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Review Article

Metabolic engineering strategies to improve microbial acetate utilization for chemical production

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Abstract

Microbial cell factories have been established as an important platform for green biomanufacturing. However, current systems remain highly dependent on food-based carbon sources such as glucose, creating an industrial dilemma of "competing with humans for food and with agriculture for land," thereby impeding large-scale industrial deployment. Acetate is considered a promising alternative carbon source that can circumvent these constraints and is broadly available from sources such as industrial off-gas conversion. In this review, the application potential of acetate as a carbon source is systematically summarized from four dimensions: acetate-assimilating microorganisms, metabolic engineering strategies for improving acetate utilization, metabolic engineering strategies for enhancing acetate tolerance, and metabolic engineering strategies for producing chemicals from acetate. In addition, the key challenges and future directions of acetate-based biomanufacturing are analyzed, providing a theoretical basis for industrial implementation.
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Keywords: Acetate; Microbial cell factories; Metabolic engineering; Synthetic biology; Chemicals

1. Introduction

Microbial cell factories offer an environmentally benign and sustainable route for producing fine chemicals, pharmaceutical intermediates, food additives, and biofuels from renewable resources [1,2]. Control of feedstock cost is critical for their industrial deployment. At present, most fermentation processes rely on food-based substrates such as sucrose, glucose, and starch, which not only increase production costs but also intensifies competition with human food supplies [3]. Therefore, replacement of current feedstock systems with lower-cost, non-food substrates is becoming imperative. With the progressive industrialization of technologies for capturing, fixing, and converting C1 gases into acetate, acetate is expected to emerge as a highly promising bulk feedstock for future industrial production [4,5].

The conversion of C1 gases to acetate can be broadly classified into biocatalytic [6] and chemocatalytic [7] approaches. Biocatalytic strategies mainly include syngas fermentation and microbial electrochemical systems (MESs). In syngas fermentation, *Clostridium ljungdahlii* is typically employed, with CO/CO_2 and H_2 serving as the carbon and energy inputs, respectively, and acetate being produced through CO_2 fixation via the Wood–Ljungdahl pathway (WLP) [6]. A key advantage of this system lies in the direct conversion of CO/CO_2 into acetyl-CoA through the WLP, followed by its further conversion into acetate, thereby

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theoretically enabling a high carbon utilization efficiency with nearly no carbon loss. In addition, CO and CO_2 are mainly derived from industrial off-gases and lignocellulosic gasification, which allows low-cost feedstock supply and facilitates the valorization of waste resources. At present, syngas fermentation has demonstrated promising industrial potential, with acetate titers ranging from 4 to 60 g/L [8,9]. For example, by optimizing the expression levels of enzymes in the WLP, an engineered strain was able to synthesize 51 g/L acetate from CO_2 and H_2 within 3.8 days [10]. MESs consist of an anode chamber and a cathode chamber separated by a proton exchange membrane. In the anode chamber, electrons are generated through the oxidation of organic substrates or water, whereas, in the cathode chamber, these electrons are utilized by microorganisms to reduce C1 gases for acetate synthesis [11]. The advantages of this system are that no external H_2 supply is required, thereby reducing mass transfer limitations and safety risks, and that CO_2 fixation and acetate production are carried out in situ within the electrolytic reactor, which lowers production costs [12]. However, the acetate titer achieved by this approach remains relatively low at present (generally below 10 g/L), indicating that a considerable gap still exists before industrial application can be realized [13,14]. The efficiency with which electrons are acquired by microorganisms is critical to MES performance. Therefore, to promote its industrial development, the electron uptake capacity of microorganisms can be further enhanced. Relevant strategies include the addition of soluble electron shuttles [15], increasing cell loading on the electrode [16], and engineering conductive cytochrome systems in the microbial chassis [17]. Depending on the mode of energy input, chemocatalysis can be further divided into electrocatalysis [18] and photocatalysis [19]. In electrocatalytic systems, an external potential is applied at the electrode surface, and electrical energy is converted into reducing power by the catalyst, thereby driving the stepwise reduction of C1 gases through multi-electron/proton-coupled reactions accompanied by C–C coupling to ultimately produce acetate. Compared with MESs, electrocatalytic systems generally exhibit higher reaction rates, greater process simplicity because cell growth does not need to be maintained, and superior operational stability. Catalysts play a pivotal role in this process. Accordingly, diverse catalyst families have been explored, including manganese-based catalysts [20], tin(II) monosulfide (SnS) nanobelts [21], copper-based catalysts [22,23], metal–organic framework-derived catalysts [24,25], and indium-based catalysts [26]. This approach has already demonstrated promising potential for industrial application. In one representative example, under laboratory conditions, a copper single-atom cluster catalyst (CuZSM SACL) achieved a current density of 1.8 A/cm^2 and an acetate Faradaic efficiency of 71% in the CO reduction reaction. Moreover, stable catalytic conversion of CO to acetate was maintained even at an industrially relevant current density of 1 A/cm^2 and a cell voltage of 2.7 V, and continuous operation for more than 1000 h was achieved under atmospheric pressure, with a Faradaic efficiency of 61% [27]. Photocatalysis employs semiconductor materials that generate electron–hole pairs upon light absorption, thereby driving redox reactions and converting photon energy into chemical energy for C1 reduction. Representative semiconductor systems include $CuPt/WO_3$ [28], In_2O_3/InP [29], and TiO_2 [19]. The principal advantage of photocatalysis lies in the direct utilization of renewable light energy, enabling carbon valorization under mild conditions. However, the reported rates of photocatalytic acetate production in the literature remain relatively low, generally at the μmol/(g h) level, indicating that substantial challenges still remain before industrial application can be realized [28]. Even in more recent representative studies, only limited performance has been achieved; for example, the Pt-based catalyst Pt-rGO exhibited an acetate production rate of only 29.63 μmol/g/h and a selectivity of 91.8% for the conversion of CO_2 to acetate [30]. To advance the industrial implementation of this technology, catalyst optimization may be pursued from several aspects: enhancement of CO_2 adsorption and activation through the introduction of defects such as oxygen vacancies [31]; improvement of charge separation efficiency and interfacial reaction capability through the construction of p–n junctions, Z-scheme, S-scheme, or interface-rich heterostructures [32]; improvement of electron extraction, CO_2 activation, and intermediate coupling through the design of single-atom, dual-atom, or bimetallic active sites [33]; and broadening of light absorption together with optimization of band alignment through semiconductor coupling, elemental doping, or the introduction of metal sites [34]. Taken together, these routes provide complementary pathways for acetate production from C1 gases, while microbial upgrading of acetate into high-value chemicals offers a sustainable platform for low-carbon biomanufacturing (Fig. 1).

As a novel feedstock for microbial cell factories, acetate offers several advantages (Table 1). (1) Low cost. Acetate is priced at approximately USD 300–450 per ton, providing a cost advantage over glucose (~USD 500 per ton) [35]. (2) High water solubility. Its high solubility can reduce feeding frequency and help preserve the usable working volume of bioreactors [36]. (3) High stability. As a non-reducing compound, acetate is unlikely to trigger Maillard reactions. Therefore, undesired by-product formation and medium darkening are minimized, and downstream separation/purification is not further complicated [37]. (4) Favorable carbon yield. Acetate can be converted to acetyl-CoA at an equimolar 1:1 ratio in one or two steps (yield coefficient for carbon, $Y_C = 1$). By contrast, the longer conversion route from glucose to acetyl-CoA via pyruvate decarboxylation incurs carbon loss, resulting in a lower Y_C of 0.67 [37]. (5) Operational convenience. Because acetate consumption increases fermentation broth pH, acetate can be used as a neutralizing agent; with online pH monitoring, acetate can be fed in a timely manner to achieve coupled feeding and pH control [38]. For example, in a recent study, *Halomonas bluephagenesis* was employed to convert acetate into poly(3-hydroxybutyrate) (PHB). In a 5000-L industrial-scale fermenter, after 68 h of cultivation, a cell dry weight (CDW) of 73.88 g/L and a PHB content of 74.33 wt% were successfully achieved. Compared with the conventional glucose-based process, the acetate-based process

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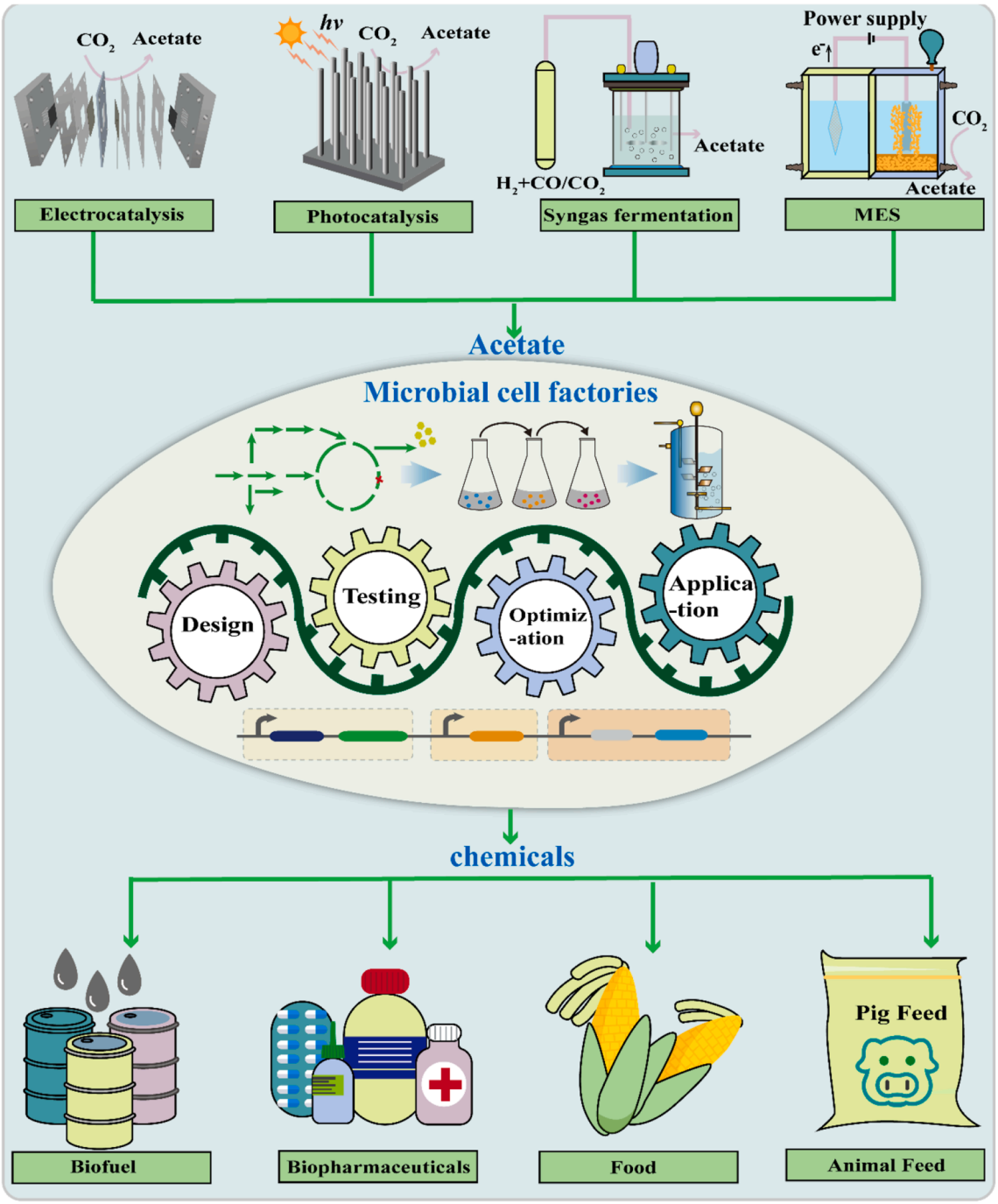

Fig. 1. Conceptual schematic of a promising platform in which microorganisms utilize acetate to synthesize chemicals, offering a low-carbon and sustainable route. C1 gases are converted to acetate via electrocatalysis, photocatalysis, syngas fermentation, and microbial electrochemical systems (MESs). Microorganisms then use acetate to produce high-value chemicals, such as biofuels, biopharmaceuticals, food products, and animal feed.

was shown to reduce the production cost of PHB by approximately 33%. This reduction was mainly attributed to the replacement of food-based glucose with low-cost acetate as the carbon source, the elimination of NaOH costs because acetate could serve as a neutralizing agent, the reduction in labor costs through coupling of feeding and pH control, and the lower separation cost resulting from the absence of Maillard reactions [38]. In this review, the potential of acetate as a feedstock for microbial cell factories is discussed from four perspectives: microorganisms capable of using acetate as a carbon source, strategies to enhance microbial acetate utilization, approaches to improve acetate tolerance, and metabolic engineering for chemical production from acetate (Fig. 2). In addition, current bottlenecks and future directions for acetate-based biomanufacturing are outlined.

2. Acetate-assimilating microorganisms

To enable the efficient conversion of acetate into diverse fine chemicals, a broad set of acetate-assimilating

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Table 1
Comparison between acetate and glucose.

Feedstocks	Price ($/ton)	Yield coefficient for carbon, Y_C	Addition of a neutralizing agent	Water solubility (g/L)	Maillard reactions	Sources
Acetate	300−450 [36]	Acetate can be converted to acetyl-CoA with $Y_C = 1$ [5].	No neutralizing agent is required, as the fermentation broth is alkaline and can itself maintain the pH [38].	1233 [36]	No Maillard reactions [37]	Sourced from the conversion of C1 waste streams and the depolymerization of lignocellulose, this approach is beneficial for the development of a green circular economy [39].
Glucose	500 [3]	Glucose can be converted to acetyl-CoA with $Y_C = 0.67$, owing to carbon loss caused by pyruvate decarboxylation [37].	A neutralizing agent is required to maintain the pH [40].	909 [36]	Maillard reactions present [9,41]	Derived from the hydrolysis of starch, potatoes, wheat, and corn, this process creates an industrial dilemma by competing with the human food supply [42].

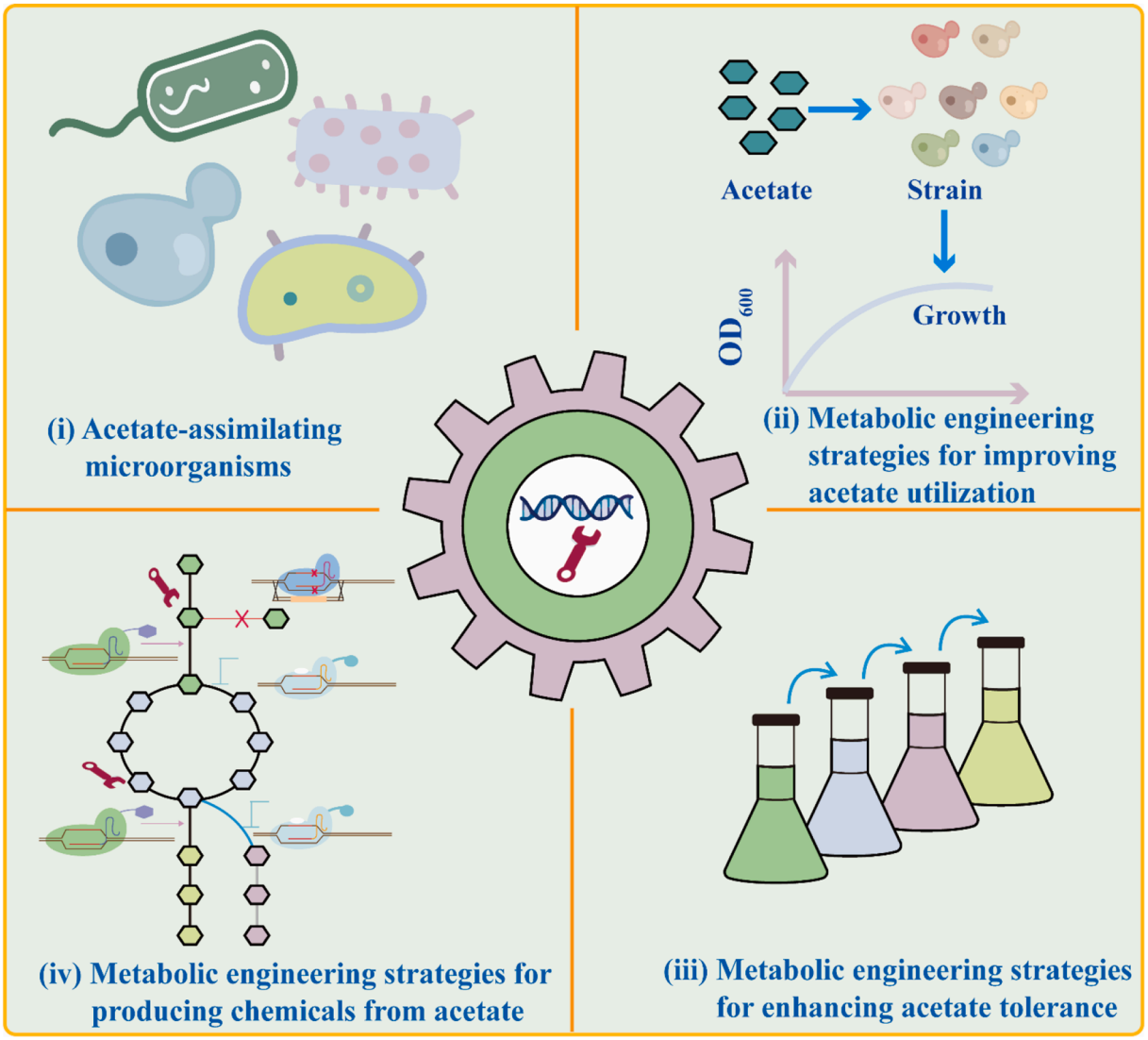

Fig. 2. **Schematic overview of this review.** This review assesses acetate as a substrate for microbial cell factories across four axes: organisms capable of acetate assimilation, strategies to boost acetate utilization, approaches to improve acetate tolerance, and metabolic engineering methods for converting acetate to chemicals.

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microorganisms has been mined and engineered, encompassing both non-model and model hosts.

The non-model microorganisms primarily include *Ralstonia eutropha* [43], *Yarrowia lipolytica* [44], *Aspergillus oryzae* [45], *Pseudomonas denitrificans* [46], *Cryptococcus curvatus* [9], and *Rhodotorula glutinis* [47]. These organisms are distinguished by high environmental adaptability and robustness, sustaining growth under extreme conditions such as elevated temperature, high salinity, and strongly acidic or alkaline media, thereby helping to lower energy consumption and costs while improving overall process efficiency. A representative case is *A. oryzae* DSM 1863, which, without genetic modification, maintained vigorous growth in 45 g/L acetate and produced 8.44 g/L malate, highlighting its promising application potential in biomanufacturing [45]. In addition, *H. bluephagenesis*, a halophilic microorganism, has been employed for the biosynthesis of PHB via the assimilation of sodium acetate, owing to its remarkable growth advantage under high-salt conditions. Through genome mining, a unique ADP-dependent acetyl-CoA synthetase (ACS_{ADP}) was identified in *H. bluephagenesis*, and its overexpression resulted in a significant enhancement of sodium acetate utilization by the strain. Under a high sodium acetate concentration (30 g/L), the engineered strain's CDW reached 7.38 g/L, with PHB accounting for 78.58 wt% of the biomass. Even when the sodium acetate concentration was increased to 100 g/L, production activity was maintained, with the CDW and PHB content reaching 4.37 g/L and 66.50 wt%, respectively [38].

Despite the promise of non-model microorganisms for acetate-based chemical synthesis, their further development is constrained by the lack of efficient genome-editing tools. By contrast, model hosts such as *Escherichia coli* [36], *Saccharomyces cerevisiae* [48], and *Corynebacterium glutamicum* [49] possess well-characterized genetic backgrounds and mature engineering toolkits and have therefore been widely applied in industrial production. For example, by deleting the hexokinase genes *GLK1*, *HXK1*, and *HXK2*, and heterologously expressing the glucose-1-phosphatase genes *agP* and *yihX*, an engineered *S. cerevisiae* strain was constructed to convert acetate into glucose, achieving a titer of 2.3 g/L, which represents a 30% increase over the control [50]. In addition, in *E. coli* W, strengthening the expression of genes involved in acetate assimilation and the glyoxylate cycle markedly improved acetate utilization, yielding 3.57 g/L itaconate—a 4.54-fold increase relative to the unmodified strain [51].

3. Metabolic engineering strategies for enhancing acetate utilization

Although diverse microorganisms have been developed to use acetate as a substrate, acetate assimilation efficiency has remained low. Accordingly, multiple strategies have been devised to enhance acetate utilization in microbes. In this section, the following three aspects are discussed: (1) acetate assimilation pathways in microorganisms; (2) enhancement of acetate-to-acetyl-CoA conversion to promote acetate utilization; and (3) optimization of the glyoxylate cycle to facilitate acetate utilization (Fig. 3).

3.1. Acetate assimilation pathways in microorganisms

The principal mode by which acetate enters microbial cells is determined by the environmental pH. Under acidic conditions, acetate is present in the environment predominantly in its undissociated form, CH_3COOH. Owing to its relatively high lipophilicity, this form can be transported into the cell through passive diffusion across the cell membrane [39]. In contrast, under neutral or alkaline conditions, acetate exists primarily as the negatively charged acetate ion, which cannot freely penetrate the cell membrane because of its polarity. Consequently, its intracellular uptake depends on specific monocarboxylate transporters, such as ActP (acetate permease) and SatP (succinate–acetate transporter protein), through an active transport process [3]. After acetate is taken up by microorganisms, it is primarily converted to acetyl-CoA via two metabolic pathways: the acetate kinase–phosphate acetyltransferase pathway (AckA–Pta) and the acetyl-CoA synthetase pathway (ACS) [37] (Fig. 3A). Subsequently, acetyl-CoA enters the glyoxylate cycle to produce malate, which is further oxidized to fumarate and oxaloacetate. This process not only provides energy to the cell but also contributes to the synthesis of high-carbon compounds through gluconeogenesis [41].

In the AckA–Pta route, AckA and Pta catalyze a reversible two-step sequence. First, AckA transfers the γ-phosphate of adenosine triphosphate (ATP) to acetate to form acetyl phosphate (acetyl-P) and adenosine diphosphate (ADP). Second, Pta condenses acetyl-P with coenzyme A (HS-CoA) to generate acetyl-CoA while releasing inorganic phosphate. The overall pathway consumes one ATP equivalent. By contrast, the ACS pathway converts acetate directly to acetyl-CoA in a single, essentially irreversible step that hydrolyzes ATP to AMP with inorganic pyrophosphate (PPi), thereby consuming two ATP equivalents. Nevertheless, ACS exhibits ~35-fold higher affinity for acetate than the AckA–Pta system, indicating a kinetic advantage for acetate assimilation [3].

3.2. Enhancement of acetate-to-acetyl-CoA conversion to promote acetate utilization

Conversion of acetate to acetyl-CoA constitutes the key initiating step in microbial acetate metabolism, and its efficiency directly determines a strain's capacity to utilize acetate. Principal strategies to enhance this step include optimization of the ACS pathway, reinforcement of the AckA–Pta pathway, introduction of heterologous acetate assimilation routes, and strengthening of intracellular ATP supply.

Optimization of the ACS pathway can be implemented in two modes: plasmid-based expression [52] and chromosomal integration [53]. Owing to their high copy number, plasmid systems enable strong gene expression. For example, regulation of *acs* on the pRSFDuet-1 vector markedly increased the cellular acetyl-CoA supply, enlarged the intracellular pyruvate

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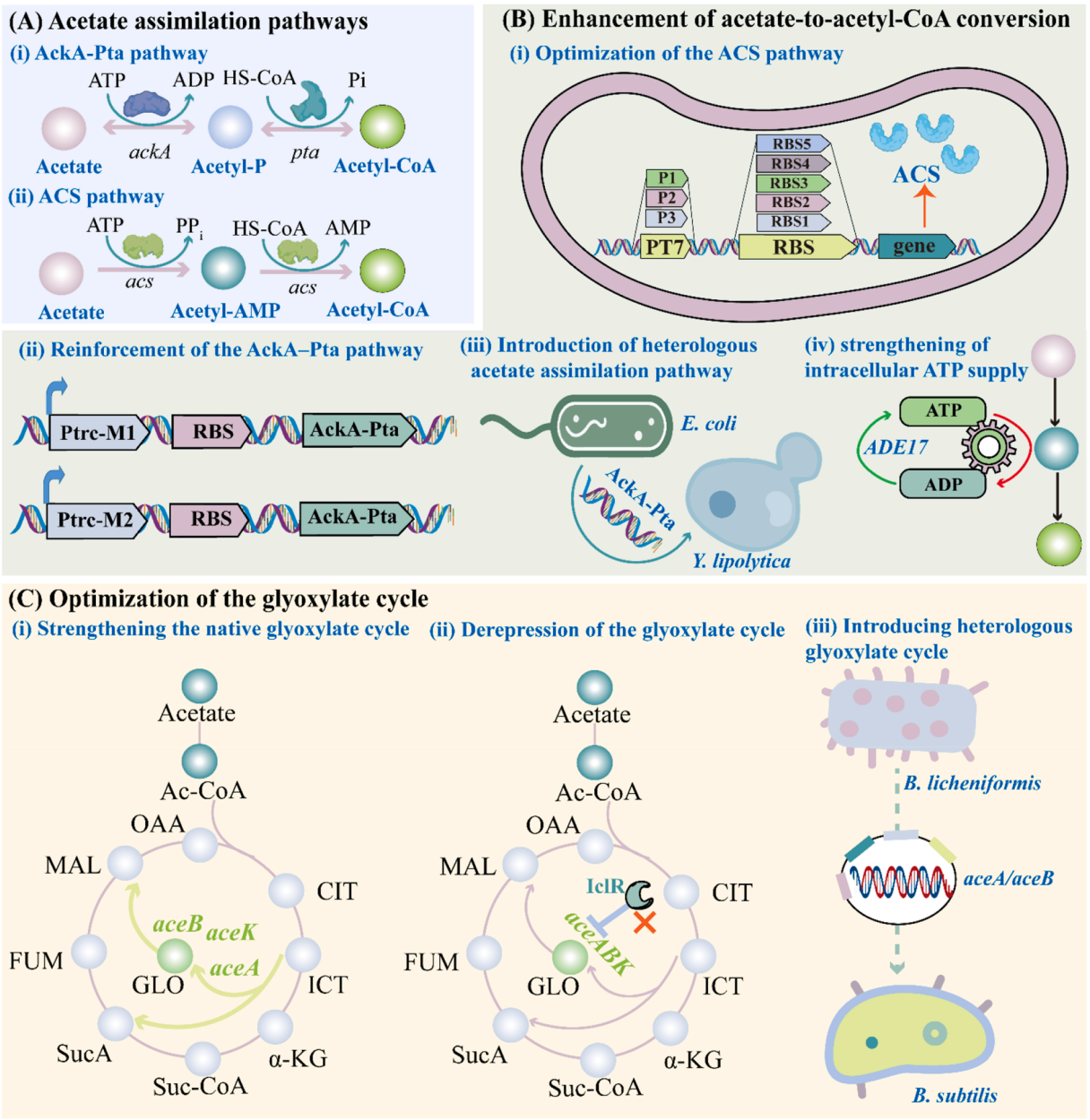

Fig. 3. **Metabolic engineering strategies for enhancing acetate utilization.** (A) Acetate assimilation pathways, including the AckA–Pta pathway and the ACS pathway. (B) Strategies to promote the conversion of acetate to acetyl-CoA, including optimization of the ACS pathway, reinforcement of the AckA–Pta pathway, introduction of heterologous acetate assimilation routes, and strengthening of intracellular ATP supply. (C) Strategies to enhance glyoxylate cycle flux, including strengthening the native glyoxylate cycle, derepression of the glyoxylate cycle, and introduction of heterologous glyoxylate cycles. Abbreviations: ATP, adenosine triphosphate; ADP, adenosine diphosphate; PPi, inorganic pyrophosphate; Pi, inorganic phosphate; P, promoter; PT7, T7 promoter; RBS, ribosome-binding site; GLO, glyoxylate; Ac-CoA, acetyl-CoA; OAA, oxaloacetate; MAL, malate; FUM, fumarate; SucA, succinate; Suc-CoA, succinyl-CoA; α-KG, α-ketoglutarate; ICT, isocitrate; CIT, citrate; *pta*, phosphotransacetylase encoding gene; *ackA*, acetate kinase encoding gene; *acs*, acetyl-CoA synthetase encoding gene; *ADE17*, the bifunctional purine biosynthesis protein ADE17 encoding gene; *aceA*, isocitrate lyase encoding gene; *aceB*, malate synthase encoding gene; *aceK*, isocitrate dehydrogenase kinase encoding gene; IclR, glyoxylate cycle inhibitor protein. The T-shaped symbol represents gene expression repression, whereas the cross (×) symbol denotes gene knockout.

pool, and raised OD_{600} by 21.0% relative to the control [52]. However, plasmids are genetically unstable and prone to loss during serial cultivation, thereby compromising production performance, and their reliance on inducers elevates manufacturing costs. By contrast, genome integration establishes plasmid-free expression directly on the chromosome, affording high genetic stability and eliminating inducer requirements, which reduces industrial costs. For example, replacing the native *acs* promoter with the strong T7 promoter increased the acetate consumption rate to 0.11 g/g dry cell weight (DCW)/h—2.2-fold higher than that of the parent strain [53] (Fig. 3B).

Nevertheless, the ACS pathway is not universally suitable and is energetically demanding. Therefore, reinforcement of

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the AckA−Pta pathway provides an alternative. In strain HB01, substitution of the native *ackA* and *pta* promoters on the chromosome with the strong Ptrc-M1 and Ptrc-M2 promoters, respectively, yielded strains HB02 and HB03. The transcription levels of *ackA* and *pta* in the two strains were substantially enhanced, acetate utilization improved, and succinate titers reached 26.69 mmol/L and 27.40 mmol/L at 72 h, 9.2% and 11.2% higher than in strain HB01 [54] (Fig. 3B).

In addition, introducing heterologous routes can act synergistically with native pathways to further increase acetate assimilation. For example, heterologous expression of the *E. coli* AckA−Pta pathway in *Y. lipolytica* significantly strengthened acetate metabolism, raising fatty alcohol production by 1.89-fold relative to the control [55] (Fig. 3B).

However, whether acetate is converted to acetyl-CoA via the ACS pathway or the AckA−Pta pathway, ATP supply is required. Accordingly, intracellular energy metabolism must also be strengthened as a key strategy to enhance acetate-to−acetyl-CoA conversion, primarily by overexpressing native energy-metabolism genes [56] and introducing heterologous energy modules [57]. For example, in *S. cerevisiae*, overexpression of the native *ADE17* gene (encoding the bifunctional purine biosynthesis protein ADE17) significantly increased intracellular ATP levels, raising the maximum biomass by 38.80% [56] (Fig. 3B). By contrast, incorporation of exogenous energy modules can broaden ATP-generation routes: heterologous expression of the *Ricinus communis* phosphoenolpyruvate carboxykinase gene (*RcPCK*) in *E. coli* markedly enhanced ATP regeneration; the engineered strain completely consumed 80 mmol/L acetate within 10 h, shortening the processing time by 20% relative to the parent [57]. Through targeted control of the energy-metabolic network, these strategies provide the driving force for efficient acetate bioconversion.

3.3. Optimization of the glyoxylate cycle to facilitate acetate utilization

After acetate is converted to acetyl-CoA, the metabolite is routed into the glyoxylate cycle, providing cellular energy and gluconeogenic precursors required for biomass synthesis. Therefore, optimization of the glyoxylate cycle is essential for enhancing acetate utilization. Engineering strategies focus on three directions: strengthening the native glyoxylate cycle, relieving its repression, and introducing heterologous glyoxylate cycles.

Strengthening the native cycle has been achieved by overexpressing glyoxylate-cycle genes [58] and attenuating the TCA cycle [59]. In *E. coli*, the *aceBAK* operon—encoding malate synthase (AceB), isocitrate lyase (AceA), and isocitrate dehydrogenase kinase (AceK)—constitutes the core of the glyoxylate cycle. Overexpression of *aceBAK* promoted acetate assimilation and increased the pyruvate titer from 0.04 g/L to 0.41 g/L [58] (Fig. 3C). Additionally, in *C. glutamicum*, expression of a catalytically weakened isocitrate dehydrogenase variant (ICD^{R453C}) diminished TCA-cycle flux to augment glyoxylate-cycle throughput, raising biomass from 8.6 to 19.1 g/L DCW [59].

In *E. coli*, IclR (isocitrate lyase regulator) functions as a transcriptional repressor primarily by regulating the expression of key genes involved in the glyoxylate cycle [60]. Specifically, IclR is able to bind to the promoter region of the *aceBAK* operon, thereby blocking the binding of RNA polymerase to the promoter or inhibiting the formation of the transcription initiation complex. As a result, the transcription of the key enzymes AceA and AceB is repressed, which in turn restricts the activation and metabolic flux of the glyoxylate cycle. By contrast, repression of the glyoxylate cycle by the IclR protein (encoded by *iclR*) can be relieved by *iclR* deletion, which avoids the metabolic burden associated with gene overexpression. For example, in *E. coli* BL21, *iclR* knockout enhanced growth on acetate and increased 3-hydroxypropionate (3-HP) production by 24.1% [61] (Fig. 3C).

By introducing heterologous glyoxylate-cycle modules, acetate-assimilation capacity can be conferred on hosts that do not naturally utilize acetate, thereby expanding the applicability of microbial platforms. For instance, expression of *aceA* and *aceB* from *Bacillus licheniformis* in *Bacillus subtilis* produced functionally active glyoxylate cycle enzymes, as confirmed by integrated transcriptomic, proteomic, and metabolomic analyses, thereby enabling acetate utilization in the engineered strain [62] (Fig. 3C).

4. Metabolic engineering strategies for enhancing acetate tolerance

Acetate exhibits cytotoxicity that markedly inhibits microbial growth even at low concentrations. Its toxicity arises primarily from three mechanisms: (1) Molecular (undissociated) acetate toxicity [3]: owing to its small size and hydrophilicity, undissociated acetic acid traverses the membrane by simple diffusion, eliciting pronounced intracellular proton and anion stress; (2) Anion-specific toxicity [63,64]: intracellular accumulation of acetate anions depletes the glutamate pool, inhibits the methionine biosynthetic pathway, and perturbs enzyme activities and acetyl-CoA homeostasis via protein acetylation, ultimately impairing normal metabolism and proliferation; and (3) Reactive oxygen species (ROS)-mediated oxidative stress [65,66]: acetate disrupts the electron transport chain, promoting electron leakage and formation of superoxide, which is further converted to hydrogen peroxide, hydroxyl radicals, and other highly oxidative ROS. Concurrently, acetate diminishes the activities of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, reducing ROS scavenging capacity and exacerbating oxidative damage. To counter these toxicities, tolerance to acetate is typically improved by membrane engineering, optimization of intracellular acid-resistance systems, and adaptive laboratory evolution (Fig. 4).

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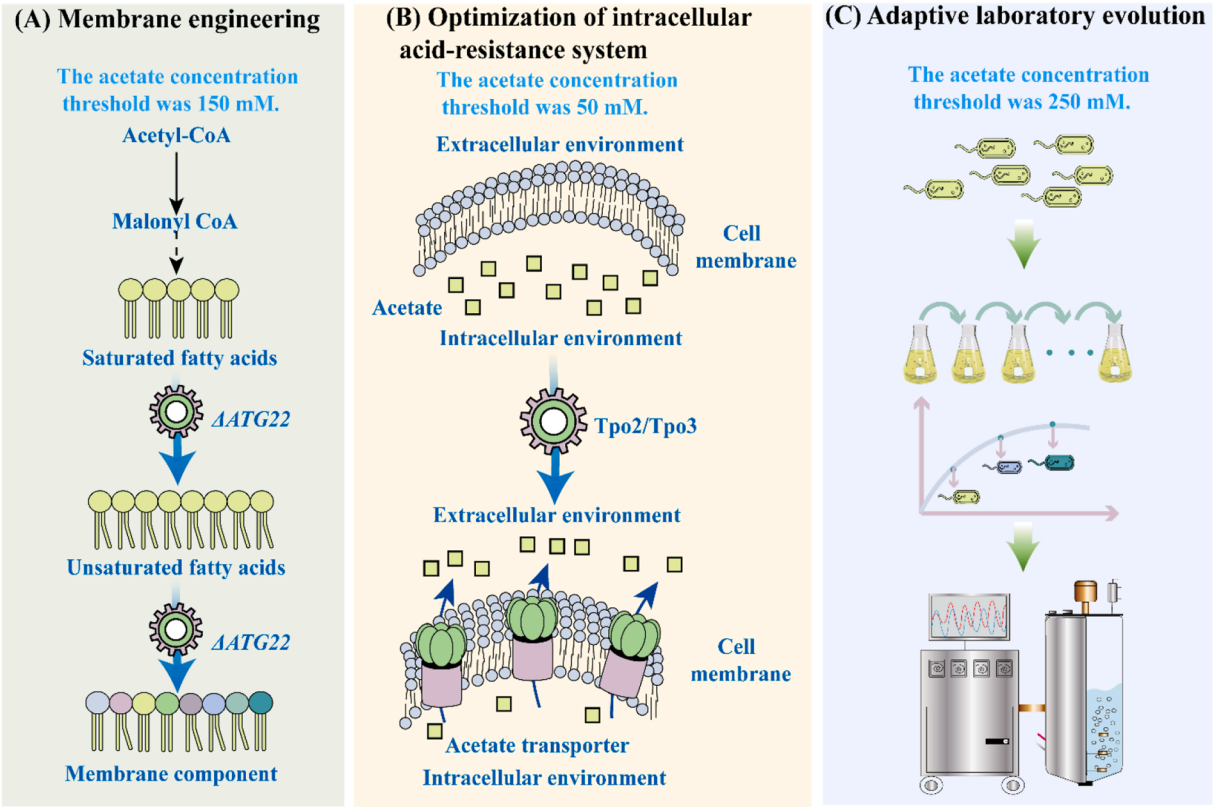

Fig. 4. **Metabolic engineering strategies for enhancing acetate tolerance.** (A) Membrane engineering improves the tolerance to acetate. (B) Optimization of intracellular acid-resistance systems enhances the tolerance to acetate. (C) Adaptive laboratory evolution increases the tolerance to acetate. Abbreviations: *ATG22*, a vacuolar membrane protein encoding gene; Tpo2/Tpo3, acetate transporter.

4.1. Membrane engineering

As a biological barrier, the cell membrane separates intracellular and extracellular environments and is critical for resisting external stresses in industrial microbial processing [67]. Membrane engineering strategies to enhance acetate tolerance primarily include modulating fatty acid composition, promoting sphingolipid biosynthesis, and strengthening sterol synthesis. Alterations in fatty acid profiles are expected to increase membrane integrity, thereby improving strain robustness. For example, deletion of Atg22p, a vacuolar membrane protein, in *S. cerevisiae* markedly increased acetate tolerance. Following *ATG22* deletion, total fatty acid content was 25% and 64% higher than in the control strain and the *ATG22*-overexpression strain, respectively; saturated fatty acids were elevated by 28% and 72%, and unsaturated fatty acids by 18% and 70%, respectively. Membrane integrity was enhanced by these changes in fatty acid composition, thereby improving acetate tolerance [68] (Fig. 4A). In addition, increasing sphingolipid synthesis has shown promise: supplementation of the medium with phytosphingosine promoted sphingolipid synthesis and conferred a significant growth advantage to *S. cerevisiae* under acetate stress [69]. Compared with the preceding strategies, sterol biosynthesis confers acetate tolerance primarily by reinforcing membrane rigidity [70]. For example, *ERG1*, which encodes squalene monooxygenase and plays a key role in ergosterol biosynthesis, is critical under acetate stress: disruption of *ERG1* expression rapidly compromises the viability of *S. cerevisiae*, whereas its upregulation increases tolerance to acetate [71].

4.2. Optimization of intracellular acid-resistance systems

Optimization of intracellular acid-resistance systems focuses on modulating the intracellular milieu to help microorganisms withstand acetate stress. The principal strategies are promoting the efflux of intracellular acetate [72] and reducing intracellular ROS levels [73]. For example, strengthening expression of the transporters Tpo2 and Tpo3 reduced intracellular acetate and increased the acetate tolerance of *S. cerevisiae* to 50 mmol/L [72] (Fig. 4B). However, the expression of transporter proteins consumes a significant amount of cellular energy, which may compete with the energy required for product synthesis pathways, thereby reducing the titer of the target product.

By contrast, decreasing intracellular ROS offers an alternative with promising applicability. Using a genomic library–based approach in *S. cerevisiae*, the key tolerance target *RCK1* (encoding a putative serine/threonine protein kinase) was identified and overexpressed. When acetate was used as the carbon source, intracellular ROS levels in the engineered strain decreased by 40% relative to the parental strain, resulting in enhanced tolerance, increased growth rate, and

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doubled ethanol productivity [74]. In addition, CRISPRi-mediated repression of *YALI1_A06958g* (encoding a Rho guanosine diphosphate dissociation inhibitor) elevated the NADPH/$NADP^+$ ratio by 40%, thereby promoting reduced glutathione synthesis to quench ROS, and lowered ROS to 61% of the control, significantly improving acetate tolerance in *Y. lipolytica* [44].

4.3. Adaptive laboratory evolution

Although the above strategies can enhance the acetate tolerance of strains, the complexity of gene interaction networks and regulatory systems renders the engineering of specific pathways or genes to obtain strains with robust phenotypes still challenging.

Adaptive laboratory evolution (ALE) addresses this challenge and can generate industrial strains with desirable properties. The advantages of ALE include [75,76]: the rapid evolution and selection of highly robust mutants, followed by systems-level analyses to identify causal genotypes that guide rational engineering; and the integration of beneficial mutations into wild-type backgrounds via reverse metabolic engineering to obtain superior strains without accumulating extraneous mutations. For example, ALE was conducted in M9 minimal medium, yielding an evolved *E. coli* strain capable of robust growth across a wide acetate concentration range (10−250 mmol/L). Whole-genome sequencing combined with reverse metabolic engineering revealed that mutations in the cold-shock protein gene *cspC* and the acetyltransferase gene *patZ* synergistically enhanced acetate metabolism and tolerance. The evolved strain was subsequently employed as a platform for acetate-based production of proteins, mevalonate, and *n*-butanol [77] (Fig. 4C). In another study, ALE was also employed to enhance acetate tolerance in *Y. lipolytica*. During serial passaging, the acetate concentration in the medium was gradually increased from 2 to 50 g/L, and an evolved strain, ACS 5.0, capable of tolerating 50 g/L acetate, was obtained. Under 22.5 g/L acetate, the evolved strain exhibited an OD_{600} of 9.2, with lipid and succinate titers reaching 0.84 g/L and 5.1 g/L, respectively. Following further optimization of the fermentation process, the OD_{600}, lipid titer, and succinate titer of ACS 5.0 were further increased to 23.5, 1.5 g/L, and 6.5 g/L, respectively. In addition, the acetate uptake rate reached 0.2 g/L/h, representing a 2.86-fold increase over that of the unevolved strain [78]. However, the above approaches often require evolutionary adaptation and screening periods spanning several months or even years. To rapidly obtain desired traits and improve breeding efficiency, accelerated microbial genome evolution systems can be rationally designed. Relevant strategies include modulation of the expression levels of proofreading-related elements involved in DNA replication [79], suppression or deletion of the mismatch repair pathway [80], interference with the translesion synthesis pathway [81], and introduction of heterologous deaminases [82]. For example, in *S. cerevisiae*, the strain yRFA2 carrying an evolution tool based on the cytidine deaminase APOBEC was able to reach an OD_{600} of 1.2 under 12 g/L acetate, whereas the control strain was almost unable to grow under the same conditions [83]. These results indicate that an increased genomic mutation rate accelerated the acquisition of acetate tolerance in the strain.

5. Metabolic engineering strategies for producing chemicals from acetate

The conversion of acetate into high-value chemicals by microorganisms provides an important route for low-carbon biomanufacturing, and the resulting products span multiple sectors, including organic acids, amino acids, biofuels, biomaterials, carbohydrates, etc. (Fig. 5 and Table 2). To enhance the synthesis of these products, a range of metabolic engineering strategies has been developed, including pathway enzyme optimization, precursor supply optimization, inactivation of competing pathways, cofactor engineering, and pathway coordination optimization (Fig. 6). Among them, precursor supply optimization, pathway enzyme optimization, and inactivation of competing pathways correspond to the Push, Pull, and Block components, respectively, of the classical “Push−Pull−Block” integrated strategy in metabolic engineering [84]. To examine the approaches developed for optimizing each component, this integrated strategy is dissected in the present article, and each part is discussed in detail.

5.1. Pathway enzyme optimization

Enzymes are fundamental elements of biosynthetic pathways. To enhance the conversion of acetate into target products, pathway enzymes have primarily been optimized from two aspects: enzyme mining and tuning the expression levels of pathway enzymes (Fig. 6A).

Enzyme mining is aimed at designing metabolic routes with higher catalytic efficiency. As a representative example, an ADP-dependent acetyl-CoA synthetase (ACS_{ADP}) was identified through genome mining and was introduced into *H. bluephagenesis* to construct a synthetic PHB biosynthesis pathway (ACS_{ADP}−PhaA−PhaB−PhaC). Using acetate as the substrate, a CDW of 82.5 g/L (with 82.6 wt% PHB) was achieved in a 7 L fermenter, and the process was successfully scaled to 100 L and 5000 L while maintaining >74 wt% PHB [38]. This strategy provided a proof-of-concept for low-cost, scalable, carbon-negative manufacturing of biodegradable plastics.

Optimization of pathway enzyme expression is primarily aimed at alleviating metabolic bottlenecks arising from insufficient or excessive expression. Representative strategies include promoter engineering [101], ribosome-binding site (RBS) engineering [92], and 5′ untranslated region (5′ UTR) engineering [61]. Promoter engineering tunes enzyme expression at the transcriptional level. For example, expression of the pathway genes *budB* (encoding acetolactate synthase), *budA* (encoding acetolactate decarboxylase), and *budC* (encoding 2,3-butanediol dehydrogenase) was strengthened

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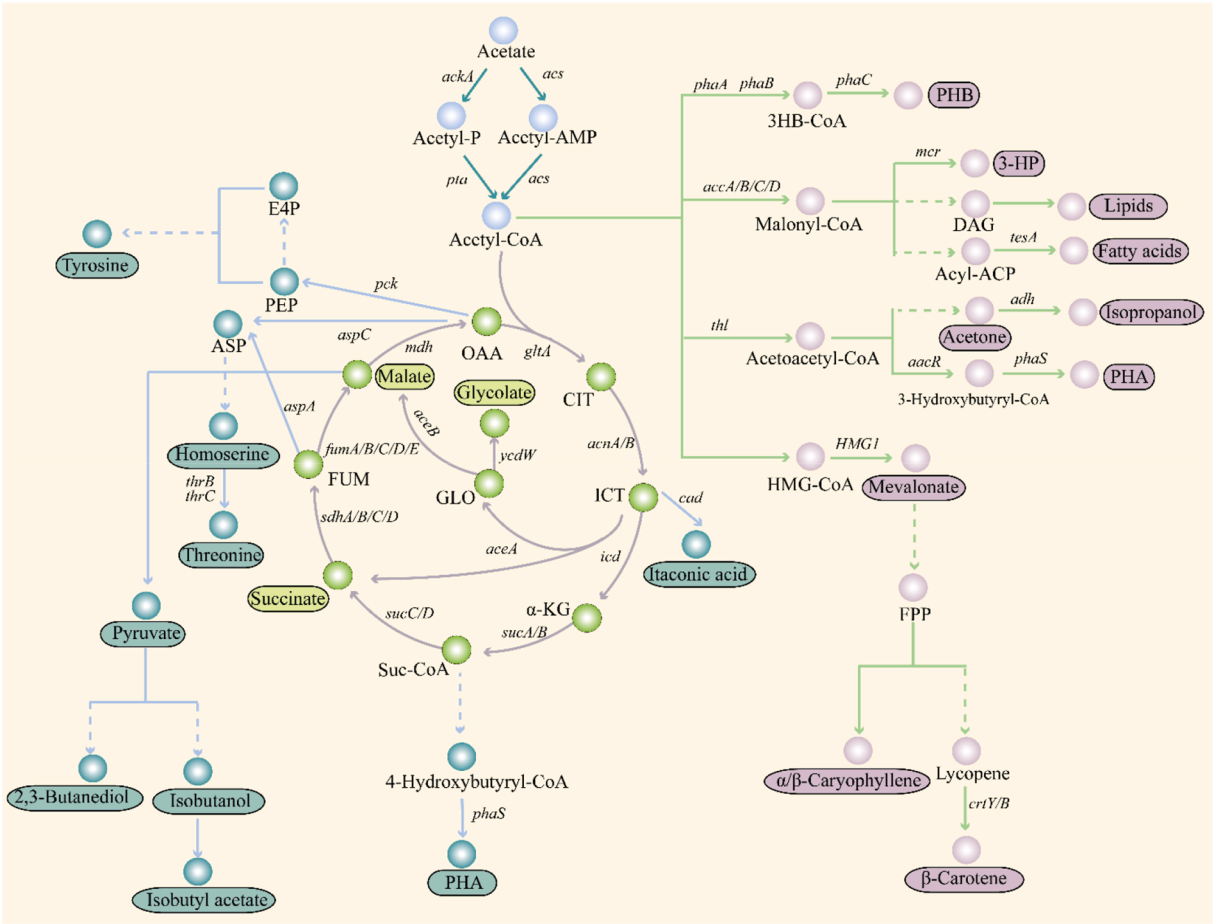

Fig. 5. **Metabolic pathway map for chemical synthesis using acetate as the substrate.** Abbreviations: E4P, erythrose-4-phosphate; PEP, phosphoenolpyruvate; ASP, aspartate; 3HB-CoA, 3-hydroxybutyryl-CoA; PHB, poly(3-hydroxybutyrate); 3-HP, 3-hydroxypropionic acid; DAG, diacylglycerol; Acyl-ACP, acyl–acyl carrier protein; PHA, polyhydroxyalkanoates; HMG-CoA, 3-hydroxy-3-methyl glutaryl CoA; FPP, farnesyl pyrophosphate; *phaA*, encoding β-ketothiolase; *phaB*, encoding acetoacetyl-CoA reductase; *phaC*, encoding PHB synthase; *accA/B/C/D*, encoding acetyl-CoA carboxylase; *mcr*, encoding malonyl-CoA reductase; *tesA*, encoding acyl-CoA thioesterase; *thl*, encoding thiolase; *aacR*, encoding acetoacetyl-CoA reductase; *adh*, encoding alcohol dehydrogenase; *phaS*, encoding PHA synthase; *HMG1*, encoding 3 -hydroxy-3-methylglutaryl-CoA reductase; *crtY/B*, encoding phytoene synthase; *gltA*, encoding citrate synthase; *acnA/B*, encoding aconitate hydratase; *cad*, encoding cis-aconitate decarboxylase; *icd*, encoding isocitrate dehydrogenase; *sucA/B*, encoding 2-oxoglutarate dehydrogenase; *sucC/D*, encoding succinyl-CoA synthetase; *sdhA/B/C/D*, encoding succinate dehydrogenase; *fumA/B/C/D/E*, encoding fumarate hydratase; *mdh*, encoding malate dehydrogenase; *pck*, encoding phosphoenolpyruvate carboxykinase; *aspC*, encoding aspartate aminotransferase; *aspA*, encoding aspartate ammonia-lyase; *thrB*, encoding homoserine kinase; *thrC*, encoding threonine synthase; *ycdW*, encoding glyoxylate reductase. Solid arrows indicate single-step metabolic reactions, whereas dashed arrows indicate multi-step metabolic reactions.

using the T7 promoter, and diols production in engineered *E. coli* was increased to 1.56 g/L [101]. In contrast, RBS engineering modulates enzyme expression at the translational level. For example, expression of *atoDA* (encoding acetate CoA/acetoacetate CoA transferase) and *adh* (encoding alcohol dehydrogenase) was tuned via an engineered RBS sequence (TACGTAAACTATAGGGAGGGAATAA), thereby increasing isopropanol production to 24.5 mmol/L with a yield of 0.56 mol/mol [92]. In addition, 5′ UTR engineering provides an alternative route for expression tuning. For example, 3-hydroxypropionic acid (3-HP) pathway flux was finely adjusted by optimizing the 5′ UTR sequences of *mcrN* (encoding malonyl-CoA reductase), *accADBC* (encoding acetyl-CoA carboxylase), and *birA* (encoding biotin–[acetyl-CoA-carboxylase] ligase), resulting in an increase of 3-HP to 72 mmol/L in the engineered strain [61].

5.2. Precursor supply optimization

A sufficient and stable precursor supply is required to sustain smooth metabolic flux, thereby increasing the final product titer. Accordingly, precursor availability has been improved from two aspects—strengthening precursor biosynthetic routes and reducing precursor consumption—to facilitate the conversion of acetate into target products (Fig. 6B).

Strengthening precursor biosynthetic pathways is most commonly achieved by promoter engineering to enhance precursor formation [87]. For example, *acs* and *pck* were overexpressed to increase the conversion of oxaloacetate into phosphoenolpyruvate, a precursor for tyrosine biosynthesis, and the tyrosine titer in the engineered strain was increased to 0.7 g/L, representing a 1.6-fold improvement over the parental strain [87]. In parallel, precursor consumption can be reduced

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Table 2
Types of chemicals synthesized from acetate.

Product	Strain	Titer (g/L)	Yield (g/g)	Productivity [g/(L h)]	Reference
Organic acids					
Succinate	*E. coli*	27.40 mM	–	–	[54]
Malate	*A. oryzae*	8.44	0.19	0.044	[45]
Glycolate	*E. coli*	73.3	1.08	1.04	[53]
3-HP	*E. coli*	4.73	0.63	0.118	[85]
Mevalonate	*E. coli*	7.85	0.27	0.13	[86]
Itaconate	*C. glutamicum*	29.2	0.21	0.63	[59]
Pyruvate	*E. coli*	0.41	–	0.009	[58]
Amino acids					
Tyrosine	*E. coli*	0.70	0.07	0.023	[87]
Homoserine	*E. coli*	44.1	0.18 mol/mol	0.85	[88]
Threonine	*E. coli*	45.8	0.22 mol/mol	0.88	[88]
Biofuels					
Ethanol	*S. cerevisiae*	45	0.414	0.51	[89]
Isobutanol	*E. coli*	0.1−0.2	–	–	[52]
Fatty acids	*E. coli*	0.9	0.064	0.0035	[90]
2,3-Butanediol	*E. coli*	1.16	0.09	0.011	[91]
Isopropanol	*E. coli*	1.47	0.56 mol/mol	0.041	[92]
Biomaterials					
PHA	*P. putida*	31.5 wt%	–	–	[93]
PHB	*H. bluephagenesis*	80.75 wt%	–	–	[38]
Carbohydrates					
Starch	*Y. lipolytica*	19.30	0.105	0.161	[94]
Glucose	*S. cerevisiae*	2.3	–	–	[50]
Others					
Lipids	*Y. lipolytica*	46	0.16	0.27	[95]
Acetone	*E. coli*	6.57	0.21	0.274	[96]
β-Caryophyllene	*E. coli*	1.05	0.021	0.015	[97]
Isobutyl acetate	*E. coli*	19.7	0.33	0.164	[98]
β-Carotene	*Blakeslea trispora*	2.13	–	0.015	[99]
Limonene	*E. coli*	105.52 mg/L	–	–	[100]

either by adding chemical inhibitors to block competing routes [102] or by deleting precursor-consuming pathways [103]. Compared with chemical inhibition, gene deletion is advantageous because no additional reagents are required, making it more cost-effective and thus more attractive for practical applications. In a glycolate production study, precursor glyoxylate consumption was reduced by deleting *aceB*/*glcB* (encoding malate synthase A/malate synthase G), *gcl* (encoding glyoxylate carboligase), and *glcD* (encoding glycolate oxidase), thereby directing metabolic flux toward the desired product and increasing glycolate production to 2.75 g/L [103].

5.3. Inactivation of competing pathways

Inactivation of competing pathways can redirect a larger fraction of metabolic flux toward the target product, thereby reducing flux waste. Approaches to suppress competing routes during acetate-to-chemical conversion mainly include blocking degradation pathways of the target product [90] and attenuating competing branch pathways [104] (Fig. 6C). A notable example is that deletion of FadE, an acyl-CoA dehydrogenase involved in fatty acid degradation, was used to increase fatty acid titer to 1 g/L, enabling efficient conversion of acetate into biofuels [90]. In contrast, repression of branch pathways can limit by-product accumulation, thereby simplifying downstream separation. For example, by knocking out the *maeB* gene (encoding malic enzyme) to reduce the metabolic flux of the branched gluconeogenesis pathway, the metabolic flux was redirected to the succinate synthesis pathway, resulting in a 2.6-fold increase in succinate production in the engineered strain [104].

Although the application of any single strategy within the "Push−Pull−Block" framework can enhance the biosynthesis of target chemicals, improved outcomes are often achieved when these strategies are implemented in an integrated manner [94,105]. For example, to enhance the conversion of acetate into 3-HP, the expression level of acetyl-CoA carboxylase (Acc) was regulated by the promoter Pfic to increase the supply of the precursor malonyl-CoA, which is subsequently converted into 3-HP by malonyl-CoA reductase (MCR). As a result, the 3-HP titer of the engineered strain reached 3.00 g/L, representing a 38.89% increase over that of the control strain. On this basis, the *fadR* gene, which encodes the activator FadR in the fatty acid biosynthetic pathway, was deleted, and cerulenin, an inhibitor of fatty acid synthesis, was added to weaken metabolic flux through the fatty acid biosynthetic pathway and thereby alleviate its competition for the precursor malonyl-CoA. Consequently, the 3-HP titer of the engineered strain was further increased to 4.73 g/L [85]. In a recent study on limonene biosynthesis from acetate, protein engineering was first performed on MvaS in the synthetic pathway to

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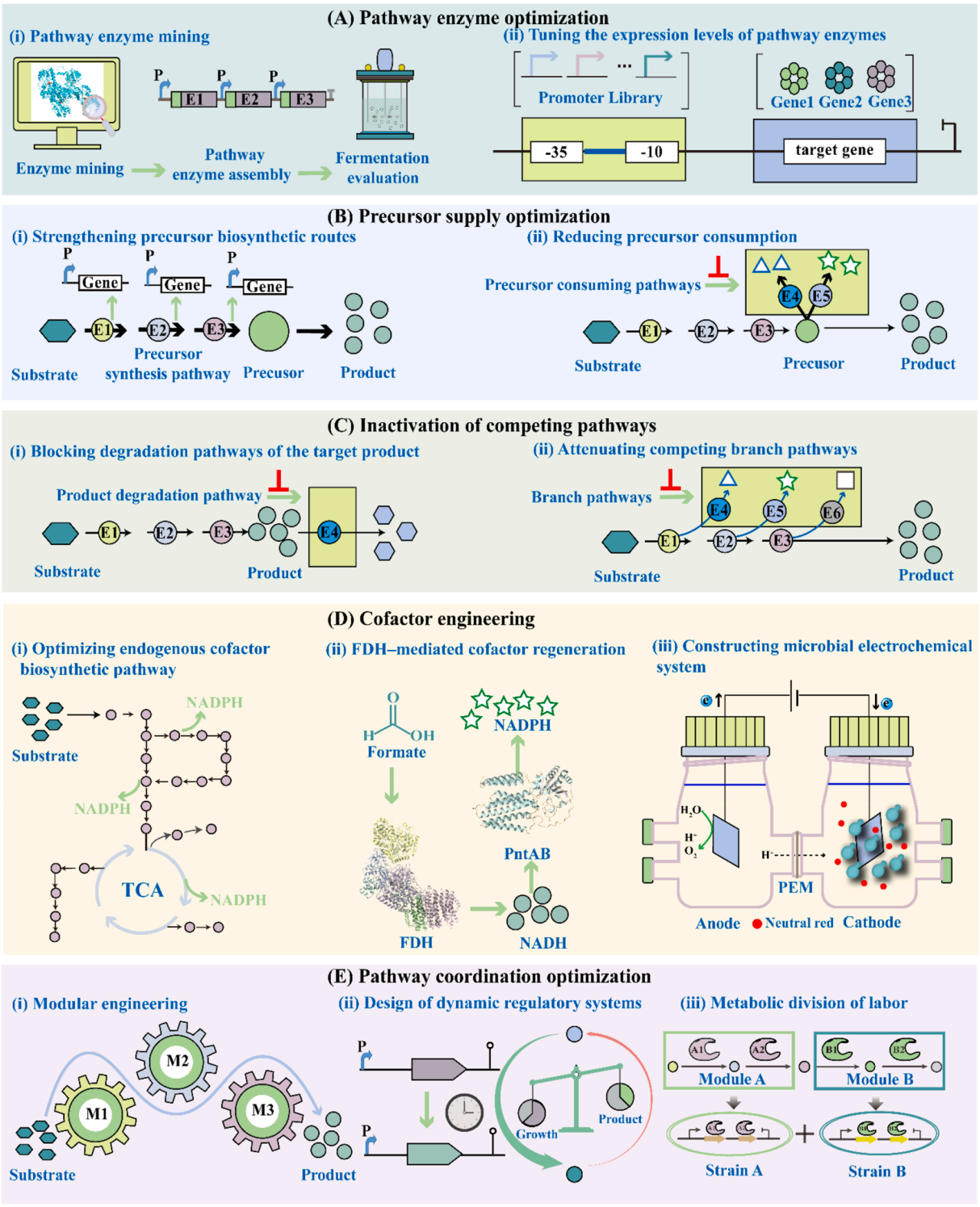

Fig. 6. **Metabolic engineering strategies for producing chemicals from acetate.** (A) Pathway enzyme optimization strategies, including enzyme mining and tuning the expression levels of pathway enzymes. (B) Precursor supply optimization strategies, including strengthening precursor biosynthetic routes and reducing precursor consumption. (C) Inactivation of competing pathways strategies, including blocking degradation pathways of the target product and attenuating competing branch pathways. (D) Cofactor engineering strategies, including optimizing endogenous cofactor biosynthetic pathways, implementing FDH-mediated cofactor regeneration, and constructing microbial electrochemical systems. (E) Pathway coordination optimization strategies, including modular engineering, the design of dynamic regulatory systems, and metabolic division of labor. Abbreviations: E, enzyme; −35, the −35 region of the promoter; −10, the −10 region of the promoter; NADPH, nicotinamide adenine dinucleotide phosphate (reduced form); TCA, tricarboxylic acid cycle; FDH, formate dehydrogenase; PntAB, pyridine nucleotide transhydrogenase; PEM, proton exchange membrane; e^-, electron; M, module; O_2, oxygen; H_2O, water. A1, A2, B1, and B2 represent different genes.

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promote the conversion of acetyl-CoA into the precursor isopentenyl pyrophosphate (IPP), yielding the mutant MvaS^{A110G}, which increased limonene production to 8.98 mg/L. Subsequently, to enhance the metabolic flux from IPP to limonene, the expression levels of limonene synthase (LS) and NPP synthase (NPPS) were strengthened using the strong promoter T7, resulting in an increase in limonene production to 40.44 mg/L. Finally, to block competing pathways and further enhance limonene biosynthesis, *fadR* and lactate dehydrogenase LdhA were deleted to reduce the conversion of acetyl-CoA into fatty acids and lactate, respectively, thereby further increasing limonene production to 105.52 mg/L [100].

5.4. Cofactor engineering

The reducing equivalents generated during microbial acetate metabolism are substantially lower than those produced from sugar substrates and are often insufficient to meet the cofactor demand required for efficient chemical biosynthesis. Accordingly, strengthening intracellular cofactor supply has been recognized as an effective approach to improve acetate-based bioproduction. The main strategies include optimizing endogenous cofactor biosynthetic pathways, implementing formate dehydrogenase (FDH)–mediated cofactor regeneration, and constructing MESs (Fig. 6D).

Strategies for optimizing endogenous cofactor biosynthesis mainly include glucose supplementation to enhance intracellular cofactor formation [53] and overexpression of the pyridine nucleotide transhydrogenase PntAB [92], which catalyzes NADH-to-NADPH conversion. For example, during glycolate production, glucose was supplemented to the medium, and *pfkAB* (encoding 6-phosphofructokinase), *ptsI* (encoding phosphoenolpyruvate–protein phosphotransferase), and *sthA* (encoding soluble pyridine nucleotide transhydrogenase) were deleted, while expression of *zwf* (encoding glucose-6-phosphate 1-dehydrogenase), *pgl* (encoding 6-phosphogluconolactonase), and *tktA* (encoding transketolase 1) was strengthened, thereby increasing NADPH supply and enabling a glycolate titer of 73.3 g/L, which represents the highest glycolate titer reported to date using acetate and glucose as co-substrates [53]. In contrast, overexpression of PntAB can avoid glucose supplementation and is therefore more cost-effective. For instance, pntAB was overexpressed in *E. coli* to supply NADPH to alcohol dehydrogenase, thereby increasing isopropanol titer by 15% [92].

An FDH-mediated energy-regeneration system refers to the use of formate as an electron donor to drive intracellular reducing-equivalent regeneration through expression of FDH [106]. This strategy is advantageous because formate, a non-food feedstock, can be exploited as the electron donor, offering sustainability and improved economic feasibility. For example, in an acetate-to-isobutanol pathway, ketol-acid reductoisomerase IlvC and alcohol dehydrogenase YqhD require NADPH as a cofactor. Accordingly, FDH (catalyzing NADH generation from formate) together with PntAB was introduced, and supplementation of the medium with 5 mmol/L formate increased isobutanol titer in the engineered strain by 7% relative to the control strain [52].

MESs are built on electron transfer between microorganisms and the extracellular environment to enable the production of target chemicals from cost-effective substrates [107]. For example, synthesis of one molecule of a C18 fatty alcohol from acetyl-CoA requires the consumption of 18 NAD(P)H equivalents. To supplement the NAD(P)H demand for fatty alcohol biosynthesis from acetate, a MES was designed. Specifically, the electrode was used as the electron donor, and neutral red was employed as an electron shuttle to facilitate electron transfer. The intracellular NADPH/NADP$^+$ and NADH/NAD$^+$ ratios were increased by 2.79-fold and 2.73-fold, respectively, thereby elevating fatty alcohol production by 6.17, fold−83.8 mg/g DCW [55]. However, the addition of electron shuttles may lead to contamination of the culture medium. Moreover, because of their short lifetime and susceptibility to dissipation, continuous supplementation is required to maintain system operation, which is unfavorable for the practical application of MES. To address this challenge, genetic engineering of microbial strains can be performed to enable direct electron uptake, thereby avoiding the need for electron shuttle addition. The main strategies include the heterologous expression of electron transfer proteins, such as the MtrABC electron transfer conduit from *Shewanella oneidensis* [108] and the OmcS protein from *Geobacter* [109], enhancement of cell membrane fluidity [110], reconstruction of the periplasmic cytochrome protein network [111], and biofilm engineering to increase cell loading on the electrode surface [112]. For example, overexpression of the *Geobacter*-derived OmcS protein in *Synechococcus elongatus* increased the affinity between electrons and the cell membrane, thereby promoting intracellular electron uptake. As a result, the faradaic efficiency of the engineered strain was increased to 23.3% [109].

5.5. Pathway coordination optimization

Pathway coordination optimization is aimed at achieving efficient, stable, and controllable synthesis of target products by balancing and regulating individual steps within a metabolic pathway. The major strategies include modular engineering, the design of dynamic regulatory systems, and metabolic division of labor (Fig. 6E).

In modular engineering, the product biosynthetic pathway is partitioned into several relatively independent units that are optimized individually and then integrated, thereby improving overall cellular performance [113]. For example, to enable efficient conversion of acetate to threonine, modular engineering was applied to coordinately optimize the acetate assimilation pathway, TCA cycle flux, the glyoxylate shunt, and pantothenate kinase activity. The engineered strain produced 45.8 g/L threonine within 52 h with a yield of 0.22 mol/mol, representing increases of 21.2% and 15.8%, respectively, over the control strain [88].

Unlike modular engineering, dynamic regulatory systems are focused on balancing metabolic flux by decoupling cellular

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growth from production, thereby improving strain performance. As a representative example, during 3-HP production, expression of succinate dehydrogenase (SDH) was controlled by a temperature-sensitive P_R promoter: at 39 °C, SDH expression was maintained and metabolic flux was directed toward growth, whereas at 25 °C, SDH expression was repressed and flux was redirected toward product biosynthesis. As a result, 3-HP production in the engineered strain was increased to 3.92 g/L [114].

Compared with the two aforementioned strategies that emphasize optimization within a single cell, a division-of-labor strategy based on co-culture construction provides a means to regulate cell–cell interactions. This strategy is advantageous because interstrain interactions can reduce the protein expression burden and resource competition within any single strain. For example, to enable efficient acetate bioconversion, a *Rhodotorula glutinis–Chlorella vulgaris* co-culture was constructed. In this system, CO_2 generated by *R. glutinis* metabolism was utilized by *C. vulgaris* to drive photosynthesis and enhance lipid synthesis, whereas O_2 produced via photosynthesis by *C. vulgaris* was consumed by *R. glutinis* to promote cell growth and lipid accumulation. In a 5 L fermenter with acetate as the substrate, 6.9 g/L biomass and 2.6 g/L lipids were accumulated by this system [47].

6. Conclusions and perspectives

In conclusion, the application potential of acetate as a carbon feedstock for industrial microorganisms was comprehensively outlined from four perspectives, namely acetate-utilizing microorganisms, strategies for enhancing acetate assimilation, approaches for improving tolerance to high acetate concentrations, and metabolic engineering for acetate-to-chemical conversion. Although progress has been achieved in acetate-based biosynthesis of chemicals, several issues remain to be addressed: (1) low pathway efficiency for target-product formation, resulting in poor yields; (2) sufficient energy supply is required for both acetate assimilation and the biosynthesis of chemicals; and (3) efficient genetic editing toolkits are lacking for non-model microorganisms.

To construct highly efficient product-biosynthesis pathways, future efforts could be pursued along three directions: (1) genome-scale metabolic network models could be introduced to enable systematic analyses of flux distributions and regulatory bottlenecks [115]; (2) integrated multi-omics datasets (e.g., transcriptomics, metabolomics, and proteomics) could be leveraged for cross-comparative interrogation to identify high-value engineering targets [116]; and (3) intelligent dynamic control strategies could be designed on the basis of intracellular metabolite signals and quorum-sensing circuits to achieve fine-tuned regulation of metabolic pathways [117].

To construct an efficient energy-regeneration system that facilitates energy supply, subsequent research can be advanced in two aspects: (1) the construction of biomimetic photosynthetic systems. Redundant components are to be eliminated and minimal photosynthetic units with essential functions are to be engineered based on the principles of natural photosynthesis [118]; semiconductor nanomaterials for light capture are to be integrated with microorganisms in a highly biocompatible manner to form artificial hybrid photosynthetic systems [119–121]. (2) The design of novel electrically driven systems. New electrode materials are to be developed to enhance charge transport, catalytic activity, and stability [122]; solid-state electrolytes and renewable biomass-based electrolytes are to be introduced to improve system selectivity and durability [123,124]; and the coupling of photo/electrocatalysis is to be promoted to increase energy-conversion efficiency, thereby facilitating the efficient synthesis of target products [125].

To design high-performance genome-editing tools for extending the applicability of non-model microorganisms, future studies may focus on two avenues: (1) Recombinase-mediated genome editing methods are to be designed based on recombinases, enabling site-specific, marker-free, and efficient integration of target gene expression cassettes into the chromosome [126]; (2) Biosensor-assisted CRISPR interference systems are to be constructed based on CRISPR-Cas technology, coupled with mismatched sgRNA libraries, to achieve simultaneous regulation of the expression levels of multiple target genes [127].

CRediT authorship contribution statement

Guangjie Liang: Writing − original draft, Methodology, Conceptualization. **Kaifang Liu:** Software, Methodology. **Cong Gao:** Validation, Investigation. **Qi Sheng:** Visualization, Resources. **Gan Qiao:** Software, Formal analysis. **Jia Liu:** Visualization, Validation. **Guipeng Hu:** Formal analysis, Investigation, Methodology. **Jens Nielsen:** Conceptualization, Project administration. **Liming Liu:** Writing − review & editing, Funding acquisition.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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