Review
Advanced Fermentation Techniques for Lactic Acid Production from Agricultural Waste

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Abstract: Lactic acid plays an important role in industrial applications ranging from the food industry to life sciences. The growing demand for lactic acid creates an urgent need to find economical and sustainable substrates for lactic acid production. Agricultural waste is rich in nutrients needed for microbial growth. Fermentative production of lactic acid from non-food-competing agricultural waste could reduce the cost of lactic acid production while addressing environmental concerns. This work provided an overview of lactic acid fermentation from different agricultural wastes. Although conventional fermentation approaches have been widely applied for decades, there are ongoing efforts toward enhanced lactic acid fermentation to meet the requirements of industrial productions and applications. In addition, agricultural waste contains a large proportion of pentose sugars. Most lactic-acid-producing microorganisms cannot utilize such reducing sugars. Therefore, advanced fermentation techniques are also discussed specifically for using agricultural waste feedstocks. This review provides valuable references and technical supports for the industrialization of lactic acid production from renewable materials.

Keywords: lactic acid; agricultural waste; fermentation; industrial production

1. Introduction

Lactic acid (LA) is an organic acid with widespread applications in the food, textile, cosmetic, chemical, polymer and pharmaceutical industries. As one of the most important platform chemicals, LA can be converted to a variety of biopolymers, green solvents, and chemicals. Polylactic acid (PLA) polymers have presented great potential for replacing petroleum-derived polymers in industry and developing biomaterials (scaffolds, implants, sutures, etc.) in biomedical engineering, owing to their biodegradability and biocompatibility [1]. LA exists in two optically active isomers, \(\text{L-LA}\) and \(\text{D-LA}\). Some specific applications require high enantiomeric purity of LA, especially when LA and its derivatives are applied in the pharmaceutical and food industries.

The globe LA market is expected to expand to 1,960 kilotons in 2025, representing USD 9.8 billion [2]. The industrial production of LA is mainly carried out by chemical synthesis or fermentation. The chemical synthesis starts with acetaldehyde, a toxic petrochemical feedstock, and always results in a racemic mixture of \(\text{D/L-lactic acid}\). On the other hand, the fermentation method generally uses corn, rice, sweet potato, and other starchy substances as raw materials and has the biggest advantage of producing an optically pure LA by selecting the appropriate microorganism [3]. Due to the milder production conditions and higher purity, about 90% of LA in the market is produced by fermentation. However, the price of the substrates and food competition remain major challenges regarding the fermentation process. There is a great need to discover sustainable and non-food-competitive substrates.

LA-producing microorganisms involve a variety of bacteria and fungi, including lactic acid bacteria (LAB), \textit{Bacillus} strains, \textit{Corynebacterium glutamicum}, \textit{Escherichia coli}, filamentous fungi, yeast, microalgae, and cyanobacteria [4]. Different strains undergo different
metabolic pathways and growth patterns, providing a basis for the fermentative production of LA through diverse pathways. Fermentation techniques have created technical support to produce LA using renewable resources, including food waste, starchy residues, and lignocellulosic materials [5]. Furthermore, appropriate fermentation technologies can further increase LA yield and productivity.

Agricultural waste is rich in various nutrients, making it a promising alternative feedstock for microbial processes [6]. Due to the accelerated growth of the population, agricultural production has increased more than three times over the past 50 years, with a daily average of 23.7 million food tons worldwide [7]. A massive amount of agricultural waste is generated along with agricultural production. Unplanned burning and filling of agricultural waste create greater pressure on the environment, negatively impacting human health and ecosystems. Using cost-effective and abundantly available agricultural waste for fermentative LA production offers a great opportunity to lower LA production costs without using food-competitive substrates as well as settling environmental issues. Some LA manufacturers have made promising attempts in this regard. Cathay Biotech Inc. (Shanghai, China) and its collaborators have successfully developed a cyclic synthesis of L-lactide from cellulosic L-LA using wheat straw, thus breaking through the major barrier for industrial production of PLA from lignocellulosic waste [8]. TripleW (Netanya, Israel), a start-up company established in 2015, produces biobased LA and PLA from food waste [9]. Bio Base Europe Pilot Plant (Gent, Belgium) has been working on the supply chain hurdles towards LA production from agricultural waste [10].

This review focuses on LA production from agricultural waste. Conventional fermentation approaches are discussed. Advanced fermentation techniques are also introduced, including cell immobilization, co-culture, simultaneous saccharification and co-fermentation. Genetic and metabolic engineering plays an important role in adapting wild-type strains to specific agricultural waste to increase LA production. This review provides future references for the industrial scale-up of LA production from agricultural waste.

2. Lactic Acid Production from Agricultural Waste

2.1. Fermentable Sugar-Rich Waste

Non-solid agricultural processing waste contains various fermentable sugars, such as glucose, fructose, and sucrose. They are relatively easier to be accessed by LA-producing microorganisms and can be directly converted into LA. The lower requirement on raw material pretreatment is beneficial to simplify the production process and further reduce production costs. Waste products from sugar manufacturing plants, such as malt, molasses, and sugar beet juice, contain a large amount of sucrose and other essential nutrients. Non-treated beet molasses was reported as the substrate for 36.79 g/L LA production by Enterococcus hirae with a productivity of 1.02 g/(L·h) and yield of 0.91 g/g [11]. The direct cultivation of Lactobacillus paracasei (L. paracasei) on sugar beet molasses combined with distillery stillage achieved the LA productivity of 1.42 g/(L·h) and yield of 0.91 g/g [12]. Without sterilization and acidification, a racemic mixture of D-LA (4.94 g/L) and L-LA (107.40 g/L) was obtained from sugarcane molasses by using a microbial consortium containing Clostridium sensustricto, Escherichia, and Enterococcus [13]. However, some LAB cannot efficiently convert sucrose to LA. It may be related to the energy balance of NADH/NAD+ and ATP/ADP [14]. In addition, other fermentable sugar-rich fruit and vegetable wastes also demonstrated their high efficiency for sustainable lactic acid production. The syrup of carrot discards was utilized by Rhizopus arrhizus to produce 1-LA (22.18 g/L) [15]. The fermentation of exacted juice from date waste by Lactobacillus casei (L. casei) reached a maximum LA level of 89.2 g/L [16]. The sugar composition of these wastes may vary due to different growing conditions for specific crops. Therefore, the selection of a suitable LA-producing microorganism should be based on the sugar component.
2.2. Starchy Waste

Starch biomass can be hydrolyzed into glucose and then fermented to produce lactic acid, or it can be directly fermented by amylolytic lactic acid bacteria to produce lactic acid. Cornstarch and potato starch processing plants generate large amounts of wastewater and waste. These wastes are rich in starch and can serve as an inexpensive carbon source to produce lactic acid. A LA concentration of 31.6 g/L and productivity of 0.46 g/(L·h) was obtained by growing *Lactobacillus amylovorus* on enzyme-hydrolyzed cassava bagasse [17]. Enzyme-hydrolyzed cassava bagasse could also be utilized by *Lactobacillus rhamnosus* (*L. rhamnosus*) and *Bacillus coagulans* (*B. coagulans*) to produce 112.5 g/L LA at a productivity of 2.74 g/(L·h) and yield of 0.88 g/g [18]. Cassava peel enzymatic hydrolysate was used as the feedstock by *Lactobacillus delbrueckii* (*L. delbrueckii*) to produce D-LA with a yield of up to 95% of the total carbon source [19]. With the addition of amylase and glucoamylase, *B. coagulans* could utilize inedible cassava and sorghum flours to produce LA. The LA concentration, productivity, and yield reached 68.72 g/L, 1.72 g/(L·h), and 0.99 g/g [20]. White rice bran was another alternative for LA production by *B. coagulans*, with a concentration of 117 g/L, productivity of 2.79 g/(L·h), and yield of 98.75% [21]. An amylolytic *Lactobacillus plantarum* (*L. plantarum*) was able to generate 28.71 g/L LA via direct fermentation of cassava starch wastewater [22]. Cultivation of *L. rhamnosus* on inedible aging paddy rice with hull resulted in robust LA production (107.8 g/L) with a productivity of 3.4 g/(L·h) and a yield of 0.89 g/g theoretical glucose [23]. *Enterococcus faecalis* (*E. faecalis*) also exhibited relatively high LA production (73.75 g/L) using aging paddy rice with hull, with a similar yield of 87% but lower productivity of 2.19 g/(L·h) [24]. The direct conversion of potato residues to LA by *Geobacillus stearothermophilus* (*G. stearothermophilus*) at 60 °C was proved to be effective, contributing to 59 g/L LA after 48 h of fermentation [25]. *G. stearothermophilus* was also observed to produce 5.65 g/L LA from direct fermentation of rice starch waste [26].

2.3. Dairy Waste

Lactose is the most abundant component in dairy waste, followed by proteins and mineral salts. Thus, dairy waste can be used as a cheap alternative source to grow LA-producing microorganisms and produce LA with or without adding extra nutrients. Fermentation of *Pediococcus pentosaceus* from supplemented paneer whey medium yielded 42.12% lactose conversion and produced 14.5 g/L LA after 48 h [27]. Mixed culture LA fermentation of cheese whey was investigated, and the maximum LA concentration of 20.1 g/L and yield of 0.37 g/g were observed [28]. *L. casei* also showed LA production from cheese whey, giving 27.58 g/L LA with a productivity of 0.17 g/(L·h) [29]. Whey permeate was the carbon source for cultivating *L. plantarum* to produce 17.69 g/L LA [30]. Among the four acid-tolerant *Pediococcus* strains, *Pediococcus acidilactici* (*P. acidilactici*) had the highest LA production of 56.22 g/L from 48 h of fermentation of hydrolysed whey [31]. *Lactobacillus bulgaricus* (*L. bulgaricus*) was cultured with protease pretreated cheese whey powder to produce 70.70 g/L D-LA with an average productivity of 1.47 g/(L·h) [32]. Moradi et al. proved the efficacy of dairy wastewater as feedstock to produce LA. *L. delbrueckii* produced 14.2 g/L LA, with a yield of 0.78 g/g and a productivity of 0.34 g/(L·h) [33]. *Weissella soli* was first reported to produce 7.21 g/L LA in a 20-h fermentation of milk whey-supplemented medium [34].

2.4. Lignocellulosic Waste

Lignocellulosic waste makes up a large proportion of agricultural waste. Various cropland and orchard residues, such as straws, husks, branches, and leaves, are rich in lignocellulose. Lignocellulose is a composite of three components: cellulose, hemicelluloses, and lignin. Cellulose is a homopolymer of glucose, while hemicellullose is a heteropolymer of different six- and five-carbon sugars. Lignin is composed of multiple phenylpropane derivatives and is highly cross-linked. Cellulose and hemicellulose are covalently bound to lignin, making it challenging for microorganisms to uncover carbohydrates [35]. Therefore, a pretreatment process is required in most cases before lignocellulosic waste can be
used for fermentation. Conventional pretreatment techniques for lignocellulosic biomass include physical and chemical methods. Physical methods decrease the material sizes by milling, grinding, and extruding, to facilitate the efficiency of subsequent treatments. Acid pretreatment and alkaline pretreatment are commonly used chemical methods to reduce lignin content and improve cellulose accessibility [36]. Biological pretreatment has been considered a promising alternative to releasing sugars from lignocellulose under milder conditions, consuming less energy and chemicals. Ligninolytic microorganisms or enzymes break down the lignin fraction, while hydrolytic enzymes hydrolyze exposed cellulose and hemicellulose into fermentable sugars [37]. Raw material pretreatment can promote the utilization of lignocellulosic feedstocks, thereby enhancing the LA yield of fermentation. However, the addition of process builds up the production costs. Table 1 summarized some recent LA productions from lignocellulosic agriculture waste.

Table 1. Microbial LA productions from lignocellulosic agriculture waste.

<table>
<thead>
<tr>
<th>Lignocellulosic Waste</th>
<th>Pretreatment</th>
<th>Microorganism</th>
<th>LA, g/L</th>
<th>Yield, g/g</th>
<th>Productivity, g/(L·h)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn stover</td>
<td>Alkaline, hot water</td>
<td><em>Lactobacillus pentosus</em></td>
<td>92.3</td>
<td>0.66</td>
<td>1.92</td>
<td>[38]</td>
</tr>
<tr>
<td>Cornstall</td>
<td>Ionic liquid, cellulase</td>
<td><em>Bacillus sp.</em></td>
<td>-</td>
<td>0.963</td>
<td>-</td>
<td>[39]</td>
</tr>
<tr>
<td>Date palm waste</td>
<td>Grinding, alkaline</td>
<td><em>Lactobacillus delbrueckii</em></td>
<td>27.8</td>
<td>0.76</td>
<td>0.386</td>
<td>[40]</td>
</tr>
<tr>
<td>Oil palm</td>
<td>Extruding, alkaline</td>
<td><em>Lactobacillus coagulans</em></td>
<td>63.3</td>
<td>0.92</td>
<td>2.64</td>
<td>[41]</td>
</tr>
<tr>
<td>Oil palm empty fruit bunch</td>
<td>Acid</td>
<td><em>Bacillus coagulans</em></td>
<td>105.4</td>
<td>-</td>
<td>9.3</td>
<td>[42]</td>
</tr>
<tr>
<td>Orange peel</td>
<td>Milling, cellulase</td>
<td><em>Lactobacillus delbrueckii</em></td>
<td>-</td>
<td>0.94</td>
<td>6.72</td>
<td>[43]</td>
</tr>
<tr>
<td>Rice straw</td>
<td>Ethylenediamine</td>
<td><em>Bacillus coagulans</em></td>
<td>92.5</td>
<td>-</td>
<td>2.01</td>
<td>[44]</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>Milling, cellulase</td>
<td><em>Lactobacillus lactis</em></td>
<td>82.2</td>
<td>0.872</td>
<td>0.61</td>
<td>[45]</td>
</tr>
<tr>
<td></td>
<td>Gridding, acid</td>
<td><em>Lactobacillus plantarum</em></td>
<td>36.75</td>
<td>-</td>
<td>0.51</td>
<td>[46]</td>
</tr>
<tr>
<td></td>
<td>Milling, steam explosion</td>
<td><em>Bacillus coagulans</em></td>
<td>26.3</td>
<td>0.709</td>
<td>0.253</td>
<td>[47]</td>
</tr>
<tr>
<td></td>
<td>Gridding, acid</td>
<td><em>Bacillus sonorensis</em></td>
<td>55.9</td>
<td>0.97</td>
<td>0.77</td>
<td>[48]</td>
</tr>
<tr>
<td></td>
<td>Milling, steam explosion</td>
<td><em>Lactobacillus pentosus</em></td>
<td>17.7</td>
<td>0.82</td>
<td>0.37</td>
<td>[49]</td>
</tr>
</tbody>
</table>

3. Conventional Fermentation Approaches

3.1. Batch Fermentation

In the batch process, all the substrates and microorganisms are added to the system from the beginning and there is no addition or removal of major components until the fermentation is complete. The closed-loop production system reduces the risk of contamination and strain mutation. It also maximizes the conversion rate of the substrate and produces a high concentration of LA. On the other hand, the accumulation of LA results in acidification of the medium, inhibiting microbial growth and LA production. Batch fermentation represents the most used and simplest model, especially for developing unknown processes. Wang et al. applied batch mode to investigate and optimize the LA production by *Lactobacillus pentosus* (*L. pentosus*) [53,54]. The sugar metabolism and LA production of *E. faecalis* was studied in batch modes using sucrose and mixed sugars [55]. Batch fermentation could also be used to evaluate the efficacy of producing LA from renewable substrates and develop the process [56]. Mathematical LA batch fermentation models were analyzed to estimate the process indicators [57]. Based on real-life growth patterns of eight LAB strains in batch fermentation, a predictive model was proposed to describe the behavior of microorganisms [58].
3.2. Continuous Fermentation

As opposed to batch fermentation, continuous fermentation is a process where nutrients are added while culture broth is removed continuously. High productivity is achieved via constant production and avoiding repeatedly setting up a batch. Production inhibitions can be minimized by continuously removing LA and other metabolic byproducts from the system. However, it is hard to maintain a steady state and avoid contamination in the long-term process. As the microorganism grows and reproduces through multiple generations, the likelihood of mutation increases. The bioreactor design for continuous fermentation tends to be more complex. Veeravalli et al. reported that increasing the dilution rate in continuous fermentation decreased the concentrations of LA and acetic acid but had minor effects on the ratio between the two acids. A cell washout was observed when the dilution rate was close to the maximum specific cell growth rate [59]. Starting with a low pH was found to have a positive effect on LA yield in the continuous LA fermentation of dairy effluent [60]. Compared with batch fermentation of food waste, continuous mode produced 38% higher LA concentration and 36% enhanced LA yield but 57% lowered LA productivity [61].

3.3. Fed-Batch Fermentation

Fed-batch fermentation is a variation in batch fermentation, to mimic the productivity of continuous fermentation. It starts with a batch process, but a media containing concentrated nutrients is fed to the vessel after a certain point. This provides better control over cell growth by controlling feed addition. The exponential and stationary phase of cell growth can be prolonged, resulting in sustained high production of metabolites. Pejin et al. obtained significantly higher LA concentration, yield, and volumetric productivity in fed-batch fermentation by 194.8%, 2.2%, and 20.7%, respectively [62]. Fed-batch fermentation of L. casei led to a 69.24% increase in LA production and 11% increase in yield [63]. In LA production from food waste by L. pentosus, fed-batch mode further increased LA concentration from 106.7 g/L to 157.0 g/L, although the overall productivity decreased from 3.09 g/(L·h) to 2.0 g/(L·h) [64]. Feeding strategies might have positive effects on the metabolism of the microorganism. A low feed rate of model medium with inhibitors and softwood hydrolysate was proved to help P. acidilactici adapt to the inhibitors presented. The strain was able to convert softwood hydrolysate and furfural into LA and partly detoxify the media [65].

4. Cell Immobilization

Cell immobilization refers to immobilizing cells on water-insoluble carriers so that they can carry out life activities (growth, reproduction, metabolism, etc.) in a certain space and can be used repeatedly. With appropriate immobilization materials and methods, immobilized cells can robust fermentation via high cell densities. Immobilized cell fermentation allows easy cell separation from the broth and efficient cell reuse in consecutive batch cycles. Cell immobilization can be categorized as entrapment, adsorption, encapsulation, and containment within synthetic polymers (Figure 1) [66]. Wang et al. encapsulated L. pentosus in sodium alginate (SA)-polyvinyl alcohol (PVA) carrier and observed stable and efficient LA production during 15 batches of repeated fermentation [67]. Under the optimum immobilization and fermentation conditions, the LA yield and productivity of immobilized L. pentosus were 13.6% and 67.3% higher than those of free cells, respectively [68]. The LA production of SV-PVA immobilized L. pentosus was further improved by film-coated with chitosan, giving a LA yield of 0.966 g/g and productivity of 2.426 g/(L·h) [69]. Immobilized L. rhamnosus cells in PVA cryogel showed stable cell activity during 12 batch fermentations, achieving a high LA yield of 97% and productivity of 2.1 g/(L·h) [70]. In addition to chemical carriers, natural materials can also serve as economical and sustainable immobilization supports. Shahri et al. proposed a lignocellulosic plant material, loofah sponge, as the support matrix for R. oryzae immobilization [71]. Sunflower seed hull, brewers’ spent grain, and sugar beet pulp were tested as surface supports for L. paracasei attachment. Sugar beet
pulp was proved to be the most effective support of the three, with an LA productivity of 1.48 g/(L·h) and a LA concentration of 80.10 g/L [72].

Figure 1. Mechanism of various immobilization technologies: (a) adsorption on a surface, (b) encapsulation, (c) entrapment within a matrix, and (d) containment within a polymer. Reprinted with permission from Lu et al. [66]. Copyright 2020 American Chemical Society.

5. Membrane-Based Cell Retention

Conventional continuous LA fermentation faces the challenge of washout of the cell culture due to continuous flow. Membrane-based cell recovery systems have shown very good potential for continuous fermentation to prevent cell loss via medium exchange and maintain maximum cell growth, thereby significantly enhancing LA production [73]. Figure 2 shows a continuous system coupled with cell retention membranes [74]. Alexandri et al. applied a microfiltration device with hollow-fiber filters for continuous fermentation and obtained a high LA productivity of 11.28 g/(L·h) from bakery waste hydrolysates [75]. Ma et al. also reported high productivity of 13.8 g/(L·h) from corn stover hydrolysate by using a similar hollow-fiber module to recycle cells back to the fermenter [76]. With the combined effect of membrane-based cell recycling and B vitamin supplement, the D-LA productivity from rice straw hydrolysate reached 18.56 g/(L·h) with an optical purity of 99.5%. The addition of yeast extract was reduced to 0.5 g/L, contributing to an 86% cost reduction in the nutrient source [74]. Continuous fermentations of various agriculture wastes demonstrated high LA productivities over 6.62 g/(L·h), and the highest productivity was 10.34 g/(L·h) when molasses was employed [77]. By adjusting the dilution rate in the continuous fermentation with membrane microfiltration cell recovery, the LA production from sugarcane molasses reached 27.6 g/(L·h), with a LA yield of more than 0.95 g/g [78]. Although the LA productivity has been greatly improved, the cost of construction and maintenance of these complex fermentation systems cannot be ignored in industrial scale-up.
Figure 2. Schematic diagram of the membrane integrated continuous fermentation system. (1) N2 storage tank; (2) feed medium storage tank; (3) neutralizer reservoir; (4) fermenter; (5) hollow-fiber microfiltration module; (6) product storage tank; (7) cleaning solution tank; (8) flow meter; (9) medium feed control pump; (10) alkali feed control pump; (11) recirculation pump; (12) feed pressure indicator; (13) retentate pressure indicator; (14) retentate flow rate indicator; (15) permeate control pump; (16) backwash pump; (17) level sensor; (18) pH sensor; (19) control host; (20) sampling (analysis of residual sugars and D-lactic acid); (21) sampling (analysis of cell growth). Reprinted with permission from Ma et al. [74]. http://creativecommons.org/licenses/by/4.0/.

6. Simultaneous Saccharification and Co-Fermentation

Simultaneous saccharification and co-fermentation (SSCF) refers to the process in which both cellulose and hemicellulose are depolymerized into fermentable sugars and simultaneously converted into products in one vessel. Since sugars are continuously consumed during release from agricultural wastes, SSCF avoids substrate inhibition in separate hydrolysis and fermentation (SHF), leading to enhanced LA yield and productivity. Besides, a one-pot operation reduces the costs of large-scale industrial processing. Zhou et al. compared the LA production in SHF and SSCF at 60 g/L cellulose loading of bagasse sulfite pulp. SSCF showed a 43.73% increased LA concentration with 25.00% less processing time and 33.3% lower fungal cellulase dosage [79]. In the SSCF of cassava bagasse, both starch and cellulosic fractions were saccharified and converted to LA with a productivity of 2.74 g/(L·h). LA yield was calculated as 0.8 g/g (of starch + cellulose + hemicellulose) [18]. L. paracasei was cultured directly on bakery waste with the presence of amylase and amyloglucosidase, producing more than 26.4 g/L LA [80]. However, enzyme costs account for one of the major costs in SSCF. Li et al. proposed an on-site enzyme production from paper mill sludge. The produced enzyme was then applied with B. coagulans in SSCF of the same inexpensive feedstock to produce optically pure L-LA [81]. Another major challenge of SSCF is the different optimal temperatures between decomposing enzymes (40–60 °C) and LA-producing microorganisms (30–37 °C). A proper combination can maintain a balanced rate between raw material saccharification and LA fermentation to achieve efficient bioconversion, rather than sacrificing one as the rate-limiting step. Zhang et al. developed a continuous SSCF to produce LA from dry acid pretreated and biodegraded wheat straw substrate. Cellulase was selected for enzymatic hydrolysis, and P. acidilactici was engineered to utilize both glucose and non-glucose sugars derived from lignocellulose. The perfect agreement of the two in temperature (~50 °C) and pH (~4.8) contributed to the high LA titer and productivity of 107.5 g/L and 2.69 g/(L·h), respectively [82].
7. Co-Culture

Microbial co-culture involves the application of two or more different organisms in the same fermentation process. Traditional microbial fermentation usually relies on a single strain or single culture, and it is difficult to further improve the production capacity of natural strains after process optimization. Co-cultivation can use the advantages and abilities of different strains to simulate synergy in nature, to complete more complex synthetic pathways and achieve higher production efficiency. Unlike pure sugar medium, agricultural waste contains many impurities, inhibitors, and even harmful substances. In addition, the amount of hexose and pentose sugars derived from cellulose and hemicellulose is abundant. Failure to convert both sugars to LA results in a decreased fermentation efficiency and low LA yield. Klongklaew et al. performed a co-culture fermentation of furfural tolerant Enterococcus mundtii (xylose-utilizing LAB) and L. rhamnosus (glucose-utilizing LAB) for LA production. The co-culture was proved to be efficient without detoxification of corn stover hydrolysate, producing 31.4 g/L of 99.9% optically pure L-LA with a yield of 0.90 g/g and productivity of 1.73 g/(L·h) [83]. Co-cultures of L. plantarum and L. paracasei showed better performance in LA fermentation from orange peel than monocultures of the same strains [84]. Although Lactobacillus brevis could ferment both xylose and glucose, the mono-cultivation of L. brevis in a mixture of xylose and glucose showed only 0.52 g/g yield and a high number of by-products. Co-cultivation of L. brevis and L. plantarum significantly improved the yield and reduced ethanol production, as L. brevis focused on xylose metabolism in the co-culture system. A LA yield of 0.78 g/g and 0.80 g/g was obtained from co-culture fermentation of polar hydrolysate and alkaline-treated corn stover, respectively [85]. Another application of mixed cultures is the simultaneous saccharification and fermentation of dual organisms: one microbe saccharifies the biomass while the other ferments the hydrolysate into LA. Aspergillus niger (A. niger) and Lactobacillus sp. were used as a mixed culture directly utilizing inulin-rich Jerusalem artichoke tubers. A. niger produced inulinase and invertase to hydrolyze inulin. Simultaneously, Lactobacillus sp. converted the released sugars into LA [86].

8. Genetic and Metabolic Engineering

Complete hydrolysates derived from agricultural wastes contain mixed sugars (glucose, xylose, arabinose, etc.). However, only a few LAB can ferment pentose sugars into LA. Carbon catabolite repression is observed in most LAB, where the consumption of preferred sugars represses the consumption of non-favorable sugars. The introduction of genes encoding non-favorable sugar consumption can improve the utilization of total fermentable sugars. Although LA is the natural major metabolite of most LAB, acetate and ethanol is formed via the phosphoketolase pathway or phosphate pathway. Modifications to carbon metabolism pathways can reduce by-product formation, leading to higher LA yields and lower downstream purification costs. As the fermentation process goes on, the accumulation of LA results in the acidification of the medium, so the acid resistance of the strain is also crucial for sustaining LA production. Certain applications in the medical and surgical fields may require optically pure L/D-LA. Deleting and replacing L/D-lactate dehydrogenase genes in the strains can alter the racemization of the produced LA. The higher optical purity of LA can be achieved by disrupting the dehydrogenase gene corresponding to the unwanted racemic isomer. In addition, multiple genetic and metabolic engineering strategies can be performed on the same strain to obtain higher efficiency. Sahoo et al. knocked out all the L-lactate genes in Lactococcus lactis and over-expressed a heterologous D-lactate dehydrogenase (ldhA) gene from L. bulgaricus to produce optically pure D-LA. The researchers also co-expressed a galactose permease (galP) gene and α-phosphoglucomutase (pgmA) gene, resulting in a 109% increase in D-LA yield from galactose. The recombinant L. lactis was applied to the co-culture batch process of whey permeate and showed an enhanced LA yield of 0.90 g/g and D-LA concentration of 45 g/L [87]. Table 2 summarizes some LA productions by engineered strains.
Table 2. Genetic and metabolic engineering in LA production.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Modifications</th>
<th>Substrate</th>
<th>Optical Purity</th>
<th>LA, g/L</th>
<th>Yield, g/g</th>
<th>Productivity, g/(L·h)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus coagulans</td>
<td>Deletion of L-lactate dehydrogenase (ldh) gene and acetoacetate synthase (alsS) gene, mutation of a growth-based suppressor, introduction of D-lactate dehydrogenase (D-LDH) gene</td>
<td>Sweet sorghum juice</td>
<td>D-LA &gt;99%</td>
<td>125</td>
<td>-</td>
<td>5</td>
<td>[88]</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Six chromosomal deletions (pfrB, ldhA, ackA, pta, frdA, adhE), over-expression of L-lactate dehydrogenase (ldhl) gene, intensification of xylose catabolism</td>
<td>Xylose medium</td>
<td>L-LA &gt;99%</td>
<td>8.12</td>
<td>0.91</td>
<td>-</td>
<td>[89]</td>
</tr>
<tr>
<td>Lactobacillus casei</td>
<td>Mutation of catabolite control protein A (ccpA) gene</td>
<td>Cheese whey</td>
<td>L-LA 94.2%</td>
<td>44.23</td>
<td>0.8</td>
<td>-</td>
<td>[29]</td>
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<tr>
<td>Lactococcus lactis</td>
<td>Replacement of L-lactate dehydrogenase (L-Ldh) gene with D-lactate dehydrogenase (D-Ldh) gene, integration of α-amylase (amyA) gene</td>
<td>Starch</td>
<td>D-LA 93.8%</td>
<td>15.0</td>
<td>-</td>
<td>0.63</td>
<td>[90]</td>
</tr>
<tr>
<td>Lactobacillus paraci</td>
<td>Disruption of D-lactate dehydrogenase (ldhD) gene</td>
<td>Wood hydrolysate</td>
<td>L-LA 98.6%</td>
<td>94.86</td>
<td>0.96</td>
<td>3.23</td>
<td>[91]</td>
</tr>
<tr>
<td>Lactobacillus paraci</td>
<td>Replacement of D-lactate dehydrogenase (ldhD) gene with L-lactate dehydrogenase 1 (ldhL1) gene, adaptive evolution at 45 °C</td>
<td>Rice straw hydrolysate</td>
<td>L-LA 99.1%</td>
<td>66.67</td>
<td>0.97</td>
<td>5.27</td>
<td>[91]</td>
</tr>
<tr>
<td>Lactobacillus plantarum</td>
<td>Deletion of D-lactate dehydrogenase (ldhA) gene, mutation of (AldhD) gene, disruption of lactate racemase operon (larA-E)</td>
<td>High glucose medium</td>
<td>L-LA 98.6%</td>
<td>221.0</td>
<td>0.96</td>
<td>7.5</td>
<td>[80]</td>
</tr>
<tr>
<td>Lactobacillus pentosus</td>
<td>Adaptive evolution at high xylose concentration and low pH</td>
<td>Wheat straw hydrolysate</td>
<td>L-LA</td>
<td>-</td>
<td>13.5</td>
<td>0.86 0.74</td>
<td>[93]</td>
</tr>
<tr>
<td>Pediococcus acidilactici</td>
<td>Disruption of phosphoketolase (pkt) gene, integration of transketolase (ktl), transaldolase (tal), xylose isomerase (xylA) and xylulokinase (xylB) genes, long-term adaptive evolution</td>
<td>Wheat straw</td>
<td>L-LA 130.8</td>
<td>130.8</td>
<td>0.95</td>
<td>1.82</td>
<td>[94]</td>
</tr>
</tbody>
</table>

9. Conclusions and Prospectives

Agricultural waste is as a sustainable alternative to expensive pure sugar or other food-competitive raw materials for the production of LA through microbial fermentation. Depending on the type and composition of agricultural waste, varying degrees of pretreatment may be required to release fermentable sugars from the feedstocks. Appropriate application of either traditional or advanced technologies can benefit LA production in different ways. Batch, fed-batch, and continuous modes are often used as the basis for process design and optimization. Cell immobilization and cell retention can increase LA productivity by high cell density. Exploring more low-cost and environmentally friendly materials for immobilization materials and microfiltration membranes is still an important topic. SSCF combines enzymatic hydrolysis of agricultural waste and bioconversion of released sugars to LA, avoiding substrate inhibition and promoting process efficiency. Cheaper enzyme sources or on-site enzyme production can help address the high enzyme cost for industrial-scale production. Co-culture exploits the synergy between different strains to improve LA yield and LA productivity. It is necessary to search extensively for suitable combinations of strains targeting specific agricultural waste. In addition, genetic and metabolic engineering are important tools to enhance strain fitness, improve substrate utilization and reduce byproduct formation in a controlled direction. However, too much modification may bring an excessive metabolic burden to the original strain, which can affect product quality and production efficiency. Although advanced fermentation techniques promise significant improvements in LA titer, yield, or productivity, careful selection
of an advanced fermentation technique needs to be made with the consideration of cost and feasibility of the industrial production.

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