield and productivity also grow by 40 % - from 0.59 to 0.83 g/g and from 0.78 to 1.09g/Lh, respectively [87].

7. CONCLUSIONS

LA is an important metabolite with application in food, pharmaceutical and cosmetic industries. A new application LA finds in polylactate synthesis.

LA can be obtained by chemical or by microbial means. The microbial fermentation is regarded as economically more feasible. Its advantages include the production of optically pure isomeric forms, depending on the organism used.

Very important is the possibility of utilizing cheap renewable raw materials like lignocellulosic, starch and inulin-containing biomass rather than purified sugars employment. But there are some problems for scientists to be solved. For example, these raw materials require specific pretreatment as they are not in appropriate for fermentation form. This results in more expensive process. A possible strategy is finding LA producers, possessing the necessary enzyme systems for conducting a process of simultaneous saccharification and fermentation. Another opportunity is applying genetic engineering methods, lending the microorganisms these qualities. The catabolite repression is another obstacle in biotechnological production, too.

On the other hand, heterofermentative organisms utilization leads to diminished LA yields due to by-products accumulation. These can also be overcome by molecular biology methods application – by inserting or deleting definite genes of microorganisms genome.

When all these challenging problems, standing in front of the scientists be solved, there can be obtained adequate final LA concentration and yields admitting its effective industrial application.

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REFERENCES

1. Benninga, H 1990, *A history of lactic acid making*, Kluwer Academic Publishers, Dordrecht, Netherlands.
2. Datta, R., Tsai, SP, Bonsignor, P, Moon, S, Frank, J. 1995, “Technological and economical potential of polylactic acid and lactic acid derivatives”, *FEMS Microbiol Rev*, vol. 16, vol 2-3, pp 221-231.
3. Abdel-Rahman, MA, Tashiro, Y, Sonomoto, K 2013, “Recent advances in lactic acid production by microbial fermentation processes” *Biotechnol Adv*, vol. 31, no 6, pp 877-902.

4. Tashiro, Y, Kaneko, W, Sun, YQ, Shibata, K, Inokuma, K, Zendo, T, Sonomoto, K 2011, "Continuous D-lactic acid production by a novel thermotolerant *Lactobacillus delbrueckii* subsp. *lactis* QU 41", *Appl Microbiol Biotechnol*, vol. 89, no 6, pp 1741-1750.
5. Akerberg, C, Hofvendahl, K, Zacchi, G, Hahn-Hägerdal, B 1998, "Modelling the influence of pH, temperature, glucose and lactic acid concentrations on the kinetics of lactic acid production by *Lactococcus lactis* sp. *lactis* ATCC 19435 in whole wheat flour", *Appl Microbiol Biotechnol*, vol. 49, no 6, pp 682-690.
6. Toyoda, Y, Erkut, C, Pan-Montojo, F, Boland, S, Stewart, MP, Müller, DJ, Wurst, W, Hyman, AA, Teymuras, V, Kurzchalia, TV 2014, "Products of the Parkinson's disease-related glyoxalase DJ-1, D-lactate, and glycolate, support mitochondrial membrane potential and neuronal survival", *Biology Open*, vol 3, pp 777-784.
7. Vijaiakumar, J, Aravindan, R, Vruthagiric, T 2008, "Recent trends in the production, purification and application of lactic acid", *Chem Biochem Eng Q*, vol. 22, no 2, pp 245-264.
8. Wang, Y, Tashiro, Y, Sonomoto, K 2015, "Fermentative production of lactic acid from renewable materials: Recent achievements, prospects, and limits", *J Biosci Bioeng*, vol. 119, no 1, pp 10-18.
9. Datta, RS, Sai, PT, Patric, B, Moon, S, Frank, J 1993, "Technological and economic potential of polylactic acid and lactic acid derivatives", International congress on chemicals from biotechnology. Hannover, Germany, pp 1-8.
10. Tsao, GT, Gao, NJ, Du, J, Gong, CS, 1999, "Production of multifunctional organic acids from renewable resources", *Adv Biochem Eng Biotechnol*, vol. 65, pp 245-277.
11. Wang, L, Xue, Z, Zhao, B, Yu, B, Xu, P, Ma, Y 2013 "Jerusalem artichoke powder: a useful material in producing high-optical-purity l-lactate using an efficient sugar-utilizing thermophilic *Bacillus coagulans* strain", *Bioresour Technol*,vol. 130, pp 174-180.
12. Christensen, CH, Rass-Hansen, J, Marsden, CC, Tarning, E, Egeblad, K 2008, "The renewable chemicals industry", *Chem Sus Chem*, vol. 1, no 4, pp 283-289.
13. Akerberg, C, Zacchi, G 2000, "An economic evaluation of the fermentative production of lactic acid from wheat flour", *Bioresour Technol*, vol. 75, no 2, pp 119-126.
14. Watanabe, M, Makino, M, Kaku, N, Koyama, M, Nakamura, K, Sasano, K 2013, "Fermentative L-(+)-lactic acid production from non-sterilized rice washing drainage containing rice bran by a newly isolated lactic acid bacteria without any addition of nutrients", *J Biosci Bioeng*, vol. 115, no 4, pp 449-452.
15. Budhavaram, NK, Fan, ZL 2009, "Production of lactic acid from paper sludge using acid-tolerant, thermophilic *Bacillus coagulans* strains", *Bioresour Technol*, vol. 100, no 23, pp 5966-5972.
16. Nguyen, CM, Kim, JS, Hwang, HJ, Park, MS, Choi, GJ, Choi, YH, Jang, KS, Kim, JC 2012, "Production of l-lactic acid from a green microalga, *Hydrodictyon reticulum*, by *Lactobacillus paracasei* LA104 isolated from the traditional Korean food, makgeolli", *Bioresour Technol*, vol. 110, pp 552-559.
17. Abdel-Rahman, MA, Tashiro, Y, Sonomoto, K 2011a, "Lactic acid production from lignocellulose-derived sugars using lactic acid bacteria: overview and limits", *J Biotechnol*, vol. 156, no 4, pp 286-301.
18. Caplice, E, Fitzgerald, GF 1999," Food fermentations: role of microorganisms in food production and preservation", Int J Food Microbiol, vol. 50, no 1-2, pp 131-149.
19. Shibata, K, Flores, DM, Kobayashi, G, Sonomoto, K 2007, "Direct L-lactic acid fermentation with sago starch by a novel amylolytic lactic acid bacterium, *Enterococcus faecium*", *Enzyme Microb Technol*, vol. 41, no 1-2, pp 149-155.

20. Hofvendahl, K, Hahn-Hägerdal, B 2000, "Factors affecting the fermentative lactic acid production from renewable resources", *Enzyme Microb Technol*, vol 26, no 1-4, pp 87-107.
21. Patel, MA, Ou, MS, Harbrucker, R, Aldrich, HC, Buszko, ML, Ingram, LO, Shanmugam, KT 2006, "Isolation and characterization of acid tolerant, thermophilic bacteria for effective fermentation of biomass-derived sugars to lactic acid", *Appl Environ Microbiol*, vol. 72, no 5, pp 3228-3235.
22. Lockwood, LB, Ward, GE, May, OE 1936, "The physiology of *Rhizopus oryzae*" *J Agric Res*, vol. 53, no 11, pp 849-857.
23. Ruengruglikit, C, Hang, YD 2003, "L(+)-Lactic acid production from corncobs by *Rhizopus oryzae* NRRL-395", *Lebensm Wiss Technol*, vol. 36, no 6, pp 573-575.
24. Guo, Y, Yan, Q, Jiang, Z, Teng, C, Wang, X 2010, "Efficient production of lactic acid from sucrose and corncob hydrolysate by a newly isolated *Rhizopus oryzae* GY 18*" J Ind Microbiol Biotechnol*, vol. 37, no 11, pp 1137-1143.
25. Lu, ZH, He, F, Shi, Y, Lu, MB, Yu, LJ 2010, "Fermentative production of L(+)-lactic acid using hydrolysed acorn starch, persimmon juice and wheat bran hydrolysate as nutrients", *Bioresour Technol*, vol. 101, no 10, pp 3642-3648.
26. Gao, MT, Hirata, M, Toorisaka, E, Hano, T 2006, "Acid-hydrolysis of fish wastes for lactic acid fermentation", *Bioresour Technol*, vol. 97, no 18, pp 2414-2420.
27. Dailey, ODJ, Dowd, MK, Mayorga, JC 2000, "Influence of lactic acid on the solubilization of protein during corn steeping" *J Agric Food Chem*, vol. 48, no 4, pp 1352-1357.
28. Kotzamanidis, C, Roukas, T, Skaracis, G 2002, "Optimization of lactic acid production from beet molasses by *Lactobacillus delbrueckii* NCIMB 8130", *World J Microbiol Biotechnol*, vol. 18, no 5, pp 441-448.
29. Timbuntam, W, Sriroth, K, Tokiwa, Y 2006, "Lactic acid production from sugar-cane juice by a newly isolated Lactobacillus sp.", *Biotechnol Lett*, vol. 28, no 11, pp 811-814.
30. Hetényi, K, Németh, Á, Sevella, B 2008, "Examination of medium supplementation for lactic acid fermentation", *Hungar J Ind Chem*, vol. 36, no 1-2, pp 49-53.
31. El-Sabaeny, AH 1996, "Influence of medium composition on lactic acid production from dried whey by *Lactobacillus delbrueckii*", *Microbiologia*, vol. 12, no 3, pp 12-20.
32. Chauhan, K, Trivedi, U, Patel, KC 2007, "Statistical screening of medium components by PlacketteBurman design for lactic acid production by *Lactobacillus* sp. KCP01 using date juice", *Bioresour Technol*, vol 98, no 1, pp 98-103.
33. Taniguchi, M, Tokunaga, T, Horiuchi, K, Hoshino, K, Sakai, K, Tanaka, T 2004, "Production of L-lactic acid from a mixture of xylose and glucose by co-cultivation of lactic acid bacteria" *Appl Microbiol Biotechnol*, vol. 66, no 2, pp 160-165.
34. Yoshida, S, Okano, K, Tanaka, T, Ogino, C, Kondo, A 2011, "Homo-D-lactic acid production from mixed sugars using xylose-assimilating operon-integrated *Lactobacillus plantarum*" *Appl Microbiol Biotechnol*, vol. 92, pp 67-76.
35. Okino, S, Suda, M, Fujikura, K, Inui, M, Yukawa, H 2008, "Production of D-lactic acid by *Corynebacterium glutamicum* under oxygen deprivation", *Appl Microbiol Biotechnol*, vol. 78, no 3, pp 449-454.
36. Yun, JS, Ryu HW 2001, "Lactic acid production and carbon catabolite repression from single and mixed sugars using *Enterococcus faecalis* RKY1", *Process Biochem*, vol. 37, no 3, pp 235-240.
37. Idris, A, Suzana, W 2006, "Effect of sodium alginate concentration, bead diameter, initial pH and temperature on lactic acid production from pineapple waste using immobilized *Lactobacillus delbrueckii", Process Biochem*, vol. 41, no 5,pp 1117-1123.

38. Guyot, JP, Calderon, M, Morlon-Guyot, J 2000, "Effect of pH control on lactic acid fermentation of starch by *Lactobacillus manihotivorans* LMG 18010", *J Appl Microbiol*, vol. 88, no 1, pp 176-182.

39. Yumoto, I, Ikeda, K 1995, "Direct fermentation of starch to L-(+)-lactic acid using *Lactobacillus amylophilus*", *Biotechnol Lett*, vol. 17, no 5, pp 543-546.

40. Salminen, S, Deighton, M, Gorbach, S 1993, "Lactic acid bacteria in health and disease" pp. 199-225, in: Salminen, S. and Wright, A. V. (Eds.), Lactic acid bacteria. Marcel Dekker, Inc., New York.

41. Panesar, PS, Kennedy, JF, Knill, CJ, Kosseva, M 2010, "Production of L(+) lactic acid using *Lactobacillus casei* from whey", *Braz Arch Biol Technol*, vol. 53, no 1, pp 219-226.

42. Qi, BQ, Yao, RS 2007, "L-Lactic acid production from *Lactobacillus casei* by solid state fermentation using rice straw", *BioResources*, vol. 2, no 3, pp 419-429.

43. Abdel-Rahman, MA, Tashiro, Y, Zendo, T, Shibata, K, Sonomoto, K 2011b, "Isolation and characterization of lactic acid bacterium for effective fermentation of cellobiose into optically pure homo L-(+)-lactic acid", *Appl Microbiol Biotechnol*, vol. 89, no 4, pp 1039-1049.

44. Wang, L, Zhao, B, Liu, B, Yu, B, Ma, C, Su, F, Hua, DL, Li, QG, Ma, YH, Xu, P 2010, "Efficient production of L-lactic acid from corncob molasses, a waste by-product in xylitol production, by a newly isolated xylose utilizing *Bacillus* sp. strain", *Bioresour Technol*, vol. 101, no 20, pp 7908-7915.

45. Sasaki, M, Jojima, T, Inui, M, Yukawa, H 2008, "Simultaneous utilization of D-cellobiose, D-glucose, and D-xylose by recombinant *Corynebacterium glutamicum* under oxygen-deprived conditions", *Appl Microbiol Biotechnol*, vol. 81, no 4, pp 691-699.

46. Wee, YJ, Kim, JN, Ryu, HW 2006, "Biotechnological production of lactic acid and its recent applications", *Food Technol Biotechnol*, vol. 44, no 2, pp 163-172.

47. Tanaka, K, Komiyama, A, Sonomoto, K, Ishizaki, A, Hall, SJ, Stanbury, PF 2002, "Two different pathways for D-xylose metabolism and the effect of xylose concentration on the yield coefficient of L-lactate in mixed-acid fermentation by the lactic acid bacterium *Lactococcus lactis* IO-1", *Appl Microbiol Biotechnol*,vol. 60, no 1-2, pp 160-167.

48. Okano, K, Yoshida, S, Tanaka, T, Fukuda, H, Kondo, A 2009, "Homo d-lactic acid fermentation from arabinose by redirection of phosphoketolase pathway to pentose phosphate pathway in L-lactate dehydrogenase gene-deficient *Lactobacillus plantarum*", *Appl Environ Microbiol*, vol. 75, no 15, pp 5175-5178.

49. Neureiter, M, Danner, H, Madzingaidzo, L, Miyafuji, H, Thomasser, C, Bvochora, J, Bamusi, S, Braun, R 2004, "Lignocellulose feedstocks for the production of lactic acid" *Chem Biochem Eng Q*, vol. 18, no 1, pp 55-63.

50. Taherzadeh, MJ, Karimi, K 2008, "Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review", *Int J Mol Sci*, vol. 9, no 9, pp 1621-1651.

51. Bungay, HR 1992, "Product opportunities for biomass refining", *Enzyme Microb Technol*, vol. 14, pp 501-507.

52. Sasaki, C, Okumura, R, Asakawa, A, Asaba, C, Nakamura, Y 2012, "Production of D-lactic acid from sugarcane bagasse using steam-explosion", *J Phys Conf Ser*, vol. 352, pp 2054-12063.

53. Kurakake, M, Ide, N, Komaki, T 2007, "Biological pretreatment with two bacterial strainsfor enzymatic hydrolysis of office paper", *Curr Microbiol*, vol. 54, pp 424-428.

54. Mousdale, DM 2008, "Biofuels: biotechnology, chemistry, and sustainable development", CRC Press, Taylor & Francis Group, Boca Raton FL, ISBN 978-1-4200-5124-7.

55. Maas, RH, Bakker, RR, Eggink, G, Weusthuis, RA 2006, "Lactic acid production from xylose by the fungus *Rhizopus oryzae*", *Appl Microbiol Biotechnol*, vol. 72, no 5, pp 861-868.

56. Ilmen, M, Koivuranta, K, Ruohonen, L, Suominen, P, Penttilä, P 2007, "Efficient production of L-lactic acid from xylose by *Pichia stipitis*", *Appl Environ Microbiol*, vol. 73, no 1, pp 117-123.

57. Nigorikawa, M, Watanabe, A, Furukawa, K, Sonoki, T, Ito, Y 2012, "Enhanced saccharification of rice straw by overexpression of rice exo-glucanase", *Rice*, vol. 5:14.

58. Sumiyoshi, M, Nakamura, A, Nakamura, H, Hakata, M, Ichikawa, H, Hirochika, H, Ishii, T, Satoh, S, Iwai, H 2013, "Increase in cellulose accumulation and improvement of saccharification by overexpression of arabinofuranosidase in rice", *PLoS One*, 8:e78269.

59. Anuradha R., Suresh A.K., Venkatesh K.V. (1999) Simultaneous saccharification and fermentation of starch to lactic acid. *Process Biochem*, 35:367-375.

60. Mehta, D, Satyanarayana, T 2013, "Biochemical and molecular characterization of recombinant acidic and thermostable raw-starch hydrolyzing α-amylase from an extreme thermophile *Geobacillus thermoleovorans", J Molec Catal, vol.* 85-86, pp 229-238.

61. Cheng, P, Muller, RE, Jaeger, S, Bajpai, R, Jannotti, EL 1991, "Lactic acid production from enzyme thinned cornstarch using *Lactobacillus amylovorus*", *J Ind Microbiol*, vol. 7, no 1, pp 27–34.

62. Petrov, K, Urshev, Z, Petrova, P 2008, "L-(+)-lactic acid production from starch by a novel amylolytic *Lactococcus lactis* subsp. *lactis* B84" ,*Food Microbiol*, vol. 25, no 4, pp 550–557.

63. Petrova, P, Petrov, K 2011, "Antimicrobial activity of starch-degrading *Lactobacillus* strains isolated from Boza", *Biotechnol. Biotech. Eq*, vol. 25 (Suppl. 1), pp 114–116.

64. Petrova, P, Petrov, K 2012, "Direct starch conversion into L (+) lactic acid by a novel amylolytic strain of *Lactobacillus paracasei* B41", *Starch – Stärke*, vol. 64, no 1, pp 10–17.

65. Petrova, P, Emanuilova, M, Petrov, K 2010, "Amylolytic *Lactobacillus* strains from Bulgarian fermented beverage Boza", *Zeitschrift für Naturforschung C*, vol. 65, no 3-4, pp 218–224.

66. Petrova, P, Petrov, K, Stoyancheva, G 2013, "Starch-modifying enzymes of lactic acid bacteria: structures, properties, and applications*", Starch- Stärke***,** vol**.** 65, no 1-2, pp 34–47.

67. Reddy, G, Altaf, M, Naveena, BJ, Venkateshwar, M, Kumar, EV 2008, "Amylolytic bacterial lactic acid fermentation – A review", *Biotechnol Adv*, vol. 26, no 1, pp 22-34.

68. Guyot, JP, Morlon-Guyot, J 2001, "Effect of different cultivation conditions on *Lactobacillus manihotivorans* OND32T, an amylolytic *Lactobacillus* isolated from sour starch cassava fermentation*", Int J Food Microbiol*, vol. 67, no 3, pp 217–225.

69. Vishnu, C, Seenayya, G, Reddy, G 2000, "Direct fermentation of starch to L(+) lactic acid by amylase producing *Lactobacillus amylophilus* GV6", *Bioprocess Eng*, vol. 23, no 2, pp 155-158.

70. Mercier, P, Yerushalami, L, Rouleau, D, Dochania, D 1992, "Kinetics of lactic acid fermentations on glucose and corn by *Lactobacillus amylophilus*", *J Chem Technol Biotechnol*, vol. 55, no 2, pp 111–121.

71. Zhang, DX, Cheryan, M 1994, "Starch to lactic acid in a continuous membrane reactor", *Process Biochem*, vol. 29, no 2, pp 145–150.

72. Giraud, E, Lelong, B, Raimbault, M 1991, "Influence of pH and initial lactate concentration on the growth of *Lactobacillus plantarum*", *Appl Microbiol Biotechnol*, vol. 36, no 1, pp 96–99.

73. Narita, J, Nakahara, S, Fukuda, H, Kondo, A 2004, "Efficient production of L-(+)-lactic acid from raw starch by *Streptococcus bovis* 148", *J Biosci Bioeng*, vol. 97, no 6, pp 423–425.

74. Thomsen, MH, Guyot, JP, Kiel, P 2007, "Batch fermentations on synthetic mixed sugar and starch medium with amylolytic lactic acid bacteria", *Appl Microbiol Biotechnol*, vol. 74, no 3, pp 540–546.

75. Giraud, E, Champailler, A, Raimbault, M 1994 "Degradation of raw starch by a wild amylolytic strain of *Lactobacillus plantarum", Appl Microbiol and Biotechnol*, vol. 60, no 12, pp 4319-4323.

76. Zhang, DX, Cheryan, M 1991, "Direct fermentation of starch to lactic acid by *Lactobacillus amylovorus*", *Biotechnol Lett*, vol. 13, no 10, pp 733–738.

77. Xiaodong, W, Xuan, G, Rakshit, SK, 1997, "Direct fermentation of lactic acid from cassava or other starch substrates", *Biotechnol Lett*, vol. 19, no 9, pp 841–843.

78. Narita, V, Okano, K, Kitao, T, Ishida, S, Sewaki, T, Moon-Hee, S, et al. 2006, "Display of α-amylase on the surface of *Lactobacillus casei* cells by use of the PgsA anchor protein, and production of lactic acid from starch", *Appl Environ Microbiol*, vol. 72, no 1, pp 269–275.

79. Chatterjee, M, Chakrabarty, SL, Chattopadhyay, BD, Mandal, RK 1997, "Production of lactic acid by direct fermentation of starchy wastes by an amylase-producing *Lactobacillus*", *Biotechnol Lett*, vol. 19, no 9, pp 73–874.

80. Rose, V 1804, "About a peculiar substance", *Neues Allgemeine Chemistry*, vol. 3, pp 217-219.

81. Franck, A 2006, "Inulin. In: Food Polysaccharides and Their Applications *CRC Press*, Boca Raton, FL, USA, pp. 335-352.

82. Dao, TH, Zhang, J, Bao, J 2013, "Characterization of inulin hydrolyzing enzyme(s) in commercial glucoamylases and its application in lactic acid production from Jerusalem artichoke tubers (Jat)". *Bioresour Technol*, vol. 148, pp 157– 162.

83. Ricca, E, Calabro, V, Curcio, S, Iorio, G 2007, "The state of the art in the production of fructose from inulin enzymatic hydrolysis" *Crit Rev Biotechnol*, vol. 27, no 3, pp 129-145.

84. Singh, RS, Singh, RP 2010, "Production of fructooligosaccharides from inulin by endoinulinases and their prebiotic potential", *Food Technol Biotechnol*, vol. 48, pp 435-450.

85. Ge, XY, Qian, H, Zhang, WG 2009, "Improvement of L-lactic acid production from Jerusalem artichoke tubers by mixed culture of Aspergillus niger and Lactobacillus sp.", *Bioresour Technol*, vol. 100, no 5, pp 1872-1874.

86. Ge, XY, Qian, H, Zhang, WG 2010, "Enhancement of L-lactic acid production in Lactobacillus casei from Jerusalem artichoke tubers by kinetic optimization and citrate metabolism", *J Microbiol Biotechnol*, vol. 20, no 1, pp 101-109.

87. Petrov, K, Popova, L, Petrova, P 2017, "High lactic acid and fructose production via Mn2+-mediated conversion of inulin by *Lactobacillus paracasei*" *Appl Microbiol Biotechnol*, vol. 101, no 11, pp 4433–4445.

88. Wee, YJ, Yun, JS, Park, DH, Ryu, HW 2004, "Biotechnological production of L(+)-lactic acid from wood hydrolyzate by batch fermentation of *Enterococcus faecalis*", *Biotechnol Lett*, vol. 26, no 1, pp 71-74.

89. Givry, S, Prevot, V, Duchiron, F 2008, "Lactic acid production from hemicellulosic hydrolyzate by cells of *Lactobacillus bifermentans* immobilized in Ca-alginate using response surface methodology". *World J Microbiol Biotechnol*, vol. 24, no 6, pp 745-752.

90. John, RP, Nampoothiri, KM, Pandey, A 2006, "Simultaneous saccharification and fermentation of cassava bagasse for L-(+)-lactic acid production using *Lactobacilli*.",*Appl Biochem Biotechnol*, vol. 134, no 3, pp 263-272.

91. Li, Z, Ding, SF, Li, ZP, Tan, TW 2006, "L-Lactic acid production by *Lactobacillus casei* fermentation with corn steep liquor-supplemented acid-hydrolysate of soybean meal", *Biotechnol J*, vol. 1, no 12, pp 1453-1458.

92. Adsul, M, Khire, J, Bastawde, K, Gokhale, D 2007, "Production of lactic acid from cellobiose and cellotriose by *Lactobacillus delbrueckii* mutant Uc-3", *Appl Environ Microbiol*, vol. 73, no 15, pp 5055-5057.

93. Dumbrepatil, A, Adsul, M, Chaudhari, S, Khire, J, Gokhale, D 2008, "Utilization of molasses sugar for lactic acid production by *Lactobacillus delbrueckii* subsp. *delbrueckii* mutant Uc-3 in batch fermentation", *Appl Environ Microbiol*, vol. 74, no 1, pp 333-335.

94. Shen, XL, Xia, LM 2006, "Lactic acid production from cellulosic material by synergetic hydrolysis and fermentation", *Appl Biochem Biotechnol*, vol. 133, no 3, pp 251-262.

95. Petrova, P, Velikova, P, Popova, L, Petrov, K 2015, "Direct conversion of chicory flour into l(+)-lactic acid by the highly effective inulinase producer *Lactobacillus paracasei* DSM 23505", *Bioresour Technol*, vol. 186, pp 329–333.

96. Sreenath, HK, Moldes, AB, Koegel, RG, Straub, RJ 2001, "Lactic acid production by simultaneous saccharification and fermentation of alfalfa fiber", *J Biosci Bioeng*, vol. 92, no 6, pp 518-523.

97. Hetényi, K, Németh, Á, Sevella, B 2011, "Investigation and modeling of lactic acid fermentation on wheat starch via SSF, CHF and SHF technology", *Per Pol Chem Eng*, vol. 55, no 1, pp 11-16.

98. Yun, JS, Wee, YJ, Kim, JN, Ryu, HW 2004, "Fermentative production of DL-lactic acid from amylase treated rice and wheat brans hydrolyzate by a novel lactic acid bacterium *Lactobacillus* sp.",*Biotechnol Lett*, vol. 26, no 20, pp 1613-1616.