Proceedings of the 6th International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/2026.37805

Synthetic Biology-Enabled Green Biomanufacturing of Bio-based Platform Chemicals: A Review of Lactic Acid, Succinic Acid, 3-Hydroxypropionic Acid, and Adipic Acid

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Abstract. Organic acids are significant bio-based platform chemicals with extensive application potential. The growing demand for biodegradable plastics, which utilize organic acids as their key monomers to mitigate plastic pollution, has been a major driver accelerating the advancement of organic acid biomanufacturing. This review systematically surveys recent progress in the biotechnological production of four representative organic acids, namely lactic acid, succinic acid, 3-hydroxypropionic acid, and adipic acid. And this review elaborates on the exploratory work directions currently being conducted by researchers on these organic acids. In particular, the applications of synthetic biology enabling strategies, such as synthetic biological components, genome editing technologies, metabolic engineering optimization, and adaptive laboratory evolution, for the development of high-performance microbial cell factories are systematically summarized. This review highlights the potential of emerging synthetic biology strategies in facilitating efficient and sustainable organic acid production and provides theoretical insights and practical guidance for the advancement of green biomanufacturing.

Keywords: Organic acids, Green biomanufacturing, Synthetic biology, Metabolic engineering, Microbial cell factories

1. Introduction

With the advancement of global sustainable development strategies and the establishment of carbon neutrality targets, conventional chemical production processes that rely heavily on fossil resources are facing increasing challenges. Organic acids represent a major class of platform chemicals that have found wide use across the food, pharmaceutical, chemical, and materials sectors. Among them, lactic acid (LA), succinic acid (SA), 3-hydroxypropionic acid (3-HP), and adipic acid (AA) are especially noteworthy because of their considerable potential in biodegradable plastics, polymeric materials, fine chemicals, pharmaceuticals, and related fields. The U.S. Department of Energy, for instance, has placed SA and 3-HP on its list of top value-added platform chemicals derived from biomass [1-4]. Currently, though, industrial production of these four organic acids continues to depend largely on petroleum-based chemical processes, which are generally energy-intensive,

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DOI: 10.54254/2753-8818/2026.37805

polluting, and reliant on nonrenewable feedstocks. These characteristics run counter to the principles of green and sustainable development [1, 2, 4].

The development of synthetic biology has created new opportunities for producing organic acids more sustainably through biomanufacturing. By combining engineered cell factories with renewable biomass feedstocks, fermentative production can reduce dependence on fossil resources and, at the same time, lower carbon emissions through optimization of carbon flux and improvement of carbon utilization efficiency [2]. However, the metabolic networks of microbial chassis are highly complex, and natural isolates often show low product yields, accumulate excessive by-products, and tolerate substrates or products poorly, which limits their direct use in efficient industrial production [5]. To overcome these limitations, researchers have investigated a variety of approaches, such as building synthetic biology components, establishing efficient genome-editing tools, applying rational metabolic engineering, and carrying out adaptive laboratory evolution (ALE). Collectively, these strategies have markedly enhanced the efficiency of organic acid production in engineered chassis and have facilitated the shift toward greener biomanufacturing processes.

This review provides an overview of recent advances in the biomanufacturing of LA, SA, 3-HP, and AA, and discusses in detail how enabling technologies from synthetic biology have contributed to improving their microbial production. It is hoped that this review will serve as a helpful reference for future work on green and sustainable biomanufacturing of these organic acids.

2. Advances in the biomanufacturing of organic acids

As bio-based feedstocks, organic acids serve as vital precursors for biodegradable plastics, and their green and efficient production has become a major research focus in the field of biomanufacturing. This section focuses on four representative organic acids—LA, SA, 3-HP, and AA (Fig. 1)—and summarizes recent advances and key technological breakthroughs in areas including chassis strain development, metabolic engineering strategies, and the selection of fermentation feedstocks (Table 1).

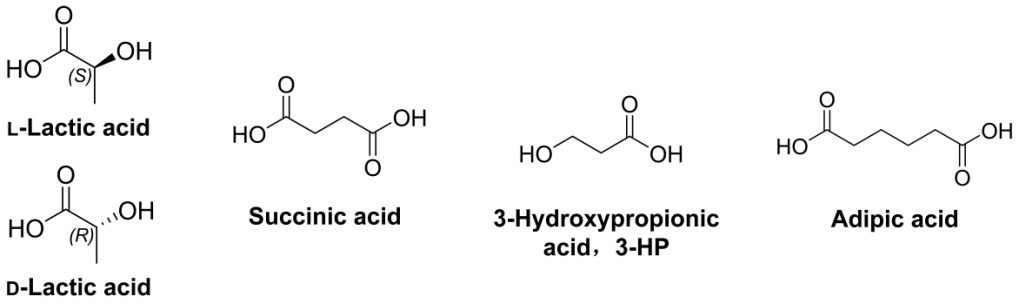

Figure 1. Chemical structures of LA, SA, 3-HP, and AA

Table 1. Advances in the biomanufacturing of four representative organic acids

Product	Chassis strain	Substrate	Titer (g/L)	Yield (g/g)	Ref.
Lactic acid	*Saccharomyces cerevisiae*	Synthetic lignocellulosic hydrolysate	29.9	0.75	[6]

DOI: 10.54254/2753-8818/2026.37805

Table 1. (continued)

d-Lactic acid	*Geobacillusthermoglucosidasius*	Glucose	153.1	93.0%	[7]
l-Lactic acid	*Geobacillusthermoglucosidasius*	Glucose	151.1	98.7%	[7]
Succinic acid	*Yarrowialipolytica*Hi-SA2	Undetoxified corncob hydrolysate	45.34	0.71	[8]
Succinic acid	*Escherichia coli*	Glucose	80.72 mM	1.54 mol/mol	[9]
3-HP	*Pichia pastoris*	Methanol	48.2	0.23	[3]
3-HP	*Saccharomyces cerevisiae*	Glucose	56.5	0.31	[10]
3-HP	*Ogataea polymorpha*	Glucose + xylose	79.6	0.35	[11]
Adipic acid	*Escherichia coli*	Glucose	68.0	0.378	[4]
Adipic acid	*Escherichia coli*	Guaiacol	3 mM	61%	[12]
Adipic acid	*Escherichia coli* (three-module co-culture)	Cyclohexane	31 mM	0.54	[13]

2.1. Advances in the biomanufacturing of lactic acid

LA is a C3 organic acid featuring both hydroxyl and carboxyl groups, which makes it useful as a building block for polylactic acid (PLA), a biodegradable polymer. It is widely used in the food, medical, and cosmetics industries [6].

At present, LA can be produced by chemical synthesis or microbial fermentation. Of the two, microbial fermentation relies on renewable biomass as the carbon source and generally operates under milder conditions. It is also more environmentally friendly and tends to provide higher optical purity. For PLA production, l-LA is the main monomer, whereas d-LA helps adjust and improve the physicochemical properties of PLA.

Traditional fermentation processes usually rely on food crops such as sugarcane and corn starch as feedstocks, and the carbon sources are mainly hexoses such as glucose and corn syrup. This raises feedstock cost. To ease the problem, researchers have turned to non-food lignocellulosic biomass, such as corn stover and rice husks, as alternative carbon sources. However, lignocellulosic biomass must undergo acid or enzymatic hydrolysis to release fermentable sugars, a step that also generates inhibitory compounds like acetic acid and furfural [6]. These compounds can reduce microbial growth and metabolic activity. A further issue is carbon catabolite repression (CCR) [1]. In many cases, microorganisms preferentially consume hexoses such as glucose first, while pentoses such as xylose are taken up more slowly or left unused. This reduces the efficiency with which lignocellulosic biomass is converted in microbial production systems.

Choi et al. [6] employed *Saccharomyces cerevisiae* as the chassis strain, strengthened the LA biosynthetic pathway, and blocked pathways leading to acetate by-product formation. The engineered yeast reached a LA yield of up to 0.75 g/g in lignocellulosic hydrolysates containing

DOI: 10.54254/2753-8818/2026.37805

mixed hexoses, pentoses, and inhibitory compounds such as acetic acid and furfural [6]. This result strongly suggests that *S.cerevisiae* is a practical host for industrial-scale LA production from lignocellulosic biomass. Liu et al. [7] metabolically engineered *Geobacillusthermoglucosidasius*, and the resulting strains fermented at 60°C, achieving l-LA and d-LA titers of 151.1 and 153.1 g/L, respectively.

Overall, LA biomanufacturing has made clear progress, especially in broadening feedstocks and selecting suitable chassis strains. Even so, several problems still need to be solved before lignocellulose-based LA production can be applied at industrial scale. Key issues include efficient removal or detoxification of inhibitors present in lignocellulosic hydrolysates, reducing carbon catabolite repression, and further engineering strains so that they can utilize lignocellulose more effectively and produce LA more efficiently.

2.2. Advances in the biomanufacturing of succinic acid

SA is a C4 dicarboxylic acid and a key intermediate in the tricarboxylic acid (TCA) cycle. It can also be used as a building block for several commercially important chemicals, including 1,4-butanediol (BDO), tetrahydrofuran (THF), γ-butyrolactone (GBL), and poly(butylene succinate) (PBS). Because SA has many industrial uses, the U.S. Department of Energy listed SA as an important candidate among the 12 most promising bio-based platform chemicals [2].

At present, industrial SA production mainly depends on a petrochemical route based on the hydrogenation of maleic anhydride. This method usually requires high energy input, creates considerable environmental pressure, and is not sustainable in the long term. Microbial fermentation offers a different route. It can produce SA from renewable carbon sources such as glucose and xylose through microbial metabolism. One benefit is that microbial SA biosynthesis can include CO_2 fixation through reductive carbon metabolism, which may help lower carbon emissions and support more sustainable production. There is another practical advantage as well. Similar to LA biomanufacturing, microorganisms can use non-food lignocellulosic biomass as a renewable carbon source, which reduces competition with food supplies.

In *Yarrowialipolytica* Hi-SA2, overexpression of the glutathione synthetase gene *YlGSH2* markedly improved tolerance to inhibitory lignocellulosic hydrolysates. After 32 h of fermentation, the engineered strain accumulated 45.34 g/L SA and reached a final pH of 3.28. This result suggests that the yeast chassis could be suitable for SA production under acidic fermentation conditions. The same strategy also reduced the negative effects of inhibitory compounds generated during lignocellulosic pretreatment, including organic acids, furfural, and aromatic compounds [8]. A different approach was reported by Meng et al. [9] They adjusted the pentose phosphate pathway in an *Escherichia coli* chassis to increase NADH availability and direct more carbon flux toward SA formation. The resulting strain achieved a SA yield of 1.54 mol/mol, or roughly 90% of the theoretical maximum.

Biological routes for SA production therefore have two main strengths: they allow more direct regulation of TCA-cycle-associated metabolic pathways, and they make use of non-food feedstocks. Recent work with chassis strains such as *Y. lipolytica* and *E. coli* shows that SA production can be improved through engineering strategies aimed at stress tolerance, cofactor balance, and metabolic pathway optimization.

DOI: 10.54254/2753-8818/2026.37805

2.3. Advances in the biomanufacturing of 3-hydroxypropionic acid

3-HP is an important organic acid with both hydroxyl and carboxyl groups. Like SA, 3-HP has been listed by the U.S. Department of Energy as one of the 12 most promising bio-based platform chemicals. In addition to serving as a key monomer for the synthesis of polyhydroxyalkanoates (PHAs), 3-HP can be converted through various chemical routes into acrylic acid, methacrylic acid, β-propiolactone, malonic acid, acrylamide, and other valuable monomers or intermediates for advanced polymeric materials, which helps explain its broad application potential [3].

Three major biosynthetic routes have been developed for 3-HP production: the glycerol pathway, the malonyl-CoA pathway, and the β-alanine pathway. For glucose-based 3-HP production, the widely used eukaryotic chassis *S. cerevisiae* has drawn considerable research interest. Yu et al. [10] employed a free fatty acid (FFA)-overproducing strain of *S. cerevisiae* as the production host. Since FFA and 3-HP biosynthesis share overlapping precursor and cofactor requirements, the researchers increased the availability of malonyl-CoA and NADPH—both critical for the pathway—and also integrated pathway genes into the genome. Ultimately, fed-batch fermentation yielded 56.5 g/L of 3-HP, which was the highest titer reported at that time for glucose-based 3-HP production.

To lessen the possible competition between bio-based feedstock production and food security or arable land resources, researchers have paid increasing attention to lignocellulose as a non-food biomass feedstock. However, lignocellulosic hydrolysates generally contain both glucose and xylose, and most microorganisms show CCR, preferring glucose while xylose utilization is suppressed. This lowers the overall efficiency of carbon source utilization. Gao et al. [11] employed the naturally xylose-utilizing yeast *Ogataea polymorpha* as the chassis and further improved its xylose utilization capacity. After enhancing the supply of both acetyl-CoA and NADPH, the engineered strain produced 79.6 g/L of 3-HP from a glucose–xylose mixture.

Methanol is an attractive one-carbon (C1) feedstock because its production does not depend on food crops and it can be generated from CO_2 through photo- or thermocatalytic conversion. Methanol therefore combines non-food-feedstock advantages with carbon-mitigation potential, which makes it especially appealing for carbon-neutral biomanufacturing. Nevertheless, efficient methanol utilization remains challenging because of the high complexity of its metabolic regulation and relatively low conversion efficiency. Wu et al. [3] employed *Pichia pastoris* as the chassis and constructed an efficient methanol-based 3-HP cell factory by overexpressing the complete malonyl-CoA pathway and increasing the supply of precursor metabolites and NADPH. When methanol was used as the sole feedstock in fed-batch operation, the engineered strain reached a 3-HP concentration of 48.2 g/L, with a product yield of 0.23 g/g.

2.4. Advances in the biomanufacturing of adipic acid

AA is a six-carbon linear dicarboxylic acid that serves as an essential building block for nylon 6,6 and various resin products. AA also finds extensive application in the production of fragrances, polymeric materials, adhesives, and macrolide antibiotics. Owing to its broad range of applications and high market demand, the U.S. Department of Energy ranked AA as an important candidate among the 12 most promising bio-based platform chemicals [4].

The current industrial route to AA relies mainly on the nitric acid-mediated oxidation of cyclohexane/cyclohexanol [12]. This conventional process involves two oxidation steps under high-temperature and high-pressure conditions and is highly energy intensive. In addition, this process releases significant amounts of N_2O, a potent greenhouse gas. The use of nitric acid can also cause

DOI: 10.54254/2753-8818/2026.37805

environmental acidification and ozone depletion. During the reaction, SA and glutaric acid are formed as by-products, which lowers product purity and reduces carbon efficiency.

Cyclohexane can also be converted into AA through biological routes. To this end, Wang et al. [13] developed a co-culture strategy based on an artificially designed multienzyme cascade system that converts cycloalkanes into α,ω-dicarboxylic acids, including AA, in one step. In their study, three modular *Escherichia coli* strains were engineered to function together for redox self-balancing and cofactor regeneration. After optimization of the module ratios and cultivation conditions, the system reached an AA titer of 31 mM.

Biological production of AA from renewable feedstocks has also been widely studied, especially with glucose as the main substrate. Among these approaches, the reverse adipate degradation pathway (RADP) has received the most attention. This pathway was first identified in *Thermobifidafusca* and converts acetyl-CoA and succinyl-CoA into AA through five sequential enzymatic reactions [4] . Still, its dependence on carbon flux distribution in central metabolism and its consumption of TCA-cycle intermediates appear to restrict both cell growth and AA yield [12]. Zhao et al. [4] addressed this problem by choosing *E. coli* as the chassis because it offers a well-developed genetic toolbox and reconstructing the RADP in this host. They then carried out metabolic engineering by overexpressing the rate-limiting enzyme Tfu_1647 and knocking out competing pathways with CRISPR/Cas9, including the lactate dehydrogenase gene *ldhA*. The result was clear. During fed-batch fermentation, strain Mad123146 produced 68.0 g/L of AA, equivalent to 93.1% of the theoretical maximum yield.

In addition to glucose, non-food feedstocks have also shown promise for AA biosynthesis. Suitor et al. [12] employed guaiacol, a lignin-derived compound, as the substrate and heterologously expressed three enzymes in *E. coli*: GcoAB (encoding guaiacol O-demethylase); CatA (encoding catechol 1,2-dioxygenase); and BcER (encoding an enoate reductase). This approach allowed the establishment of a one-pot whole-cell biocatalytic route for direct AA production from guaiacol. Following process optimization, the recombinant strain attained an AA conversion of 61%. Although further improvement in production performance is still required, this study provided the first proof of concept for AA biosynthesis from a lignocellulose-derived aromatic monomer and opened a new avenue for expanding the feedstock spectrum of AA biomanufacturing.

3. Synthetic biology enabling technologies accelerating organic acid biomanufacturing

Although different organic acids are synthesized through distinct native metabolic pathways, their industrial production remains constrained by metabolic bottlenecks, chassis performance, and limited regulatory capacity. For these reasons, the development of synthetic biology tools that can precisely manipulate microbial metabolic networks has become a practical way of improving organic acid production. In this section, we focus on four main enabling technologies, namely the design of synthetic biology elements, genome editing tools, rational metabolic engineering, and ALE (Fig. 2). We discuss how these tools enable the precise engineering of strains that produce organic acids, with particular attention to their contributions to making biomanufacturing more efficient and sustainable.

DOI: 10.54254/2753-8818/2026.37805

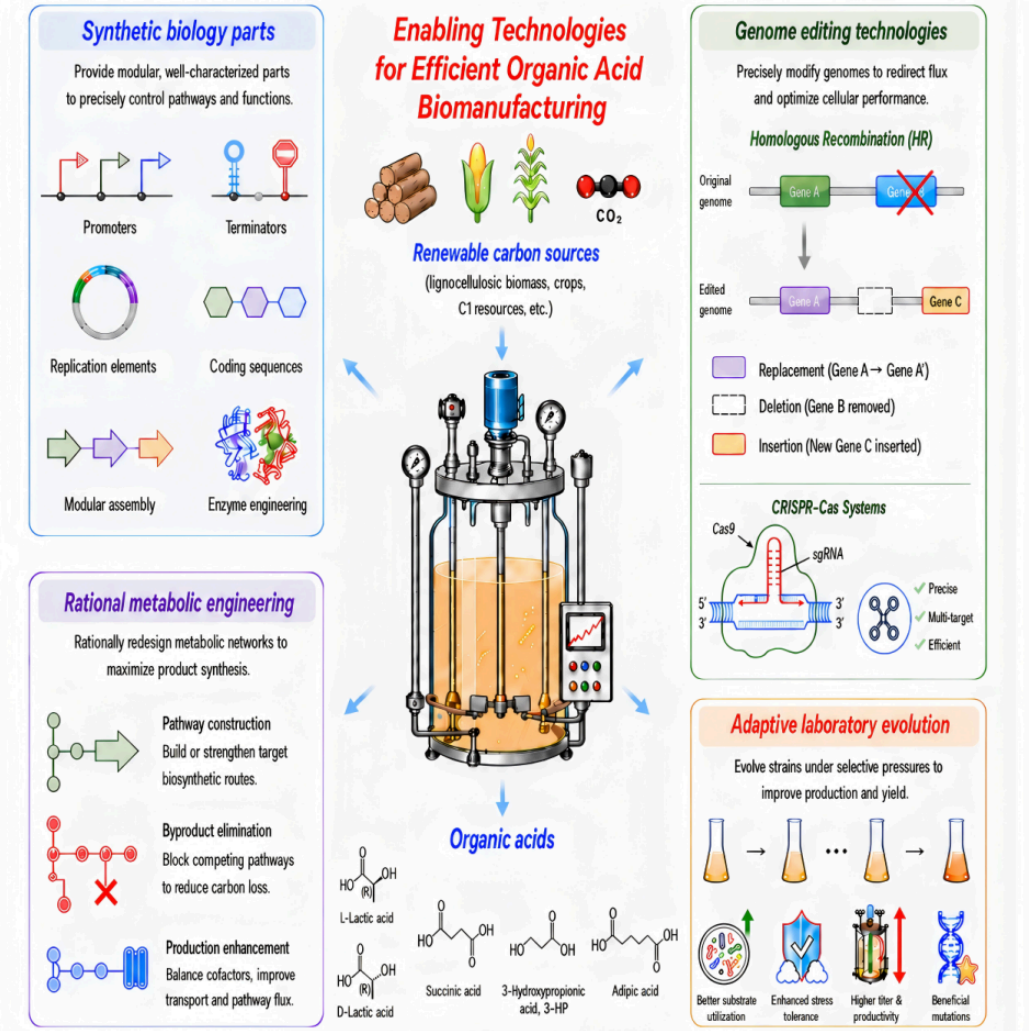

Figure 2. Synthetic biology enabling technologies for efficient organic acid biomanufacturing

3.1. Development and application of synthetic biology elements

Synthetic biology elements are functional DNA sequences that allow precise regulation of gene expression through modular assembly [5]. By function, they can be grouped into several broad categories. Promoters and terminators are basic parts of gene expression systems and control transcription initiation and termination. Autonomous replication sequences (ARSs) and centromeres (CENs) support stable plasmid replication and segregation in host cells, while coding sequences (CDSs) encode key pathway enzymes or regulatory proteins [5].

Yu et al. [10] optimized malonyl-CoA reductase (MCR) in Saccharomyces cerevisiae. MCR is a key enzyme in the malonyl-CoA pathway and catalyzes the two-step reduction of malonyl-CoA to 3-HP. In this pathway, the first step, catalyzed by the C-terminal domain (MCR-C), limits the overall conversion rate. The researchers therefore expressed the two MCR domains separately, using high-level expression of MCR-C and lower expression of MCR-N. This change mattered. After introducing this optimized enzyme module into a high-performance yeast chassis, shake-flask cultivation produced 2.4 g/L of 3-HP. During fed-batch cultures, however, severe plasmid loss reduced expression stability. To address this, the researchers integrated the MCR-C gene into the

DOI: 10.54254/2753-8818/2026.37805

genome so the enzyme module would be maintained more reliably. The resulting strain produced 56.5 g/L 3-HP with a yield of 0.31 g/g [10].

In another case, researchers expressed the hexose transporter mutant *HXT1* in *Ogataea polymorpha* under either the strong constitutive promoter P_{GAP} or the moderately strong promoter P_{PGDI} [11]. When xylose was used alone, the moderate-strength promoter supported both cell growth and xylose utilization. The result changed under mixed sugars. In a glucose–xylose mixture, only *PGAP* gave *HXT1* expression high enough to maintain sufficient xylose transport and allow simultaneous use of both sugars [11]. Based on this result, the researchers further integrated the *MCR* gene into the genome under the control of the P_{GAP} promoter. After promoter engineering and broader metabolic pathway engineering, the engineered strain reached a record 3-HP titer of 79.6 g/L in fed-batch fermentation using a glucose–xylose mixture, the highest reported for lignocellulose-based production [11] .

Overall, these examples indicate that regulatory elements like promoters offer a way to tune gene expression levels, while enzyme-encoding elements directly govern the key catalytic steps within a pathway. Optimizing the two together can thus strengthen metabolic flux control and make organic acid biomanufacturing more efficient.

3.2. Development and application of microbial genome editing technologies

Genome editing technologies are now widely used to make precise changes in microbial genomes [14]. In organic acid biomanufacturing, these tools can be used to delete pathways that produce by-products, insert heterologous genes for key enzymes, and replace genes that encode rate-limiting enzymes. This shifts metabolic flux toward the target product and can increase product accumulation. So far, the main genome engineering strategies used for organic acid-producing strains are conventional homologous recombination-based methods and CRISPR-Cas systems [2].

Liu et al. [7] used the thermophilic bacterium G. *thermoglucosidasius* as the chassis and applied a homologous recombination-based genome engineering strategy step by step. They amplified approximately 0.8-kb flanking homology regions, fused them by splicing overlap extension PCR, then constructed recombinant plasmids and used electroporation-mediated transformation to carry out precise gene deletion and insertion. They first deleted the native ldh gene and introduced a heterologous *d-ldh* gene, producing strain GTD1. After that, they deleted *acdh1/acdh2*, which encode acetaldehyde dehydrogenases, and *aadh*, which encodes aldehyde-alcohol dehydrogenase, to remove the ethanol-forming branch, producing strain GTD6. They then integrated a second copy of codon-optimized *d-ldh* at the aadh locus to further increase d-LA biosynthesis. This produced strain GTD7, with a titer of 86.1 g/L. Across these successive rounds of homologous recombination-mediated engineering, d-LA production increased from undetectable levels to 86.1 g/L. The result is clear. In this case, homologous recombination provided an effective way to re-route metabolism.

Zhao et al. [4] used the CRISPR-Cas9 system in *E. coli* BL21(DE3) to remove competing by-product pathways and improve precursor availability for AA biosynthesis. Their dual-plasmid system, consisting of pCas and pTargetF, was used to precisely delete*ldhA*,*atoB*,and*sucD*. Deletion of *ldhA* eliminated lactate formation and increased the AA yield from 49.5% to 61.7% of the theoretical maximum. Knocking out *atoB* blocked the butyrate-forming pathway and raised the yield to 68.5% of the theoretical maximum, while deletion of *sucD*reduced consumption of the precursor succinyl-CoA and brought the yield to 67.6%. The triple-knockout strain performed even better. It reached 93.1% of the theoretical maximum yield in shake flasks and produced 68.0 g/L AA in fed-batch fermentation.

DOI: 10.54254/2753-8818/2026.37805

Taken together, these studies show a shift in organic acid biomanufacturing from random mutagenesis to targeted strain engineering. The two approaches do not solve exactly the same problem. CRISPR-Cas systems work especially well in model microorganisms with established genetic tools, whereas homologous recombination remains necessary in non-conventional microbial chassis where editing systems are less mature. As genome editing methods continue to improve, they will likely make strain construction for organic acid production more precise and more efficient.

3.3. Rational metabolic engineering for enhanced biomanufacturing efficiency

Rational metabolic engineering improves the biosynthesis of a target product through precise changes to selected metabolic targets, based on an understanding of the host's metabolic network. This differs from semi-rational strategies such as adaptive laboratory evolution. In practice, rational design usually includes three main approaches: pathway construction, by-product elimination, and production enhancement [7].

In their study of SA production in *E. coli*, Meng et al. [9] applied these three approaches to address limited NADH supply. For pathway construction, they introduced *zwf243* and *gnd361*, which increased the SA yield from 1.01 to 1.16 mol/mol. When *pgl*, *tktA*, and *talB* were also overexpressed, the yield reached 1.21 mol/mol. Adding *pepck* together with *sthA* raised it further to 1.31 mol/mol. They then turned to by-product formation. Inactivation of the *ackA-pta* pathway blocked acetate formation and increased the SA yield to 1.40 mol/mol. For production enhancement, they overexpressed the succinate exporters *dcuB/dcuC* so that more product could leave the cells. This likely reduced feedback inhibition caused by intracellular accumulation of succinate. After all modifications were combined, the yield reached 1.54 mol/mol.

Pathway construction mainly works by building or strengthening the biosynthetic route to the target product through the introduction of heterologous genes or the overexpression of endogenous genes that encode key enzymes. By-product elimination redirects more carbon flux and reducing equivalents to the desired product by attenuating or deleting key genes in competing metabolic branches. Production enhancement then improves product formation by balancing cofactors, strengthening product export, and reducing the negative effects that product accumulation can have on cellular metabolism or product-associated cytotoxicity. When applied together, these strategies can substantially improve the efficiency of organic acid biomanufacturing.

3.4. Adaptive laboratory evolution for accelerating organic acid biomanufacturing

As a semi-rational approach, ALE allows strain modification with adaptability to the user-defined experimental requirements. In industrial production, engineered strains may face issues such as insufficient substrate utilization efficiency and harsh environmental conditions—including low pH, high osmotic pressure, and elevated temperature. ALE offers a practical solution to address these issues [15, 16]. The application of ALE can accelerate the development of biomanufacturing.

Liu et al. [7] applied ALE to the engineered *G. thermoglucosidasius* strains GTD7 and GTD17 after rational metabolic engineering. The evolution of GTD7 lasted 251 days and was carried out in three stages (Table 2). And the evolution of GTD17 lasted 110 days (Table 2). The evolutionary strains exhibit excellent LA production performance. Several genetic variants were detected by whole-genome resequencing, namely *ptsG* (G137S), *tpiA*(A249V), *gap* (D137E), and a disrupted *cggR*. These genetic variants may have led to an improvement in the fermentation performance of the strain.

DOI: 10.54254/2753-8818/2026.37805

Table 2. The ALE of GTD7 and GTD17

Strain	Stage	Glucose (g/L)	Fermentation time (h)	Titer (g/L) Yield
GTD7	Generations 1–45	80	24	GTD7-144, d-LA: 153.1, 93.0%
	Generations 46–74	80	48	
	Generations 75–144	100	48	
GTD17	Generations 1–55	100	48	GTD17-55, l-LA: 151.1, 98.7%

When rational design is hard to carry out because the metabolic network of a given chassis is not well understood, ALE offers a useful semi-rational option. The same applies when repeated rounds of targeted engineering do not produce the phenotype required. Another advantage is that evolved strains can then be examined in more detail through whole-genome resequencing and multi-omics analyses, including transcriptomics, proteomics, and metabolomics. This makes it possible to identify mutations and regulatory points linked to the improved phenotype. In that sense, ALE is not separate from rational metabolic engineering. It can feed back into it by providing clearer engineering targets and a better basis for later design steps.

4. Conclusions and perspectives

Organic acids represent a key class of bio-based platform chemicals, and they also serve as building blocks for sustainable materials. Their potential in food, pharmaceuticals, chemicals, and biodegradable polymers is considerable. Recent progress in synthetic biology, metabolic engineering, and fermentation engineering has considerably accelerated the development of biomanufacturing for organic acids. In this review, we systematically outline the principal biosynthetic strategies used for four representative organic acids: LA, SA, 3-HP, and AA. At the same time, synthetic biology enabling technologies have been widely used in the precise engineering of microbial chassis. They have made great progress in metabolic pathway reconstruction, genome editing, metabolic network regulation, and adaptive laboratory evolution. These developments have promoted organic acid titers, yields, and productivities.

Even so, large-scale biomanufacturing of organic acids is still restricted by feedstock costs, strain performance, and fermentation process control. In the future, the continued diversification and optimization of substrate utilization systems will be important for promoting industrial development. Shifting feedstocks from conventional sugars to renewable alternatives such as lignocellulosic biomass, agricultural residues, industrial waste streams, and C1 feedstocks can lower production costs while improving carbon utilization efficiency. However, because of insufficient co-utilization of mixed carbon sources, the presence of pretreatment-derived inhibitors, and limited strain adaptability, the efficient use of complex substrates is still difficult. Thus, engineering next-generation chassis strains will play an important role in converting non-food resources into high-value organic acids by improving multi-carbon utilization and systematically optimizing their metabolic networks.

Integrating artificial intelligence with metabolic engineering is expected to establish a new paradigm for the design of organic acid-producing cell factories. Machine learning, multi-omics analysis, and intelligent computational algorithms can help predict key metabolic nodes, screen high-performance enzyme elements, and optimize metabolic pathways, thereby reducing the trial-and-error burden commonly in conventional strain engineering [17] . Overall, as non-food carbon

DOI: 10.54254/2753-8818/2026.37805

feedstocks and emerging enabling technologies such as AI-assisted design continue to advance together, organic acid biomanufacturing is likely to become more efficient, intelligent, and sustainable, providing important technological support for green chemical production and for the growth of the bioeconomy.

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