Engineering Microbiology 2 (2022) 100036

Contents lists available at ScienceDirect

Engineering Microbiology

journal homepage: www.elsevier.com/locate/engmic

Review

Metabolic engineering using acetate as a promising building block for the production of bio-based chemicals

Guiping Gong[#,*], Bo Wu[#], Linpei Liu, Jianting Li, Qili Zhu, Mingxiong He, Guoquan Hu

Biomass Energy Technology Research Centre, Key Laboratory of Development and Application of Rural Renewable Energy (Ministry of Agriculture and Rural Affairs), Biogas Institute of Ministry of Agriculture and Rural Affairs, Chengdu 610041, China

ARTICLE INFO

Keywords:
Acetate
Microbial cell factories
Metabolic engineering
Bio-based chemicals

ABSTRACT

The production of biofuels and biochemicals derived from microbial fermentation has received a lot of attention and interest in light of concerns about the depletion of fossil fuel resources and climatic degeneration. However, the economic viability of feedstocks for biological conversion remains a barrier, urging researchers to develop renewable and sustainable low-cost carbon sources for future bioindustries. Owing to the numerous advantages, acetate has been regarded as a promising feedstock targeting the production of acetyl-CoA-derived chemicals. This review aims to highlight the potential of acetate as a building block in industrial biotechnology for the production of bio-based chemicals with metabolic engineering. Different alternative approaches and routes comprised of lignocellulosic biomass, waste streams, and C1 gas for acetate generation are briefly described and evaluated. Then, a thorough explanation of the metabolic pathway for biotechnological acetate conversion, cellular transport, and toxin tolerance is described. Particularly, current developments in metabolic engineering of the manufacture of biochemicals from acetate are summarized in detail, with various microbial cell factories and strategies proposed to improve acetate assimilation and enhance product formation. Challenges and future development for acetate generation and assimilation as well as chemicals production from acetate is eventually shown. This review provides an overview of the current status of acetate utilization and proves the great potential of acetate with metabolic engineering in industrial biotechnology.

1. Introduction

The energy crisis and environmental pollution have triggered and accelerated the development of green and sustainable production of biofuels and biochemicals [1]. An appealing technique with the benefits of environmental friendliness and fewer by-products is a biological conversion with microbial fermentation for the production of chemicals [2,3]. However, the cost of fermentation substrates is still one of the main limiting bottlenecks for the development of industrial fermentation processes, which facilitates the research of low-cost, sustainable, and alternative feedstocks in future biorefineries [4,5].

Acetate, as a typical two-carbon molecule, is the ideal and promising feedstock for the production of biochemicals from various microorganisms. Exploration and use of inexpensive substrates are crucial because the cost of the carbon source accounts for 50%–70% of the overall expenditures for microbial operations [1]. Compared with glucose or other conventional carbon sources, acetate (300–450 $ per ton) with a demand of over 18 million tons (by 2020) is less expensive than glucose (500 $ per ton) [6], and the assimilation pathway of acetate as a carbon source is much more effective. Acetate can be converted to acetyl-CoA, a critical intermediate metabolite, by one (ACS, acetyl-CoA synthetase, EC 6.2.1.1) or two steps (AK, acetate kinase, EC 2.7.2.1 and PTA, phosphotransacetylase, EC 2.3.1.8) of catalysis and subsequently participates in the subsequent metabolic pathways and biological processes for cell development and product creation [7]. This suggests that the conversion of acetate only had one or two rate-limiting steps, whereas the conversion of glucose has a long conversion route and numerous rate-limiting processes, which is not advantageous in terms of atom economy and energy consumption. Furthermore, acetate could be derived both from biological and chemical methods, including lignocellulosic biomass hydrolysis (LBH), anaerobic digestion (AD), syngas fermentation (SF), microbial electrosynthesis (MES), and chemical catalysis (CC) [6–8], implying that acetate could be used as a possible building block for biochemicals production. In particular, these acetate generation routes could depend on the waste streams (LBH and AD could use waste lignocellulosic biomass, industrial or agricultural wastes as substrates) and C1 gas conversion (like SF, MES and CC use CO_2 as raw material), which shows enor-

* Corresponding author.
E-mail address: gongguiping@caas.cn (G. Gong).
# These authors contributed equally to this work.

https://doi.org/10.1016/j.engmic.2022.100036
Received 23 May 2022; Received in revised form 13 July 2022; Accepted 13 July 2022
Available online 26 July 2022

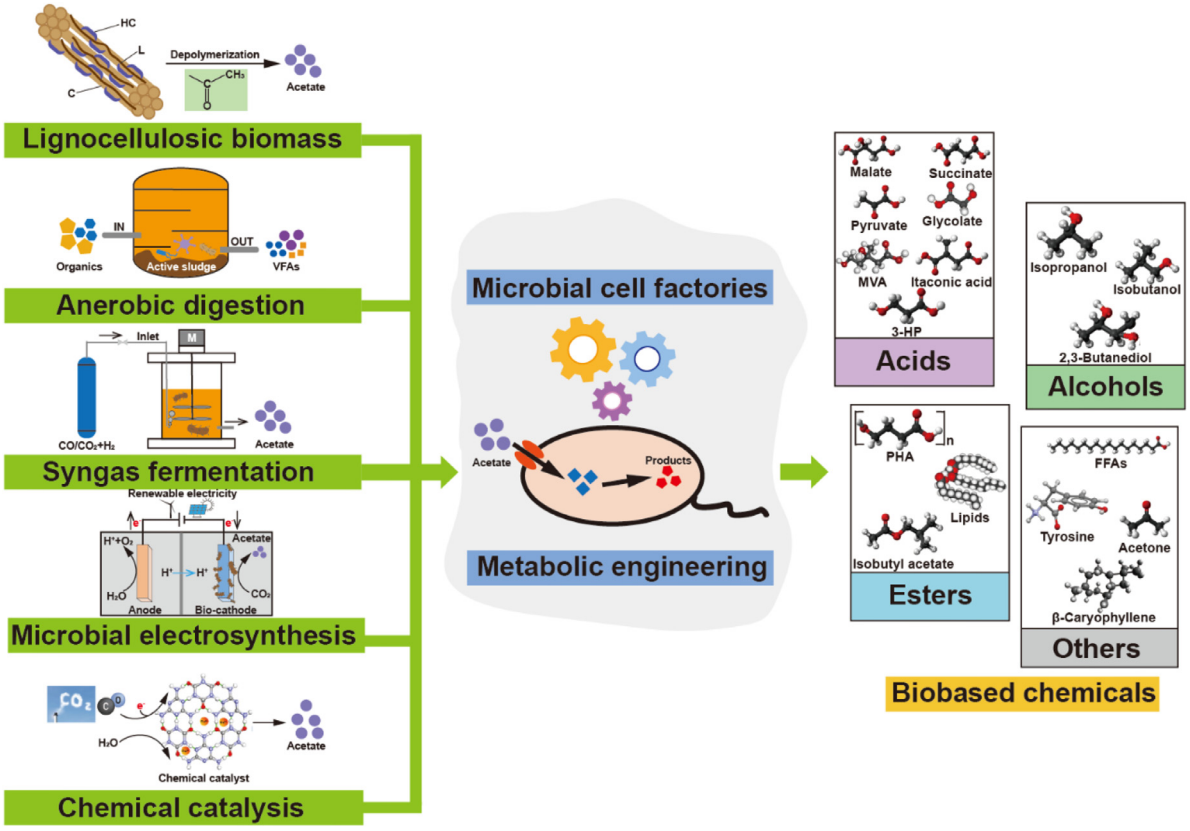

Fig. 1. Graphical representation of acetate generation approaches and bio-based chemicals production from acetate. C: cellulose; HC: hemicellulose; L: lignin; MVA: mevalonate; PHA: polyhydroxyalkanoates; 3-HP: 3-hydroxypropionic acid; FFAs: free fatty acids.

mous potential in eco-friendliness and sustainability for carbon neutrality nowadays.

This review focuses on the metabolic engineering of microorganisms with acetate as a carbon source for the production of bio-based chemicals. At first, different promising approaches and routes for acetate generation (both biological and chemical methods) are described (Fig. 1). Secondly, recent advances in using acetate as a feedstock to produce various bio-based chemicals (such as acids, alcohols, esters, and other chemicals) by metabolic engineering are summarized. Finally, the challenges and future perspectives for acetate generation, utilization, and conversion are also presented. This review provides a systematic and comprehensive overview of the current research progress of using acetate as a potential building block for bio-based chemical production.

2. Different approaches for acetate generation

Acetate synthesis has been studied for a very long time and originated from the manufacturing of vinegar in the food sectors [9]. For the traditional production of vinegar, the whole process could be generally divided into two stages: alcoholic fermentation of sugars from corns by yeasts and acetate generation from incomplete oxidation by acetic acid bacteria [6,9]. Acetic acid bacteria represent a group of strictly aerobic bacteria, which are capable of converting sugars, ethanol, or alcohols to acetic acid as an end-product [10]. Several methods from alternative sources have been used to produce acetate for industrial conversion in a sustainable manner (Fig. 1). AD and lignocellulosic biomass depolymerization are two potential methods for producing acetate while recovering energy from waste streams. Another alternative method of producing acetate is based on the utilization of abundant and low-cost C1 gas, such as CO and CO_2, which is highly interesting and attractive for waste capture and reduction of greenhouse gas emission in recent years [6,8,11]. The potential approaches for the conversion of C1 gas into acetate mainly consist of SF, MES, and CC. Significant development has already been made in acetate-producing technology using both biological and chemical approaches, as previously stated. This section summarizes and describes the different acetate manufacturing routes and approaches that are both affordable and environmentally friendly, as well as the obstacles and challenges associated with each process.

2.1. Lignocellulosic biomass depolymerization

Lignocellulosic biomass is the most abundant renewable resource in the world, with a composition of cellulose, hemicellulose, and lignin, and has been recognized as the most potential and alternative raw material for the production of biofuels and biochemicals [12]. Due to the limitation of the compact structure of lignocellulosic biomass, pretreatment of lignocellulosic biomass (like dilute acid or alkaline treatment) is essential to disrupt the crystallization structure for the release of sugars (C6 and C5) [12]. Acetate is primarily produced during the pretreatment process as a result of the breakdown of acetylated hemicelluloses under extremely strict pretreatment conditions (high temperature, acidic or alkaline conditions), and has historically been thought of as a by-product that is toxic to cells as a major inhibitor present in lignocellulosic hydrolysates during the fermentation process [12,13]. According to the biomass type and pretreatment method, it was reported that the concentration of generated acetate during the pretreatment process ranged from 1 to 18 g/L. This made it a good idea to simultaneously convert C6/C5 sugars and acetate to produce chemicals using lignocellulosic hydrolysates as substrates [8]. However, acetate cannot be assimilated by a wide range of microorganisms, and high acetate concentrations constantly hinder cell development, which is the principal barrier to acetate consumption. To solve these problems, a lot of researches were engaged in the development of acetate-utilized microbial cell factories through metabolic engineering (heterologous expression of key genes for ac-

etate utilization) and screening of tolerance strains (such as random mutagenesis and laboratory adaptation evolution) [6,7,14,15]. Moreover, considering the techno-economic feasibility, using waste lignocellulosic biomass as the raw material to obtain biomass-derived green acetate could be a promising alternative to achieve the aim of waste disposal.

2.2. Anaerobic digestion

AD has been proven to be a successful and sustainable method for the recovery of renewable energy using a variety of organic streams (industrial, agricultural, and rural waste streams) as substrates. In typical AD, the process could be divided into four stages, including hydrolysis (stage I), acidogenesis (stage II), acetogenesis (stage III), and methanogenesis (stage IV) [16,17]. In the first stage, complicated macromolecules (such as cellulose, proteins, polysaccharides, and lipids) and insoluble organics are degraded into micromolecules (simple sugars, peptides, amino acids, fatty acids, and glycerol) and soluble organics, catalyzed by exoenzymes secreted by some anaerobic fermentative microbes, which provides essential carbon source and nutrient substance, and then converted to volatile fatty acids, such as acetate, butyrate, propionate, lactate, and other weak acids, thus making the products as a mixture of organic acids [18]. During the acetogenesis stage, acetate, ammonia, and H_2 are generated by hydrogen-producing acetogenic bacteria and it was reported that the concentration of acetate produced by AD is up to 15 g/L [8,17]. When using AD to generate acetate, several issues and factors should be considered as follows: (1) a mixture of various short-chain organic acids is generated during the AD process, and the composition, as well as the individual toxicity for each acid, should be investigated when using as substrates; (2) the generation rate of acetate from AD is relatively slow and the process of acetogenesis and methanogenesis commonly simultaneously happens, resulting in difficulty and obstacle for the collection of acetate from AD.

2.3. Syngas fermentation

Industrially available gaseous substrates in significant quantities are promising sources for acetate synthesis in addition to acetate produced from biomass or waste streams. Syngas has attracted a lot of attention as a feedstock for the creation of biofuels and biochemicals since it is a mixture of CO, CO_2, and H_2 and is mostly obtained from industrial waste gas or the gasification of waste streams and lignocellulosic biomass [19,20]. Acetogenic bacteria, also called acetogens, are anaerobic organisms that have recently gained the interest of researchers due to their ability to fix CO or CO_2 through the reductive acetyl-CoA pathway (Wood-Ljungdahl pathway) [20]. The function of the Wood-Ljungdahl pathway includes: (1) reduction of CO/CO_2 to form acetyl-CoA; (2) CO_2 fixation into intracellular; (3) energy transformation or storage. In the pathway, CO or CO_2 is converted to the intermediate metabolite, acetyl-CoA, and then forms acetate through the ACK-PTA pathway. The reaction of acetate generation during anaerobic cultivation provides ATP for cell growth by substrate-level phosphorylation, which is crucial for cells grown on C1 compounds. Some studies have employed industrial CO_2 or syngas as substrates to produce acetate and demonstrated the possibility of generating high-concentration acetate from syngas with a concentration of 4–60 g/L [8,21,22]. Therefore, using low-cost industrial C1 gas for the production of acetate seems an alternative and attractive route in the future, which is promising both in contaminants disposal and energy transformation.

One of the hottest areas of research in the fields of metabolic engineering and synthetic biology right now is synthetic microbial consortia, also known as artificial synthetic consortia. Compared with monoculture, synthetic microbial consortia showed superior advantages in terms of reducing metabolic burden, impairing feedback inhibition, and enhancing substrate conversion [23,24]. Due to the toxicity of syngas to the majority of microbes and the difficulty in genetically modifying acetogens, microbial consortia, with synergetic and mutualistic interaction, is a potential alternative for chemicals production from SF. These consortia have been designed for the production of various chemicals, such as 3-hydroxypropionic acid, itaconic acid, and butyrate [25,26]. For instance, a mutualistic microbial consortium was constructed by the cocultivation of *Eubacterium limosum* and genetically engineered *Escherichia coli* [25]. In the consortia, *E. limosum*, as a CO-assimilating acetogen, was responsible for converting CO to acetate, while the engineered *E. coli* utilized the generated acetate as a carbon source for the synthesis of 3-hydroxypropionic acid and itaconic acid. The authors found that the mutualistic interaction between these two microbes in the consortium was beneficial for the stability of CO consumption as well as the successful production of chemicals [25]. These studies revealed that microbial consortia are an alternative powerful and promising platform for the efficient conversion of syngas to produce value-added biochemicals.

2.4. Microbial electrosynthesis

MES is a novel and versatile option for the conversion and recycling of CO_2 to form acetate and has drawn extensive attention due to the great potential of surplus electrical energy storage and carbon neutrality in waste biorefineries [27,28]. To produce acetate, the MES system primarily consists of an anode and a bio-cathode that are separated by a proton or ion exchange membrane [28]. The oxidation reaction of H_2O occurs in the area of the anode and provides electrons for the reaction in the cathode. In the cathode, microbial catalysts (electrochemically active bacteria) accept the electron from the anode as reducing power thus producing acetate with CO_2 as substrate and the highest concentration of acetate was up to 10 g/L [29]. Generally, pure acetogenic microorganisms or a mixture of microbial communities were applied to trigger electroacetogenesis while the mixed consortia showed superior advantages with higher biomass concentration and acetate production rates as well as easier for long-term operation [30]. However, several challenges in both the economic and technological view are required to be solved before the scale-up of the MES system. The most crucial element that must be assessed for commercial large-scale implementations of MES is its economic viability. Technological challenges include anode modification with higher electronic conductivity; renewable energy supply for driving the electron transfer; construction of microbial communities for efficient chemolithoautotrophy growth and CO_2 reduction [30,31].

Recent studies for acetate generation with MES are focused on the direct supplement of electrons to acetogens by photocatalysis. A hybrid system containing acetogens and semiconducting nanoparticles was constructed to generate acetate from CO_2 with nanoparticles providing the photogenerated electrons, and the maximum yield reached almost 90% [32]. Therefore, a potential and appealing technique for MES in the future could be the coupled microbial synthesis-photocatalysis for acetate formation from CO_2 reduction.

2.5. Chemical catalysis

With the fast development of CO_2 capture or immobilization technologies from the air, the conversion of CO_2 to produce acetate by CC becomes a feasible solution. Researchers discovered over the past few decades that acetate could be created directly from the reaction of CH_4 and CO_2, despite the fact that a higher energy barrier had to be overcome to start this reaction since CH_4 and CO_2 are inactive and thermostable [33]. Inspired by natural photosynthesis, metal-organic frameworks or semiconductor hybrid materials were utilized as catalysts for the synthesis of acetate from CO_2. Yu et al., 2021 [34] prepared a hierarchically porous composite nanomaterial (d-UiO-66/MoS) for the reduction of CO_2 to form acetate through photocatalysis with a reduced energy barrier. The prepared nanomaterial realized the direct photocatalytic conversion of CO_2 and H_2O to produce acetate under visible light irradiation conditions (39.0 μmol g^{-1} h^{-1}). This study provides an innovative approach for the photolysis of H_2O to generate acetate without the addition of any other electron sacrifice agent or hydrogen

source. In addition, electrochemical CO_2 reduction and conversion is another promising strategy for upcycling CO_2 into value-added compounds [27,35]. Based on the developed electrocatalysts and powered by renewable electricity, highly selective acetate synthesis might be accomplished by an electrochemical CO_2 reduction reaction. In a recent study, through coupling CO_2 electrolysis with yeast fermentation, a hybrid electro-biosystem was assembled and reported to efficiently converts CO_2 to long-chain products, such as glucose and free fatty acids [36]. In this system, CO_2 was first converted to pure acetic acid electrochemically catalyzed by a nanostructured copper catalyst in the solid-electrolyte reactor (resulting in a concentration of 31.05 g/L acetate), which provides the electrogenerated acetic acid as a carbon source for chemicals synthesis from genetically engineered *Saccharomyces cerevisiae*, suggesting a fascinating alternative for manufacturing industry driven by renewable energy. Although these studies have demonstrated the possibility of acetate production from CO_2 by CC, there are still some challenges and issues that remained to be addressed, for instance, improving the catalytic efficiency of electrocatalysts, promoting CO_2 capture and immobilization technologies, and taking into account CO_2 reduction under mild conditions are all necessary.

3. Acetate assimilation and conversion

3.1. Acetate transport and tolerance

The entire process of utilizing and metabolizing acetate to produce biochemicals may be broken down into three steps: acetate transport (from the external environment to the intracellular space), acetate assimilation (from acetate to acetyl-CoA), and chemicals formation (from acetyl-CoA to products) [7]. As acetate is highly miscible and completely ionized in the medium, it's convenient and easy for mass transfer into the broth. Acetate in the medium is mostly transferred across the cell membrane through passive and active transport into the intracellular. For passive transport, an undissociated acetate molecule is transported into the cells driven by the concentration gradient or potential difference across the membrane without the consumption of energy. During active transport, symporters are responsible for acetate transport, such as the sodium:solute symporter, acetate permease (ActP), and the H^+/monocarboxylic acid symporter (PMCT) [7,37]. Acetate transport plays a crucial role in acetate assimilation and conversion because we always expect acetate could be transported quicker and faster, which is helpful for the scale-up and industrialization of chemical products based on acetate.

In general, even at concentrations lower than 5 g/L, acetic acid is toxic to microbial cells [7]. Acetate is easily transported through the cell membrane because of its small size through simple diffusion into the cytoplasm, with both an anion and a proton acting as toxic elements. There were several hypotheses for the inhibition effect of acetate on cell growth [6,7,13], including (1) uncoupling: when the pH value of the culture medium is lower than the pKa, acetate exists in the form of free undissociated molecule, which could penetrate into cell membrane through simple diffusion, resulting in the potential difference across the membrane thus causing cell death; (2) anion imbalance: the weak acid dissociates intracellularly and proton is pumped into outer environment, while acetate accumulates thus inhibiting cell growth; (3) membrane permeability and integrity: it was found that organic acids destroy cell membrane permeability in *E.coli* and *S. cerevisiae* [13,38], and demonstrated that the undissociated or free form of organic acids are the main inhibition factor; (4) amino acid metabolism: it was proposed that weak acids inhibit the utilization of aromatic amino acids and the synthesis of methionine in some microorganisms [7], which accumulates the toxic cysteine in cells; (5) programmed cell death: acetate with high- concentration induces reactive oxygen species accumulation and the energy metabolism in mitochondria was impaired, which leads to the imbalance of cell homeostasis and denaturation of chromatin and nuclear DNA structure, resulting in the programmed cell death [13]. Microbes have evolved several mechanisms for acetate defense, which could be classified into five categories [7]: (1) rapid acetate metabolism; (2) acetate export; (3) amino acid decarboxylation or protonation; (4) change of cell morphology or membrane composition; (5) expression of stress-response genes or stress-signaling pathways. These approaches are all aimed to reduce intracellular acidification or limit acetate uptake to relieve acetate inhibition.

To solve the acid inhibition, various solutions were also applied to enhance acid tolerance, such as adaptive laboratory evolution, directed mutagenesis, and metabolic engineering or synthetic biology methods [13,39]. Adaptive laboratory evolution has been recognized as an efficient and powerful approach for improving stress tolerance capacity (such as organic acids, lignocellulosic hydrolysate inhibitors, high temperature, and low pH) in strain engineering. With the emergence of omics analysis and sequencing, the combination of adaptive laboratory evolution with reverse engineering provides a more reliable methodology to study tolerance mechanisms and acid-resistant strains. Additionally, with the rapid advancement of synthetic biology and metabolic engineering, it is now possible to create strains quickly and precisely with specified characteristics by manipulating genes or pathways. This accelerates research on strain creation and stress tolerance.

3.2. Acetate assimilation and metabolism

When using acetate as the only carbon source, acetate is first transformed to acetyl-CoA and then assimilated primarily by two pathways, the ACS pathway and the ACK-PTA pathway (Fig. 2), which are encoded by the enzymes: acetate kinase-phosphotransacetylase (ACKA-PTA) and acetyl-CoA synthetase, respectively [6,7]. ACS could irreversibly convert acetate to acetyl-CoA; however, ACKA-PTA is responsible for a reversible two-step route to generate acetyl-CoA. Acetate is initially activated to create the intermediate acetyl-adenosine monophosphate (acetyl-AMP), which is subsequently converted to acetyl-CoA during the assimilation phase for the ACS pathway. For the ACK-PTA pathway, acetylphosphate, as the intermediate, is first generated with the catalysis of ACKA and further converted to acetyl-CoA by PTA. ATP is necessarily required during the process of acetate activation both for the ACS and ACK-PTA pathways. The difference is that only 1 mol ATP is consumed for the ACK-PTA pathway while ACS requires 2 mol ATP due to the formation of AMP and ADP (AMP is further converted to ADP via 1 mol ATP consumption), indicating that the ACS pathway requires more energy than the ACKA-PTA pathway. However, the ACK-PTA pathway is an acetate uptake system with low affinity, whereas the ACS pathway has been demonstrated with approximately 35-fold higher affinity [6], which proved the ACS pathway is a more effective route for acetate utilization and the ACK-PTA pathway is physiologically more important for acetate generation rather than the consumption. Moreover, succinyl-CoA:acetate CoA-transferase (SCACT, EC 2.8.3.18) catalyzes the conversion of acetate to acetyl-CoA in some microbes (Fig. 2). SCACT is responsible for providing CoA from succinyl-CoA to acetate to form acetyl-CoA and succinate. It was reported that, an *E. coli* strain lacking both ACS and ACKA-PTA may grow on a medium containing acetate as a carbon source via heterologous production of SCACT [40]. Therefore, the SCACT pathway is an important substitute or supplement other than ACS or ACK-PTA for acetate assimilation.

As a precursor for carbon length extension, acetyl-CoA produced from acetate enters the tricarboxylic acid (TCA) cycle or glyoxylate shunt (from C2 to C4). It was reported that the glyoxylate shunt has a great effect on cell growth with acetate as a carbon source [7]. In the glyoxylate shunt, isocitrate is split into glyoxylate and succinate by isocitrate lyase (ICL), and glyoxylate is then converted to malate with another acetyl-CoA molecule as a reactant. Succinate and malate participate in the TCA cycle and are further oxidized to form fumarate and OAA, which contribute to energy production and higher carbon compound synthesis. Among many microorganisms, the enzymes involved in the glyoxylate shunt are negatively or positively regulated by *IclR* and

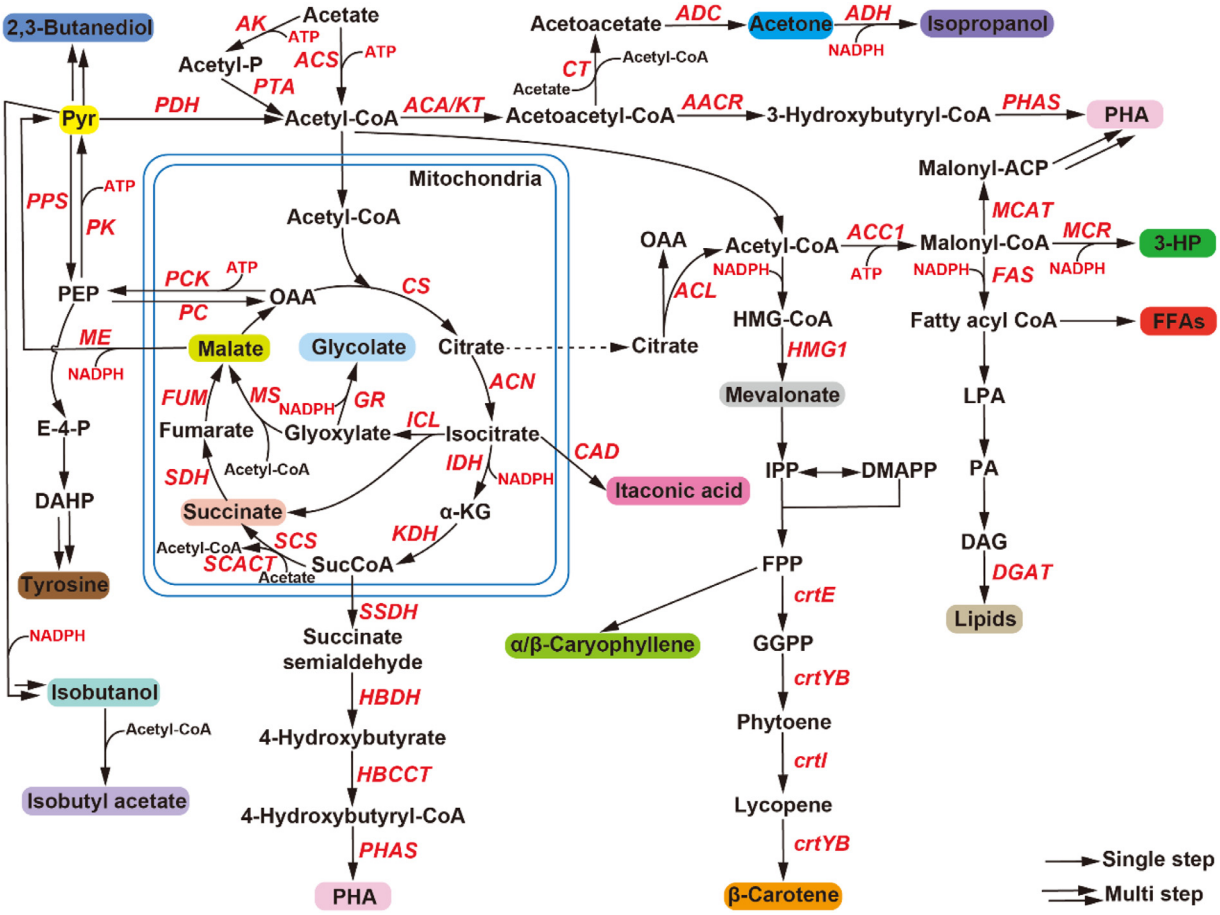

Fig. 2. Metabolic pathway and key genes involved in the production of bio-based chemicals from acetate. AK: acetate kinase; ACS: acetyl-CoA synthase; PTA: phosphotransacetylase; KT: *β*-ketothiolase; AACR: acetoacetyl-CoA reductase; PHAS: PHA synthase; IDH: SSDH: succinate semialdehyde dehydrogenase; HBDH: 4-hydroxybutyrate dehydrogenase; HBCCT: 4-hydroxybutyryl-CoA:CoA-transferase; ICL: isocitrate lyase; MS: malate synthetase; CS: citrate synthase; ACN: aconitase; KDH: *α*-ketoglutarate dehydrogenase SCS: succinyl-CoA synthetase; SDH: succinate dehydrogenase; FUM: fumarase; MDH: malate dehydrogenase; CAD: cis-aconitate decarboxylase; ME: NADP-dependent malic enzyme; PCK: phosphoenolpyruvate carboxykinase; PC: phosphoenolpyruvate carboxylase; PPS: phosphoenolpyruvate synthase; PK: pyruvate kinase; PDH: pyruvate dehydrogenase; GR: glyoxylate reductase; ADC: acetoacetate decarboxylase; CT: coenzyme A transferase; ACA: acetyl-CoA acetyltransferase; ADH: alcohol dehydrogenase; SCACT: succinyl-CoA:acetate CoA-transferase; MCAT: malonyl-CoA-ACP transacylase; ACC1: acetyl-CoA carboxylase; HMG1: 3-hydroxy-3-methylglutaryl-CoA reductase; crtE: geranylgeranyl diphosphate synthase; crtYB: phytoene synthase; crtI: phytoene desaturase; ACL: ATP citrate lyase; DGAT: acyl-CoA: diacylglycerol acyltransferase; FAS: fatty acyl-CoA synthetases; MCR: malonyl-CoA reductase; Pyr:pyruvate; PEP: phosphoenolpyruvate; E-4-P: erythrose 4-phosphate; DAHP: 3-deoxy-D-arabinoheptulosonate-7-phosphate; OAA: oxaloacetate; *α*-KG: *α*-ketoglutarate; SucCoA: succinyl-CoA; HMG-CoA: 3-hydroxy-3-methyl glutaryl CoA; IPP: isopentenyl pyrophosphate; DMAPP: Dimethylallyl pyrophosphate; FPP: farnesyl pyrophosphate; GGPP: geranylgeranyl diphosphate; LPA: lysophosphatidic acid; PA: phosphatidic acid; DAG: diacylglycerol.

FadR, suggesting alternative candidates and targets for the regulation of the glyoxylate shunt [7,41]. In the TCA cycle, the reaction from isocitrate to *α*-ketoglutarate, catalyzed by isocitrate dehydrogenase (IDH), is an important node, which competes with ICL and influences the carbon flux distribution. However, due to the creation of energy and the movement of carbon molecules, the glyoxylate shunt and TCA cycle are both critical for acetate absorption [42]. For the majority of acetate-consuming bacteria, acetate is a less favored carbon source when generating NADPH and ATP than glucose. According to reports, acetate only produces 10 ATPs per 1 mol, compared to 38 ATPs per mol for glucose [14]. Therefore, one of the factors limiting acetate absorption and cell development may be its low energy content. More importantly, as most chemicals synthesized from acetate requires ATP or NADPH (shown in Fig. 2), efficient energy supplement by metabolic engineering or other methods is crucial for the assimilation of acetate and biochemical production.

For the biological production of chemicals from acetate, the ability to grow with acetate as a carbon source and efficient conversion of acetate for microbes is required. Various potential microbes, both for natural acetate consumers or genetically modified microbes, have been reported to utilize acetate, including *E. coli, Corynebacterium glutamicum, Pseudomonas putida, S. cerevisiae, Cryptococcus curvatus, Rhodotorula glutinis, Yarrowia lipolytica*, and *Aspergillus oryzae* [43–50], etc. The following traits are necessary for strains to produce biochemicals from acetate effectively: (1) higher tolerance to acetate; (2) enhanced activation of acetate to acetyl-CoA; (3) improvement of acetate assimilation; (4) effective production of chemicals. In recent years, a variety of microbes were metabolically engineered to produce biochemicals with acetate as a carbon source, including acids, alcohols, esters, and other chemicals (Fig. 2).

4. Metabolic engineering for the production of various biochemicals from acetate

Due to the toxicity and inhibition of acetate on cell growth for the majority of microbes, the utilization of high-concentration acetate for the production of biochemicals was still a huge challenge. In most studies, pure acetic acid was not frequently used as a carbon source because of the low pH and strong inhibition, and acetate in turn was generally applied as a carbon source, such as sodium acetate and ammonium ac-

etate. When utilizing pure acetic acid as a carbon source, it's essential to adjust the pH of the medium, therefore, acetic acid was converted to acetate for utilization. On account of the low energy content of acetate as well as the requirement of ATP during acetate assimilation, many studies applied glucose (or other carbon sources) and acetate as cosubstrates to supply more energy and reduce power. Several general strategies involved in strain engineering and fermentation mode were also used in these studies to alleviate acetate inhibition and improve acetate utilization. Overexpression of *acs* or *ackA-pta* (or both *acs-ackA-pta*) as well as cofactor engineering (ATP supplement and NADPH regeneration) was a commonly used option. Furthermore, many studies used various fermentation strategies to improve utilization efficiencies, such as continuous/repetitive feeding of acetate, pH-coupled fed-batch fermentation, two-stage cultivation (glucose for cell growth, and acetate added after the depletion of glucose for products synthesis), high cell density strategy, and preculture-sequencing batch culture. All the strategies were aimed to improve the utilization efficiency of acetate thus increasing biochemicals productivity from acetate. This section detailed various metabolic engineering strategies and recent advances in the microbial conversion of acetate to various biochemical production.

4.1. Acids

4.1.1. Malate

Malate (malic acid or 2-hydroxybutanedioic acid), as a C4 dicarboxylic acid, has been widely applied in food, pharmaceutical, and chemical industries and classified as one of the top 12 building block chemicals by the US Department of Energy with a market volume exceeding 400,000 tons per year [51]. Furthermore, as an intermediate metabolite in the TCA cycle, malate plays an important role in amino acid synthesis. Several mature approaches have been developed for malate production, including CC, enzymatic synthesis, and biological fermentation [51–53]. Compared with CC or enzymatic synthesis, biological fermentation for malate production attracted considerable interest because pure L-malate could be generated with sustainable and low-cost feedstocks. In recent years, metabolic engineering approaches, as well as process optimization used in some models, strains for malate production, primarily with starch or sugars as substrates. However, few studies have reported the use of acetate for the production of malate. Some filamentous fungi, such as *Aspergillus, Ustilago,* and *Rhizopus* [51], have been regarded as efficient natural malate producer through the TCA cycle or the glyoxylate shunt pathway and have the ability of acetate assimilation. Kövilein et al., 2021 [50] assessed the potential of acetate as a carbon source for producing malate with A. oryzae DSM 1863 in a recent study. With 45 g/L of acetic acid as the sole carbon source, a concentration of 8.44 g/L of malate was obtained [50], and the titer and productivity of malate were further improved by the addition of 15 g/L glucose to acetate medium as a cosubstrate (Table 1), resulting in a production of 14.18 g/L malate [50], demonstrating the potential of acetate as a carbon source to produce malate.

4.1.2. Succinate

Succinate, also named succinic acid or butanedioic acid, occurs in the TCA cycle and is classified as one of the top 12 building block chemicals by the US Department of Energy [54]. Because of its numerous applications in the food, agricultural, pharmaceutical, and chemical industries, succinate has a high market demand as a valuable bulk and platform chemical, particularly as a precursor to biodegradable polyesters (PBAT or PBS) [54,55]. Succinate is traditionally produced by petrochemical synthesis but with the concerns of limited fossil resources, environmental issues, and emissions of CO_2 [54]. To achieve sustainable succinate production while lowering costs, biological succinate production by fermentation from renewable resources has enormous potential due to environmental friendliness, high conversion yield, and low-cost, which is competitive with traditional petrochemical synthesis [55]. Succinate biosynthesis has used acetate as a nontraditional carbon source. As shown in Fig. 2, succinate could be generated both by the TCA cycle and the glyoxylate shunt (without carbon emission), which is different in the maximum theoretical yield. *E. coli* MG1655 was engineered for succinate production with acetate as the sole carbon source by Li et al., 2016 [56]. With the deletion of *sdhAB* (encoding the succinate dehydrogenase), *maeB* (encoding the malic enzyme), and *iclR* (ICL regulator), and overexpression of *gltA* (encoding the citrate synthase), the engineered strain could produce 7.29 g/L succinate with 5 g/L sodium acetate as a sole carbon source [56] (Table 1). This engineered *E. coli* was further applied as a host strain to improve the production of succinate [57]. Through central pathway engineering of reducing OAA decarboxylation (phosphoenolpyruvate carboxykinase, *pckA* gene deletion), TCA cycle manipulation (IDH, *icdA* gene deletion), enhancing of acetate assimilation pathway (overexpression of *ackA* and *pta*) and cofactor engineering of increasing ATP supply (formate addition and expression of IDH, *fdh* gene), the final succinate titer with high cell density strategy reached 22.91 g/L (yield: 0.87 g/g, productivity: 1.43 g/L/h) by resting cells feeding with 10 g/L sodium acetate [57] (Table 1).

4.1.3. Glycolate

The simplest α-hydroxy acid, glycolate, has the properties of both a carboxylic acid and an alcohol. Due to its widespread use in the manufacturing of biological materials (as a precursor to synthesize PGA), food processing, skin care products, and textiles, the market demand for glycolate is predicted to reach over 415 million US dollars by 2025 [42]. Traditional manufacture of glycolate was dependent on the chemical carbonylation of formaldehyde or the hydrolysis of glycolonitrile, which requires harsh conditions and generates toxic materials [58]. Therefore, a more economically feasible and environmentally friendly routine of microbial fermentation was developed. The intermediate metabolite of glycolysis and the glyoxylate bypass pathway, glyoxylate, could be converted to glycolate by the catalysis of glyoxylate reductase. In the last decades, various microorganisms, including *S. cerevisiae, C. glutamicum*, and *E. coli* [59–61], were engineered to produce glycolate with glucose and xylose from renewable sources. Acetate has also been applied as a carbon source to produce glycolate by metabolic engineered microbes in recent years (Table 1). Li et al. [58] engineered *E. coli* to produce glycolate with acetate as a sole carbon source. The strategies include: heterologous expression of glyoxylate reductase (*ycdW*); overexpression of ICL (*aceA*) and IDH (*aceK*) to strengthen the native glyoxylate shunt; inactivation of the competitive reactions by deletion of malate synthase (*aceB* and *glcB*), glyoxylate carboligase (*gcl*), and glycolate oxidase (*glcD*) to lead the flux; overexpression of citrate synthase (*gltA*), *ackA* and *pta* to reinforce the TCA cycle thus improving acetate utilization, glycolate production was improved to 2.75 g/L with a yield of 0.58 g/g in shake flask [58]. Another study altered *E. coli* to produce glycolate while simultaneously utilizing acetate (which provides a carbon backbone) and glucose (provide NADPH), taking the energy source into consideration [62]. The whole engineering system was divided into two modules, with module I for the conversion of acetate to glycolate and module II for NADPH generation by glucose. In module I, *acs, gltA, aceA*, and *ycdW* were first activated. To increase NADPH supply, module II was further engineered by the deletion of *pfkA, pfkB, ptsI*, and *sthA* and upregulation of *zwf, pgl*, and *tktA*. The final strain produced 73.3 g/L glycolate with a productivity of 1.04 g/L/h and the yield reached 1.08 g/g (for acetate) and 2.58 g/g (for glucose) with fed-batch fermentation, which is the highest reported glycolate titer from acetate [62].

4.1.4. Pyruvate

Pyruvate, which is the primary main metabolite involved in cell metabolism, is a precursor to many biochemical reactions that result in the production of amino acids, pesticides, and medicines, all of which are essential for life. In the last few decades, fermentation-based pyruvate synthesis has matured with glucose as a substrate [63]. There

Table 1
Metabolic engineering for the production of acids from acetate.

Products	Strain	Strategies	Fermentation	Titer (g/L)	Yield (g/g)	Productivity (g/L/h)	Reference
Malate	*A. oryzae* DSM 1863	–	shake flask; 45 g/L NaAc as sole carbon source or 45 g/L NaAc+15 g/L Glu-as co-substrate	8.44–14.18	0.19 0.24	0.122 0.138	[50]
Succinate	*E. coli* MG1655	Δ*pckA*Δ*sdhAB*Δ*iclR*Δ*maeB*; overexpression of *gltA*	shake flask; 5 g/L NaAc as sole carbon source	7.29	0.59	0.101	[56]
	E. coli BW25113	Δ*pckA*Δ*sdhAB*Δ*iclR*Δ*maeB*Δ*icdA*Δ*frdAB* Δ*sucC*; overexpression of *gltA, ackA, pta* and *fdh*	shake flask; 10 g/L NaAc feeding	22.91	0.87	1.43	[57]
Glycolate	*E. coli* MG1655	Δ*aceB*Δ*glcB*Δ*gcl*Δ*glcD*; overexpression of *ackA, pta, gltA, aceK, aceA* and *ycdW*	shake flask; 5 g/L NaAc as sole carbon source	2.75	0.58	0.057	[58]
	E. coli ATCC 8739	Δ*pfkA*Δ*pfkB*Δ*ptsI*Δ*sthA*; activation of *acs, gltA, aceA* and *ycdW*; upregulation of *zwf, pgl* and *tktA*	5 L bioreactor; fed-batch; Glu-and NaAc as co-substrate	73.3	1.08 (Ac) 2.58 (Glu)	1.04	[62]
Pyruvate	*E. coli* K12	Δ*poxB*Δ*pflB*Δ*aceEF*Δ*ldhA*Δ*mgsA*	shake flask; 10 g/L Glu-and 5 g/L NaAc as co-substrate	9.61	–	0.20	[64]
Itaconate	*E. coli* ATCC 9637	Overexpression of *acs, ackA, pta, gltA, aceA* and *cad*; Δ*iclR*	5 L bioreactor; fed-batch; 38.7 g/L NaAc consuming	3.57	0.092	0.041	[68]
	C. glutamicum ATCC 13,032	Overexpression of *cad; icd* mutation	42 L bioreactor; acetate as sole carbon source; fed-batch; a pH and DO-coupled feeding strategy	29.2	0.21	0.63	[69]
3-HP	*E. coli* BL21	Heterologous expression *mcr* from *Chloroflexus aurantiacus*; overexpression of *acs*; Δ*iclR*; inhibition of fatty acid synthesis by adding cerulenin	shake flask; 10 g/L NaAc as sole carbon source	3.00	0.33	0.063	[74]
	E. coli BL27	Heterologous expression *acc* from *Corynebacterium glutamicum* and *mcr* from *Chloroflexus aurantiacus*; overexpression of *ackA* and *pta*; Δ*fadR*; temperature-controlling the expression of *sdh*	shake flask; 20 g/L ammonium acetate as sole carbon source; whole-cell biocatalysis	15.8	0.71	0.329	[73]
	E. coli BL21	Δ*poxB*Δ*ldhA*Δ*pflB*Δ*sfcA*Δ*adhE*Δ*pckA*Δ*iclR* Δ*aceA*Δ*aceK*; overexpression of *accADBC_birA* and *mcr*; inhibition of fatty acid synthesis by adding cerulenin	2.5 L bioreactor; fed-batch; two-stage cultivation; Glu-and Ac as co-substrate	7.3	–	–	[43]
	Pseudomonas denitrificans ATCC13867	Δ*3hpdh*Δ*3hibdhIV*Δ*3hibdhI*Δ*pta*Δ*ackA* Δ*fabB/fabF*Δ*iclR*Δ*aceK*; heterologous overexpression of *mcr* and *acc*ABCD; inhibition of fatty acid synthesis by adding cerulenin	shake flask; 10 g/L NaAc as sole carbon source	3.6	0.25	0.16	[41]
	C. glutamicum ATCC 13,032	Optimization of the heterologous expression level of *mcr*; Δ*gltA*; deregulation of the expression of *acc* by eliminating the *fasO* sequences; inhibition of fatty acid synthesis by adding cerulenin	5 L bioreactor; fed-batch; NaAc as sole carbon source	17.1	0.10	0.143	[44]
Mevalonate	*E. coli* W3110	Heterologous expression of *mvaE, mvaS* and *acs*	5 L bioreactor; fed-batch; two-stage aerobic fermentation; Glu-and Ac as co-substrate	7.85	0.27	0.13	[76]

"-": not found in the reference.

haven't been many studies on the synthesis of pyruvate from acetate. Da et al. [64] applied engineered *E. coli* to produce pyruvate by synergetic utilization of glucose and acetate. By the deletion of *poxB, pflB, aceEF, ldhA*, and *mgsA*, the engineered *E. coli* could produce 9.61 g/L pyruvate from 10 g/L glucose and 5 g/L sodium acetate as cosubstrate (Table 1).

4.1.5. Itaconate

Itaconate (itaconic acid, C5 unsaturated dicarboxylic acid), a potential platform chemical and one of the top 12 bio-based platform chemicals, is produced in quantities of about 40,000 tons annually, primarily through the fermentation of glucose by the filamentous fungus *Aspergillus terreus*. It has numerous uses in the polymer, bioplastics, textile, and pharmaceutical industries [65,66]. Citric acid, an intermediate metabolite of the TCA cycle, is first transformed into cis-aconitate during the biosynthesis of itaconate, and itaconate is subsequently produced by the catalysis of cis-aconitate decarboxylase (*cad*) [67]. Until now, several studies have performed genetic engineering of some model microorganisms to produce itaconate from acetate (Table 1). For example, a suitable and superior *E. coli* strain with high tolerance and efficient utilization of acetate (10 g/L) was selected as the host for engineering to produce itaconate from acetate [68]. Overexpression of *acs, ackA, pta, gltA, aceA*, and *cad*, as well as deletion of *iclR*, resulted in an engineered strain with a production of 3.57 g/L itaconate through consuming 38.7 g/L sodium acetate in a reactor-scale fed-batch fermentation [68]. Furthermore, *C. glutamicum*, an important industrial platform bacterium, which has demonstrated its assimilation capacity with a moderate concentration of acetate, was also used as a host for itaconate production by metabolic engineering [69]. A strain with heterologous overexpression of *cad* and *icd* mutation for reducing IDH activity was constructed, resulting in a maximum titer of 29.2 g/L itaconate with an integrated pH-coupled acetate feeding control strategy in a fed-batch process [69]. These studies demonstrated the possibility and potential of using acetate as a carbon source to produce itaconate with metabolically engineered

Table 2
Metabolic engineering for the production of alcohols from acetate.

Products	Strain	Strategies	Fermentation	Titer (g/L)	Yield (g/g)	Productivity (g/L/h)	Reference
Ethanol	*S. cerevisiae* SR8	Overexpression of *xyl1, xyl2, xks1*, alcohol dehydrogenase (ADH) and acetylating acetaldehyde dehydrogenase (AADH) genes; evolutionary engineering; Δ*ALD6*	shake flask; cellulosic hydrolysates as substrate (20 g/L glucose, 80 g/L xylose and 2 g/L acetic acid)	45	0.414	0.51	[46]
	Ralstonia eutropha H16	Overexpression of *adhE*; Δ*phaCAB*	shake flask; repetitive feeding of acetate	0.35	–	0.004	[83]
Isopropanol	*E. coli* MG1655	Heterologous expression of *thlA, adc, atoDA* and *adh*; cofactor engineering by expression of *nadK* and *pntAB*; enhancement of the ACK-PTA pathway (*ack-pta*)	shake flask; Ac as sole carbon source	1.47	0.41	0.041	[21]
Isobutanol	*E. coli* MG1655	Heterologous overexpression of *alsS, ilvCD, kivD, yqhD, acs, pckA*, and *maeB*; addition of TCA cycle intermediates; introduction of additional NADPH regeneration system and acetate scavenging pathway genes (*pntAB, fdh* and *pta-ackA*)	shake flask; Ac as sole carbon source	0.1–0.2	–	–	[88]
2,3-Butanediol	*E. coli* W	Δ*ldhA*Δ*adhE*Δ*pta*Δ*frdA*; overexpression of *budA, budB* and *budC*	bioreactor with a working volume of 200 mL; modification of medium with addition of amino acids and vitamins; pulsed fed-batches	1.16	0.09	0.011	[89]

"-": not found in the reference.

microbes, resulting in a novel and attractive strategy for itaconate production.

4.1.6. 3-Hydroxypropionic acid

With two functional groups (hydroxyl and carboxylic group), 3-hydroxypropionic acid (3-HP) is a significant platform compound (one of the top 12 bio-based platform chemicals) that may be used as a raw material for production of various chemicals, including acrylate, 1,3-propanediol, propiolactone, and malonic acid, which have broad applications in chemical and polymer industries [70]. Biosynthesis of 3-HP has been explored for many years with various genetically engineered microbes, such as *E. coli, Klebsiella pneumonia, S. cerevisiae, Pseudomonas denitrificans*, and *C. glutamicum* [41,43,44,71,72], and a variety of raw materials have been extensively applied for 3-HP production, including acetate. To improve the utilization of acetate, the ACS or ACK-PTA pathway was constructed in some microorganisms that could not assimilate acetate. During the biological production of 3-HP, malonyl-CoA reductase (*mcr*) is responsible for the conversion from malonyl-CoA to 3-HP. Due to the lack of malonyl-CoA reductase, heterologous expression of *mcr* is necessary for 3-HP formation in most microbes. To improve the supply of acetyl-CoA as a precursor to produce 3-HP, acetyl-CoA carboxylase (*acc*), which converts acetyl-CoA to malonyl-CoA, was overexpressed in some engineered microbes [41,43,44,73]. Some competitive and by-products formation pathways, such as the glyoxylate shunt (*iclR* and *aceA* deletion) [41,43,74] and the TCA cycle (*gltA* and *aceK* deletion, *sdh* regulation), have also been impaired or down-regulated to increase 3-HP production [41,43,44,73]. In addition, the lipid synthesis pathway, also with acetyl-CoA and malonyl-CoA as precursors, was also inhibited by the deletion of key genes (such as *fadR, fabB*, and *fabF*) [41,73] and the addition of inhibitor (cerulenin) to the medium [41,44,68,74]. The final titer of 3-HP varied from several g/L to almost 20 g/L, which has the potential for further improvement in large-scale production.

4.1.7. Mevalonate

Mevalonate (β-hydroxy-β-methyl-δ-valerolate), as the key metabolite involved in the mevalonate (MVA) pathway, is an essential precursor for the biosynthesis of terpenes and steroids like isoprene, carotenoid, and artemisinin, which find widespread use in the production of materials, fuels, and pharmaceuticals [75]. An acetate-derived mevalonate biosynthesis pathway was created by Xu et al., 2018 [76], to investigate a mevalonate manufacturing approach using non-food substrates. The mevalonate biosynthetic pathway, which began with acetate, was first constructed by expressing *acs* and two MVA pathway genes (*mvaE* and *mvaS*) from *Enterococcus faecalis* [76]. Fed-batch fermentation with pH control was performed to further improve productivity. In a two-stage aerobic fermentation process with glucose and acetate as cosubstrate (glucose for cell growth during the first stage and acetate added in the medium until the depletion of glucose for product conversion in the second stage), the maximum concentration of mevalonate reached 7.85 g/L, providing a successful example of mevalonate bioconversion from less expensive acetate.

4.2. Alcohols

4.2.1. Mono alcohols (ethanol, isopropanol, and isobutanol)

Alcohols, in addition to various acids, have a large market demand in the industries of biofuels, biopolymers, solvents, detergents, and chemicals [77]. Traditional alcohol production is primarily based on the petrochemical method, which uses fossil resources as raw material. Given the depletion of fossil fuels and the environmental pollution problem, the biological production of various alcohols attracted a wide range of interests and has been largely developed from renewable sources, such as lignocellulosic biomass [78]. These alcohols include ethanol, isopropanol, butanol, propanediol, butanediol, etc. Among the renewable sources used for alcohol production, acetate is recognized as an alternative feedstock and has also been utilized for several alcohol production in recent years (Table 2).

The most studied alcohol component, ethanol, is typically made from fermentative sugars, including glucose, xylose, sucrose, and fructose [79,80]. Ethanol production from lignocellulosic biomass has been intensively investigated and applied in the last decades [81,82], although still with obstacles in a techno-economic view. Some, but not many,

studies have attempted to use acetate to create ethanol. In *S. cerevisiae*, by the combination of NADH-consuming acetate consumption pathway (overexpression of alcohol dehydrogenase and acetylated acetaldehyde dehydrogenase), xylose utilization pathway (heterologous expression of *xyl1, xyl2,* and *xks1*), and evolutionary engineering, the engineered strain could produce 45 g/L ethanol with a mixture of sugars (20 g/L glucose and 80 g/L xylose) and acetic acid (2 g/L) as substrates in an anaerobic shake flask cultivation [46]. Another research applied genetically modified *Ralstonia eutropha* as a host strain for ethanol production from acetate [83]. By the deletion of the *phaCAB* operon encoding pathway from acetyl-CoA to polyhydroxybutyrate (PHB) and overexpression of *adhE* (encoding alcohol dehydrogenase), this strain generated approximately 0.35 g/L ethanol through repetitive feeding of acetate [83].

Isopropanol (IPA) is an important chemical product and ingredient and is mainly utilized for the production of pharmaceuticals, cosmetics, plastics, and coating. The market demand for IPA steadily expands at a rate of 7% per year due to the multipurpose application [21]. Although the chemical synthesis of propylene is still the most common method for producing IPA, biomanufacturing exhibits enormous potential for utilizing renewable materials and mild reaction conditions. Yang et al. [21] and coworkers used a genetically modified *E. coli* strain to produce IPA from acetate. The IPA biosynthetic pathway from acetyl-CoA was first constructed by a combined expression of multiple genes (*thlA, adc, atoDA*, and *adh*). A strategy of enhancing the acetate assimilation pathway (exchange the promoter of *ack-pta*) coupled with cofactor engineering (expression of *nadK* and *pntAB*) was further adopted to enhance the production of IPA, which resulted in a titer of 1.47 g/L IPA [21].

Isobutanol, a C4 alcohol, is crucial as a fuel additive and a starting material for the manufacture of rubber. Isobutanol is more suited for gasoline blends than ethanol because it is less hygroscopic, has a higher energy capacity, and is compatible with existing engines [77]. The biological production of isobutanol was developed with engineered platform organisms, such as *E. coli, S. cerevisiae, C. glutamicum*, and *Bacillus subtilis* [84–87], with glucose as the common substrate. The feasibility of using acetate to produce isobutanol by metabolic engineering was investigated in *E. coli* [88]. A heterologous pathway for isobutanol production from pyruvate was constructed with the expression of a gene cluster (*alsS, ilvCD, kivD*, and *yqhD*). Then, the overexpression of *acs* and introduction of *pta-ackA* for acetate scavenging enhanced the acetate assimilation. To guide the flux to isobutanol production, the pathways for pyruvate generation and the glyoxylate shunt were further activated by overexpression of *pckA, maeB*, and *aceA* and the addition of several intermediates in the TCA cycle. In consideration of energy supplements during the synthesis of isobutanol, the authors introduced an NADPH regeneration system by the introduction of *pntAB* and *fdh*. The final engineered strain resulted in a production of 0.1–0.2 g/L isobutanol [88]. This study represented the first approach to produce isobutanol from acetate through flux optimization.

4.2.2. 2,3-Butanediol

Except for mono alcohols, acetate also has been explored to produce diol, for instance, 2,3-butanediol, which is used as absorbent, plasticizer, solvent, and raw material for the production of synthetic resin [89]. In an *E. coli* strain, several strategies were employed to produce 2,3-butanediol from acetate [89], including (1) construction of 2,3-butanediol biosynthetic pathway (overexpression of *budA, budB* and *budC*); (2) genes deletion of mixed-acid fermentation pathways (*ldhA, adhE, pta* and *frdA*); (3) medium modification by the addition of amino acids and vitamins. Cultivation based on pulsed fed-batches resulted in a production of 1.16 g/L 2,3-butanediol from acetate alone [89].

These studies suggested a potential strategy and showed the feasibility of the biochemical production of alcohols with acetate as the only carbon source, even though the titer of these alcohols produced from the acetate introduced above is still very low and needs further investigation and improvement.

4.3. Esters

4.3.1. Polyhydroxyalkanoates (PHA)

Polyhydroxyalkanoates (PHA), an important and promising biodegradable material or biopolymer, have gradually developed as a replacement for traditional plastics with the concepts of reducing white pollution and protecting the environment [90]. This is due to the properties of biodegradability, biocompatibility, renewability, and diverse structure [90]. PHA is generally produced by some microbes in cells for carbon and energy storage, making it a promising alternative for PHA production through microbial fermentation [90]. However, production efficiency and cost are still the most important limitation factors, which restricted further application and development [91]. Numerous studies have been conducted in recent years to build various microbial cell factories using model hosts and natural PHA producers to increase PHA productivity [92–94]. In addition, to decrease the cost of PHA and make it economically competitive and feasible, low-cost substrates have also been evaluated for PHA production. Currently, glucose is still the main carbon source for PHA synthesis but some other nontraditional feedstocks also have been applied. In recent years, acetate was investigated as an alternative carbon source for a potential cost-effective PHA production strategy (Table 3).

Chen et al., 2018 [95] reported the production of P3HB (1.27 g/L), P3HB4HB (2.15 g/L), and PHBV (1.09 g/L) from acetate as the main carbon source through the engineered *E. coli* [95]. The performance of *pta-ackA* and *acs* overexpression on acetate assimilation and P3HB production was first compared. A P3HB4HB synthesis pathway was constructed both from acetyl-CoA and succinyl-CoA as a precursor by heterologous expression of *phaCAB, sucD, 4hbD*, and *orfZ*. For the production of PHBV, the assimilation pathway of propionate was also introduced by expression of *pct* and *prpP* to supply 3-hydroxyvalerate. This study demonstrated the possibility of effectively producing various types of PHA from acetate as the main carbon source. In *P. putida*, mcl-PHA (medium-chain length PHA) was generated (0.21 g/L) by overexpression of *acs* and the construction of the *ackA-pta* pathway [45]. In addition, another study applied a wild-type *Aeromonas hydrophila* as a natural PHA producer for the improvement of PHA by metabolic engineering [96]. Heterologous overexpression of a series of genes including *acs, pta-ackA, pct, acsA*, and *phaCAB* resulted in a production of PHB and PHBV with 0.55 g/L and 0.57 g/L, respectively [96]. These results above supplied new approaches for the utilization of acetate as a low-cost carbon source to produce high-value-added biopolymers.

4.3.2. Microbial lipids

Microbial lipids, also called single cell oils, are accumulated in cells (over 20% of dry cell weight) by a wide range of oleaginous microorganisms consisting of yeasts, fungi, microalgae, and bacteria [97]. Microbial lipids are considered an attractive and promising feedstock for the production of biofuels (such as biogasoline, biodiesel, and biolubricant) [1]. It has been discovered that the primary barrier to the industrialization and commercialization of microbial lipids is the cost of carbon sources used during growth, which makes up almost 50%–70% of the total cost of producing microbial lipids [97]. Therefore, exploiting and applying more economical low-cost substrates for the production of microbial lipids is required [97]. Compared with glucose or other carbon sources, acetate is a superior and ideal substrate for the production of microbial lipids because of its shorter and more efficient assimilation pathway, which is beneficial to reduce carbon loss and accumulate more precursors (acetyl-CoA). Many microorganisms have proved their potential as natural lipids producers for utilizing acetate as a substrate to accumulate lipids through various regulation and control strategies during fermentation, including, *C. curvatus* [47,98], *Chlorella pyrenoidosa* [99], *Y. lipolytica* [100], *R. glutinis* [48,101], *Cryptococcus podzolicus* [102], and *Trichosporon porosum* [102] (Table 3). Besides, these oleaginous microbes were also genetically engineered for the improvement of lipids production from acetate. In a recent study, the ability of *Y. lipolytica* to

Table 3
Metabolic engineering for the production of esters from acetate.

Products	Strain	Strategies	Fermentation	Titer (g/L)	Yield (g/g)	Productivity (g/L/h)	Reference
P3HB, P3HB4HB and PHBV	*E. coli* JM109	Heterologous overexpression of *pta-ackA, acs, prpP, pct, phaCAB, sucD, 4hbD, orfZ*	shake flask; acetate as a main carbon source (5 g/L)	P3HB: 1.27 P3HB4HB: 2.15 PHBV: 1.09	0.254 0.358 0.218	0.026 0.045 0.023	[95]
mcl-PHA	*P. putida* KT2440	Overexpression of *acs* and construction of *ackA-pta* pathway	5 L bioreactor; fed-batch; NaAc as sole carbon source	0.21	0.03	0.02	[45]
PHB and PHBV	*Aeromonas hydrophilia* 4AK4	Heterologous overexpression of *acs, pta-ackA, pct, acsA* and *phaCAB*	shake flask; acetate as a main carbon source (10 g/L)	PHB: 0.55 PHBV: 0.57	0.055 0.0475	0.023 0.0238	[96]
Lipids	*C. curvatus* ATCC 20,509	–	shake flask; Glu (40), Xyl (20) and Ac (20) as co-substrate	14.5	0.18	0.13	[98]
	Y. lipolytica	–	2 L stirred tank bioreactor; fed-batch; integrated conversion comprising a two-stage system	46	0.16	0.27	[100]
	C. curvatus MUCL 29,819	–	shake flask; preculture and sequencing batch culture strategy	5.43	–	–	[47]
	Chlorella pyrenoidosa FACHB-9	–	shake flask; 2–10 g/L NaAc as carbon source; mixotrophic cultivation	-	–	13.48 (mg/L/d)	[99]
	R. glutinis CGMCC 2.703	–	5 L bioreactor; fed batch; two-stage pH regulation strategy	35.8	–	0.186	[101]
	Cryptococcus podzolicus DSM 27,192 and *Trichosporon porosum* DSM 27,194	–	2.5 L fermentor; continuous self-provided fermentation; continuous feeding; Glu-and Ac as co-substrate	10.4 11.5	–	0.024 0.027	[102]
	Rhodotorula glutinis CGMCC 2258	–	shake flask; 20 g/L NaAc as sole carbon source; light exposure	1.97	0.099	0.018	[48]
	Yarrowia lipolytica PO1f	Overexpression of *acs, acc1* and *fas*	5 L stirred tank reactor; fed-batch; glycerol and acetate as cosubstrate	Lipid content: 41.7%	–	–	[49]
Isobutyl acetate	*E. coli* JCL260	Heterologous overexpression of *ackA, pta, alsS, ilvCD, kivd, adhA* and *atf1*	Glu-and Ac as co-substrate; acetate-feeding	19.7	0.33	0.164	[103]

"-": not found in the reference.

use acetate and accumulate lipids was successfully increased by overexpressing the essential genes *acs, acc1*, and *fas*, which are involved in acetate absorption and lipid biosynthesis, using both acetate and glycerol as cosubstrates in a fed-batch method [49].

4.3.3. Isobutyl acetate

Isobutyl acetate (IBA), as the esterification product of isobutanol and acetate, is mainly used for solvent and chemical synthesis [103]. As stated in Section 4.2.1, isobutanol could be generated from acetate as a carbon source, which provides the possibility for the production of isobutanol-related products. In a previous study, glucose and acetate were used as cosubstrates in *E. coli* to build a biosynthetic route for the manufacture of IBA [103]. The route contains two independent pathways, the first for producing isobutanol from glucose and the other for acetyl-CoA generation from acetate, which are combined to produce IBA. For this purpose, *alsS, ilvCD, kivd*, and *adhA* were overexpressed for isobutanol production from glucose, and the *ackA-pta* pathway was activated for acetate assimilation. *Atf1*, as alcohol-O-acetyl transferase from *S. cerevisiae*, was then introduced, which is responsible for the reaction of isobutanol and acetyl-CoA for IBA production. The final engineered strain could produce 19.7 g/L IBA with an acetate feeding strategy [103]. During the biosynthesis process, acetate serves as the carbon source for acetyl-CoA generation through the acetate assimilation pathway without carbon loss or redox cofactor imbalance, which is a promising attempt of ester production for reducing carbon emission.

4.4. Others

Other compounds that were not classified as acids, alcohols, or esters were also reported to be produced from acetate as a carbon source. Free fatty acids (FFAs), β-carotene, β-caryophyllene, tyrosine, and acetone are some of these substances (Table 4).

FFAs are essential precursors for the synthesis of microbial lipids, as well as precursors of advanced biofuels (alkanes, fatty alcohols, waxes, and esters), which have recently gained a lot of attention [104]. In *E. coli*, several genetic strategies were adopted to generate FFAs with acetate as substrate, including deletion of *fadE* and overexpression of *acs* and *tesA* [105]. In a pH-coupled fed-batch fermentation, the titer of FFAs reached almost 1 g/L with acetate as the sole carbon source [105]. As previously mentioned, the MVA pathway is an important route for various chemical production with acetyl-CoA as a precursor. As the representatives, it has been noted that acetate can be used to create β-carotene and β-caryophyllene [106,107]. As a natural β-carotene producer, *Blakeslea trispora* was reported to produce 2.13 g/L β-carotene using glucose and acetate as cosubstrate, and the author demonstrated that acetate was beneficial to *de novo* synthesis of β-

Table 4
Metabolic engineering for the production of some other chemicals from acetate.

Products	Strain	Strategies	Fermentation	Titer (g/L)	Yield (g/g)	Productivity (g/L/h)	Reference
FFAs	*E. coli* BL21	Δ*fadE*; overexpression of *acs* and *tesA*	1 L bioreactor; pH-coupled fed-batch fermentation; Ac as sole carbon source	0.9	0.064	0.0035	[105]
β-Carotene	*Blakeslea trispora* ATCC 14271 and 14272	–	Glu-and Ac (35 mM) as co-substrate	2.13	–	0.015	[106]
β-Caryophyllene	*E. coli* BL21	Heterologous expression of *mvaE, mvaS, erg12, erg8, erg19, idi1, acs, qhs1, gpps2, ispA* and *nphT7*	5 L bioreactor; pH-coupled fed batch fermentation	1.05	0.021	0.015	[107]
Tyrosine	*E. coli* SCK1	Overexpression of *acs* and *pck*; varying the expression of *aceA*	shake flask; 10 g/L NaAc as sole carbon source	0.70	0.07	0.023	[108]
Acetone	*E. coli* MG1655	Heterologous overexpression of *thlA, adc, ctfAB, atoB, atoDA* and *ackA-pta*; Δ*pckA*Δ*maeB*Δ*icdA*	mini-reactor with automatic pH control system; 20 g/L NaAc as sole carbon source	6.57	0.21	0.274	[109]

"-": not found in the reference.

carotene through upregulation of key genes in carotenogenesis [106]. For the first time, an engineered *E. coli* strain was used to construct a novel *β*-caryophyllene biosynthesis route based on the MVA system, which converts acetic acid to make *β*-caryophyllene [107]. The hybrid pathway for a *β*-caryophyllene generation was divided into three parts: the upper pathway from acetyl-CoA to MVA (heterologous expression of *mvaE* and *mvaS*), the lower pathway responsible for converting MVA to DMAPP (heterologous expression of *erg12, erg8, erg19*, and *idi1*) and *β*-caryophyllene biosynthetic pathway (integration of *gpps2, ispA*, and *qhs1*). To enhance the precursor supply and acetate utilization, *acs* and acetoacetyl-CoA synthase (*nphT7*) were also coexpressed. The final modified strain accumulated 1.05 g/L *β*-caryophyllene through pH-coupled fed-batch fermentation in a 5 L bioreactor [107]. Another successful attempt from acetate is the efficient production of tyrosine in *E. coli*. Tyrosine is an important precursor for flavonoids and alkaloids synthesis, which has wide applications in the food, cosmetic, and pharmaceutical industries [108]. In the study, *acs* and *pck* were overexpressed to increase the flux, which activated the acetate uptake and gluconeogenesis pathways in the tyrosine-producing strain [108]. Moreover, the glyoxylate shunt pathway's flux distribution was accurately controlled by adjusting the expression level of *aceA*, and the final strain produced 0.7 g/L tyrosine using 10 g/L acetate as the sole carbon source [108]. Acetone, as an important organic solvent, could also be produced from acetate. In a study, a novel acetone biosynthesis pathway was assembled by the expression of gene clusters including *thlA, atoB, ctfAB, atoDA*, and adc (for acetone biosynthesis from acetyl-CoA), *ackA-pta* (for acetate assimilation) and the deletion of *pckA, maeB*, and *icdA* (block the TCA cycle) [109]. As a result, the concentration of acetone reached 6.57 g/L with 20 g/L of sodium acetate as the sole carbon source [109]. These studies above expand the diversities of acetate-derived biochemicals and indicate the potential of acetate replacing glucose as a carbon source for producing various chemicals.

5. Perspectives

Even though acetate has emerged as a promising substrate for microorganisms to manufacture various value-added bioproducts, and significant efforts have been made in constructing microbial cell factories with metabolic engineering, problems and difficulties in the elements of acetate formation, acetate assimilation, and chemicals production from acetate continue to be addressed (Fig. 3). First and foremost, industrial biotechnology requires an adequate and effective supply of acetate with low-cost feedstocks. This might lower the cost, making the downstream process (from acetate to biochemicals) more feasible. Many technical challenges, including the creation of an effective acetate producer, the capture and immobilization of CO_2, the creation of a highly efficient and selective catalyst, the construction of bioreactors, and process optimization, must be resolved quickly among the choices for producing acetate both biologically and chemically.

Furthermore, for acetate assimilation, the challenges are mainly reflected in the toxicity of acetate to cell growth for most microbes, which generally causes a slow uptake rate and low efficiency. The current bioindustry cannot produce many bulk goods using fermentation due to the lower concentration and utilization efficiency of substrate. The coculture of acetogens and acetate-utilizing microbes appears to be a promising approach to reduce toxicity and improve utilization efficiency, with acetogens continuously and stably producing acetate at a certain level and acetate-utilizing microbes assimilating the produced acetate with stabilized rate thus reducing the toxicity and inhibition [25,26]. Every step for acetate assimilation, including the transfer into the cell, the conversion to acetyl-CoA, and the further utilization of acetyl-CoA, is equally important for the acceleration of acetate utilization. To overcome this difficulty, screening new chassis or appropriate strains with outstanding acetate assimilation capacity as well as exploring mechanisms underlying the stress tolerance should be a good option. Superior fermentation mode or strategy is also crucial for reducing acetate inhibition and increasing the utilization and that is the reason for most studies reported an acetate feeding (fed-batch or pH-coupled feeding control) or two-stage (more favorable carbon sources [like glucose] are used for cell growth during the first stage, and acetate is then added into the medium after the complete consumption of carbon source in the first stage and converted for targeting compound synthesis in the second stage) strategy during the fermentation process. Considering the demand for ATP or other energy, efficient energy supply during the acetate assimilation is still an important aspect to consider.

As most platform microbes were mainly engineered with carbohydrates or other substrates for a long time, reconstruction and optimization of pathways in the upstream processing for acetate assimilation and compound biosynthesis seem to become the major challenge. Acetate has a lower energy content than other conventional carbon sources because of its oxidized nature. This is not advantageous for the production of highly reduced products with long chains because the biosynthesis of these compounds typically requires a lot of NADPH or energy for the linking of carbon-carbon bonds. This issue seriously limits the ranges of acetate-derived target products, and as a result, less energy-intensive and more oxidized products (3-HP, succinate, or itaconate as ideal candidates) are preferred for commercialization from acetate conversion, which is desired for the establishment of simple pathways by pathway

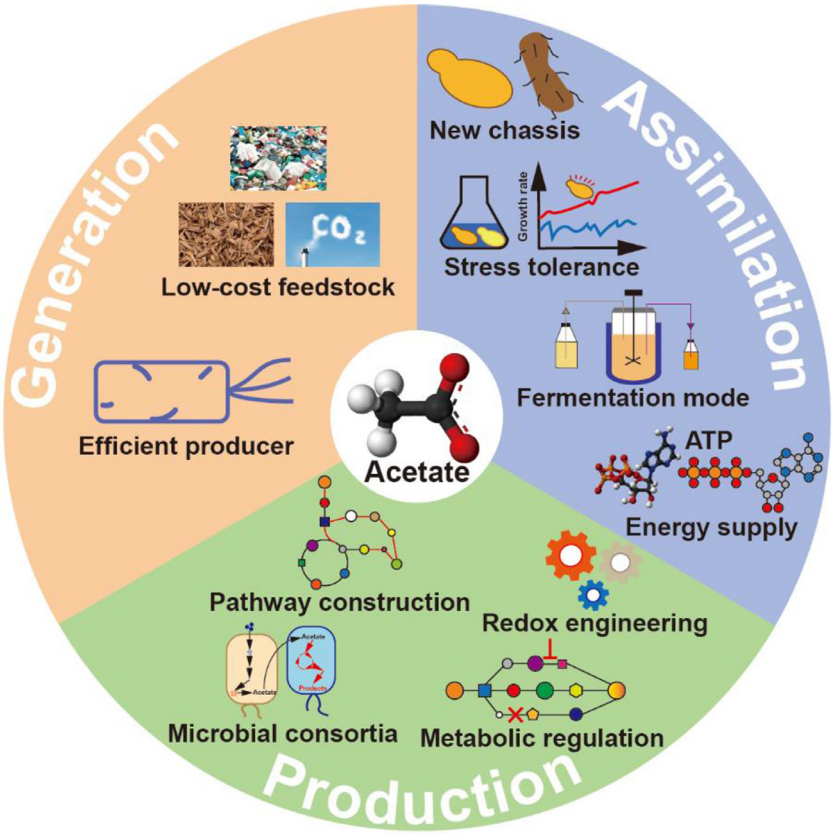

Fig. 3. Future perspectives and development of biochemical production from acetate.

construction and energy supplement with redox engineering to broaden the products spectrum. A prospective and perfect alternative for the use of acetate to manufacture value-added chemicals in the future is to use microbial consortia, a growing field of metabolic engineering. Design of mutualistic microbial consortia for stable conversion of acetate to value-added chemicals was an attractive option (for example, the construction of two mutualistic microbes both assimilate acetate as a carbon source for chemical synthesis, or one microbe converts substrate (like CO or CO_2) to acetate and provides acetate as a carbon source to the other microbe for chemicals production), which is advantageous for reducing acetate inhibition and enhancing products productivity. In addition, as cell growth and products synthesis both employ acetyl-CoA as a precursor, the flux balance between these two processes is required for precise regulation and control at the acetyl-CoA node, simultaneously ensuring proper carbon distribution in the glyoxylate shunt, TCA cycle, and gluconeogenesis, both for the cell growth and the supply of a carbon skeleton for the compound synthesis. The improvement of titer, rate, and yield, which makes it similar to those advantageous carbon sources, requires global regulation and control of the entire metabolism for bacteria ingesting acetate and creating chemicals. Overall, although many biological and technological issues remained unsolved, acetate should be considered an important option and alternative for bioprocessing and biotransformation, which has bright prospects and great potential to replace the traditional carbon sources for value-added chemicals production in future industrial biotechnology.

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.

Acknowledgments

The work is supported by Agricultural Science and Technology Innovation Project of Chinese Academy of Agricultural Sciences (CAAS-ASTIP-2016-BIOMA), Central Public-Interest Scientific Institution Basal Research Fund (1610012022001_03104), Central Public-Interest Scientific Institution Basal Research Fund (1610012022009-03102), Sichuan Provincial Science and Technology Research Project (2022YFN0062) and Sichuan Science and Technology Program (2022YFS0460).

References

[1] F. Long, W. Liu, X. Jiang, et al., State-of-the-art technologies for biofuel production from triglycerides: a review, Renew. Sust. Energ. Rev. 148 (2021) 111269.
[2] Y.-S. Ko, J.W. Kim, J.A. Lee, et al., Tools and strategies of systems metabolic engineering for the development of microbial cell factories for chemical production, Chem. Soc. Rev. 49 (14) (2020) 4615–4636.
[3] V. Kumar, P. Binod, R. Sindhu, et al., Bioconversion of pentose sugars to value added chemicals and fuels: recent trends, challenges and possibilities, Bioresour. Technol. 269 (2018) 443–451.
[4] L.R. Lynd, G.T. Beckham, A.M. Guss, et al., Toward low-cost biological and hybrid biological/catalytic conversion of cellulosic biomass to fuels, Energ. Environ. Sci. 15 (3) (2022) 938–990.
[5] S.H. Kee, J.B.V. Chiongson, J.P. Saludes, et al., Bioconversion of agro-industry sourced biowaste into biomaterials via microbial factories - A viable domain of circular economy, Environ. Pollut. 271 (2021) 116311.
[6] D. Kiefer, M. Merkel, L. Lilge, et al., From acetate to bio-based products: underexploited potential for industrial biotechnology, Trend. Biotechnol. 39 (4) (2021) 397–411.
[7] Y. Kim, S. Lama, D. Agrawal, et al., Acetate as a potential feedstock for the production of value-added chemicals: metabolism and applications, Biotechnol. Adv. 49 (2021) 107736.
[8] K. Novak, S. Pflügl, Towards biobased industry: acetate as a promising feedstock to enhance the potential of microbial cell factories, FEMS Microbiol. Lett. 365 (2018) fny226.
[9] Y. Wu, M. Xia, X. Zhang, et al., Unraveling the metabolic network of organic acids in solid-state fermentation of Chinese cereal vinegar, Food Sci. Nutr. 9 (8) (2021) 4375–4384.

[10] I.Y. Sengun, S. Karabiyikli, Importance of acetic acid bacteria in food industry, Food Control 22 (5) (2011) 647–656.
[11] J. Bae, Y. Song, H. Lee, et al., Valorization of C1 gases to value-added chemicals using acetogenic biocatalysts, Chem. Eng. J. 428 (2022) 131325.
[12] A.R. Mankar, A. Pandey, A. Modak, et al., Pretreatment of lignocellulosic biomass: a review on recent advances, Bioresour. Technol. 334 (2021) 125235.
[13] S. Wang, X. Sun, Q. Yuan, Strategies for enhancing microbial tolerance to inhibitors for biofuel production: a review, Bioresour. Technol. 258 (2018) 302–309.
[14] W. Seong, G.H. Han, H.S. Lim, et al., Adaptive laboratory evolution of *Escherichia coli* lacking cellular byproduct formation for enhanced acetate utilization through compensatory ATP consumption, Metab. Eng. 62 (2020) 249–259.
[15] D.B. Tovilla-Coutino, C. Momany, M.A. Eiteman, Engineered citrate synthase alters acetate accumulation in *Escherichia coli*, Metab. Eng. 61 (2020) 171–180.
[16] Y. Li, Y. Chen, J. Wu, Enhancement of methane production in anaerobic digestion process: a review, Appl. Energy. 240 (2019) 120–137.
[17] M. Rasapoor, B. Young, R. Brar, et al., Recognizing the challenges of anaerobic digestion: critical steps toward improving biogas generation, Fuel 261 (2020) 116497.
[18] T.C. D' Silva, A. Isha, R. Chandra, et al., Enhancing methane production in anaerobic digestion through hydrogen assisted pathways – A state-of-the-art review, Renew. Sust. Energ. Rev. 151 (2021) 111536.
[19] E.T. Liakakou, B.J. Vreugdenhil, N. Cerone, et al., Gasification of lignin-rich residues for the production of biofuels via syngas fermentation: comparison of gasification technologies, Fuel 251 (2019) 580–592.
[20] X. Sun, H.K. Atiyeh, R.L. Huhnke, et al., Syngas fermentation process development for production of biofuels and chemicals: a review, Bioresour. Technol. Rep. 7 (2019) 100279.
[21] H. Yang, C. Zhang, N. Lai, et al., Efficient isopropanol biosynthesis by engineered *Escherichia coli* using biologically produced acetate from syngas fermentation, Bioresour. Technol. 296 (2020) 122337.
[22] C. Liu, G. Luo, W. Wang, et al., The effects of pH and temperature on the acetate production and microbial community compositions by syngas fermentation, Fuel 224 (2018) 537–544.
[23] X. Qian, L. Chen, Y. Sui, et al., Biotechnological potential and applications of microbial consortia, Biotechnol. Adv. 40 (2020) 107500.
[24] K. Jawed, S.S. Yazdani, M.A. Koffas, Advances in the development and application of microbial consortia for metabolic engineering, Metab. Eng. Commun. 9 (2019) e00095.
[25] S. Cha, H.G. Lim, S. Kwon, et al., Design of mutualistic microbial consortia for stable conversion of carbon monoxide to value-added chemicals, Metab. Eng. 64 (2021) 146–153.
[26] X. Li, M.A. Henson, Metabolic modeling of bacterial co-culture systems predicts enhanced carbon monoxide-to-butyrate conversion compared to monoculture systems, Biochem. Eng. J. 151 (2019) 107338.
[27] N. Chu, Q. Liang, Y. Jiang, et al., Microbial electrochemical platform for the production of renewable fuels and chemicals, Biosens. Bioelectron. 150 (2020) 111922.
[28] F. Kong, H.-.Y. Ren, S.G. Pavlostathis, et al., Overview of value-added products bioelectrosynthesized from waste materials in microbial electrosynthesis systems, Renew. Sust. Energ. Rev. 125 (2020) 109816.
[29] M. Roy, R. Yadav, P. Chiranjeevi, et al., Direct utilization of industrial carbon dioxide with low impurities for acetate production via microbial electrosynthesis, Bioresour. Technol. 320 (Pt A) (2021) 124289.
[30] B. Bian, S. Bajracharya, J. Xu, et al., Microbial electrosynthesis from CO_2: challenges, opportunities and perspectives in the context of circular bioeconomy, Bioresour. Technol. 302 (2020) 122863.
[31] L. Jourdin, T. Burdyny, Microbial electrosynthesis: where do we go from here? Trend. Biotechnol 39 (4) (2021) 359–369.
[32] K.K. Sakimoto, A.B. Wong, P. Yang, Self-photosensitization of nonphotosynthetic bacteria for solar-to-chemical production, Science 351 (2016) 74–77.
[33] S. Wang, S. Guo, Y. Luo, et al., Direct synthesis of acetic acid from carbon dioxide and methane over Cu-modulated BEA, MFI, MOR and TON zeolites: a density functional theory study, Catal. Sci. Technol. 9 (23) (2019) 6613–6626.
[34] F. Yu, X. Jing, Y. Wang, et al., Hierarchically porous metal–organic framework/MoS_2 interface for selective photocatalytic conversion of CO_2 with H_2O into CH_3COOH, Angew. Chem. Int. Ed. 60 (47) (2021) 24849–24853.
[35] W. Luc, X. Fu, J. Shi, et al., Two-dimensional copper nanosheets for electrochemical reduction of carbon monoxide to acetate, Nat. Catal. 2 (5) (2019) 423–430.
[36] T. Zheng, M. Zhang, L. Wu, et al., Upcycling CO_2 into energy-rich long-chain compounds via electrochemical and metabolic engineering, Nat. Catal. (2022).
[37] R. Borghese, D. Zannoni, Acetate permease (ActP) Is responsible for tellurite ($TeO3^{2-}$) uptake and resistance in cells of the facultative phototroph *Rhodobacter capsulatus*, Appl. Environ. Microbiol. 76 (3) (2010) 942–944.
[38] Y. Qi, H. Liu, X. Chen, et al., Engineering microbial membranes to increase stress tolerance of industrial strains, Metab. Eng. 53 (2019) 24–34.
[39] N. Guaragnella, M. Bettiga, Acetic acid stress in budding yeast: from molecular mechanisms to applications, Yeast 38 (7) (2021) 391–400.
[40] C. Zhang, C. Ottenheim, M. Weingarten, et al., Microbial utilization of next-generation feedstocks for the biomanufacturing of value-added chemicals and food ingredients, Front. Bioeng. Biotechnol. 10 (2022) 874612.
[41] S. Zhou, S. Lama, J. Jiang, et al., Use of acetate for the production of 3-hydroxypropionic acid by metabolically-engineered *Pseudomonas denitrificans*, Bioresour. Technol. 307 (2020) 123194.
[42] Y. Deng, N. Ma, K. Zhu, et al., Balancing the carbon flux distributions between the TCA cycle and glyoxylate shunt to produce glycolate at high yield and titer in *Escherichia coli*, Metab. Eng. 46 (2018) 28–34.
[43] S. Lama, Y. Kim, D.T. Nguyen, et al., Production of 3-hydroxypropionic acid from acetate using metabolically-engineered and glucose-grown *Escherichia coli*, Bioresour. Technol. 320 (Pt A) (2021) 124362.
[44] Z. Chang, W. Dai, Y. Mao, et al., Enhanced 3-hydroxypropionic acid production from acetate via the malonyl-CoA pathway in *Corynebacterium glutamicum*, Front. Bioeng. Biotechnol. 9 (2021) 808258.
[45] S. Yang, S. Li, X. Jia, Production of medium chain length polyhydroxyalkanoate from acetate by engineered *Pseudomonas putida* KT2440, J. Ind. Microbiol. Biotechnol. 46 (6) (2019) 793–800.
[46] N. Wei, J. Quarterman, S.R. Kim, et al., Enhanced biofuel production through coupled acetic acid and xylose consumption by engineered yeast, Nat. Commun. 4 (2013) 2580.
[47] X.F. Huang, Y. Shen, H.J. Luo, et al., Enhancement of extracellular lipid production by oleaginous yeast through preculture and sequencing batch culture strategy with acetic acid, Bioresour. Technol. 247 (2018) 395–401.
[48] G. Gong, X. Zhang, T. Tan, Simultaneously enhanced intracellular lipogenesis and beta-carotene biosynthesis of *Rhodotorula glutinis* by light exposure with sodium acetate as the substrate, Bioresour. Technol. 295 (2020) 122274.
[49] L. Chen, W. Yan, X. Qian, et al., Increased lipid production in *Yarrowia lipolytica* from acetate through metabolic engineering and cosubstrate fermentation, ACS Synth. Biol. 10 (11) (2021) 3129–3138.
[50] A. Kovilein, J. Umpfenbach, K. Ochsenreither, Acetate as substrate for L-malic acid production with *Aspergillus oryzae* DSM 1863, Biotechnol. Biofuels. 14 (1) (2021) 48.
[51] Z. Dai, H. Zhou, S. Zhang, et al., Current advance in biological production of malic acid using wild type and metabolic engineered strains, Bioresour. Technol. 258 (2018) 345–353.
[52] Z. Chi, Z.P. Wang, G.Y. Wang, et al., Microbial biosynthesis and secretion of L-malic acid and its applications, Crit. Rev. Biotechnol. 36 (1) (2016) 99–107.
[53] A. Kövilein, C. Kubisch, L. Cai, et al., Malic acid production from renewables: a review, J. Chem. Technol. Biot. 95 (3) (2019) 513–526.
[54] Z. Dai, F. Guo, S. Zhang, et al., Bio-based succinic acid: an overview of strain development, substrate utilization, and downstream purification, Biofuels Bioprod. Bior. 14 (5) (2019) 965–985.
[55] J. Lu, J. Li, H. Gao, et al., Recent progress on bio-succinic acid production from lignocellulosic biomass, World J. Microbiol. Biotechnol. 37 (1) (2021) 16.
[56] Y. Li, B. Huang, H. Wu, et al., Production of succinate from acetate by metabolically engineered *Escherichia coli*, ACS Synth. Biol. 5 (11) (2016) 1299–1307.
[57] B. Huang, H. Yang, G. Fang, et al., Central pathway engineering for enhanced succinate biosynthesis from acetate in *Escherichia coli*, Biotechnol. Bioeng. 115 (4) (2018) 943–954.
[58] W. Li, J. Chen, C.X. Liu, et al., Microbial production of glycolate from acetate by metabolically engineered *Escherichia coli*, J. Biotechnol. 291 (2019) 41–45.
[59] L. Salusjarvi, M. Toivari, M.L. Vehkomaki, et al., Production of ethylene glycol or glycolic acid from D-xylose in *Saccharomyces cerevisiae*, Appl. Microbiol. Biotechnol. 101 (22) (2017) 8151–8163.
[60] T. Zhu, D. Yao, D. Li, et al., Multiple strategies for metabolic engineering of *Escherichia coli* for efficient production of glycolate, Biotechnol. Bioeng. 118 (12) (2021) 4699–4707.
[61] S.S. Lee, J. Park, Y.B. Heo, et al., Case study of xylose conversion to glycolate in *Corynebacterium glutamicum*: current limitation and future perspective of the CRISPR-Cas systems, Enzyme. Microb. Technol. 132 (2020) 109395.
[62] Y. Yu, M. Shao, D. Li, et al., Construction of a carbon-conserving pathway for glycolate production by synergetic utilization of acetate and glucose in *Escherichia coli*, Metab. Eng. 61 (2020) 152–159.
[63] W.C. Moxley, M.A. Eiteman, Pyruvate production by *Escherichia coli* by use of pyruvate dehydrogenase variants, Appl. Environ. Microbiol. 87 (13) (2021) e0048721.
[64] Y.-.Y. Da, Z.-.H. Liu, R. Zhu, et al., Coutilization of glucose and acetate for the production of pyruvate by engineered *Escherichia coli*, Biochem. Eng. J. 170 (2021) 107990.
[65] S. Krull, A. Hevekerl, A. Kuenz, et al., Process development of itaconic acid production by a natural wild type strain of *Aspergillus terreus* to reach industrially relevant final titers, Appl. Microbiol. Biotechnol. 101 (10) (2017) 4063–4072.
[66] A.H. Hossain, A. Li, A. Brickwedde, et al., Rewiring a secondary metabolite pathway towards itaconic acid production in *Aspergillus niger*, Microb. Cell Fact. 15 (1) (2016) 130.
[67] B.C. Saha, Emerging biotechnologies for production of itaconic acid and its applications as a platform chemical, J. Ind. Microbiol. Biotechnol. 44 (2) (2017) 303–315.
[68] M.H. Noh, H.G. Lim, S.H. Woo, et al., Production of itaconic acid from acetate by engineering acid-tolerant *Escherichia coli* W, Biotechnol. Bioeng. 115 (3) (2018) 729–738.
[69] M. Merkel, D. Kiefer, M. Schmollack, et al., Acetate-based production of itaconic acid with *Corynebacterium glutamicum* using an integrated pH-coupled feeding control, Bioresour. Technol. 351 (2022) 126994.
[70] F. de Fouchécour, A.-.K. Sánchez-Castañeda, C. Saulou-Bérion, et al., Process engineering for microbial production of 3-hydroxypropionic acid, Biotechnol. Adv. 36 (4) (2018) 1207–1222.
[71] J. Jiang, B. Huang, H. Wu, et al., Efficient 3-hydroxypropionic acid production from glycerol by metabolically engineered *Klebsiella pneumoniae*, Bioresour. Bioprocess. 5 (1) (2018) 34.
[72] R.Y. Ji, Y. Ding, T.Q. Shi, et al., Metabolic engineering of yeast for the production of 3-hydroxypropionic acid, Front. Microbiol. 9 (2018) 2185.

[73] N. Lai, Y. Luo, P. Fei, et al., One stone two birds: biosynthesis of 3-hydroxypropionic acid from CO_2 and syngas-derived acetic acid in *Escherichia coli*, Synth. Syst. Biotechnol. 6 (3) (2021) 144–152.
[74] J. Lee, S. Cha, C. Kang, et al., Efficient conversion of acetate to 3-hydroxypropionic acid by engineered *Escherichia coli*, Catalysts 8 (11) (2018) 525.
[75] Y.R. Ma, K.F. Wang, W.J. Wang, et al., Advances in the metabolic engineering of *Yarrowia lipolytica* for the production of terpenoids, Bioresour. Technol. 281 (2019) 449–456.
[76] X. Xu, M. Xie, Q. Zhao, et al., Microbial production of mevalonate by recombinant *Escherichia coli* using acetic acid as a carbon source, Bioengineered 9 (1) (2018) 116–123.
[77] T. Schubert, Production routes of advanced renewable C1 to C4 alcohols as biofuel components-a review, Biofuel Bioprod. Bior. 14 (4) (2020) 845–878.
[78] Y. Li, S. Yang, D. Ma, et al., Microbial engineering for the production of C2-C6 organic acids, Nat. Prod. Rep. 38 (8) (2021) 1518–1546.
[79] A.K. Gombert, A.J. van Maris, Improving conversion yield of fermentable sugars into fuel ethanol in 1st generation yeast-based production processes, Curr. Opin. Biotechnol. 33 (2015) 81–86.
[80] F.V. Gambacorta, J.J. Dietrich, Q. Yan, et al., Rewiring yeast metabolism to synthesize products beyond ethanol, Curr. Opin. Chem. Biol. 59 (2020) 182–192.
[81] C.G. Liu, Y. Xiao, X.X. Xia, et al., Cellulosic ethanol production: progress, challenges and strategies for solutions, Biotechnol. Adv. 37 (3) (2019) 491–504.
[82] L.R. Lynd, X. Liang, M.J. Biddy, et al., Cellulosic ethanol: status and innovation, Curr. Opin. Biotechnol. 45 (2017) 202–211.
[83] H.-.M. Lee, B.-.Y. Jeon, M.-.K. Oh, Microbial production of ethanol from acetate by engineered *Ralstonia eutropha*, Biotechnol. Bioproc. E. 21 (3) (2016) 402–407.
[84] P. Gu, L. Liu, Q. Ma, et al., Metabolic engineering of *Escherichia coli* for the production of isobutanol: a review, World J. Microbiol. Biotechnol. 37 (10) (2021) 168.
[85] Y. Su, W. Shao, A. Zhang, et al., Improving isobutanol tolerance and titers through EMS mutagenesis in *Saccharomyces cerevisiae*, FEMS Yeast Res 21 (2) (2021) foab012.
[86] S.A.E. Heider, V.F. Wendisch, Engineering microbial cell factories: metabolic engineering of *Corynebacterium glutamicum* with a focus on non-natural products, Biotechnol. J. 10 (8) (2015) 1170–1184.
[87] S.A. Park, S.K. Bhatia, H.A. Park, et al., *Bacillus subtilis* as a robust host for biochemical production utilizing biomass, Crit. Rev. Biotechnol. 41 (6) (2021) 827–848.
[88] H.S. Song, H.M. Seo, J.M. Jeon, et al., Enhanced isobutanol production from acetate by combinatorial overexpression of acetyl-CoA synthetase and anaplerotic enzymes in engineered *Escherichia coli*, Biotechnol. Bioeng. 115 (8) (2018) 1971–1978.
[89] K. Novak, R. Kutscha, S. Pflugl, Microbial upgrading of acetate into 2,3-butanediol and acetoin by *E. coli* W, Biotechnol. Biofuels. 13 (2020) 177.
[90] V.C. Kalia, S.K. Singh Patel, R. Shanmugam, et al., Polyhydroxyalkanoates: trends and advances toward biotechnological applications, Bioresour. Technol. 326 (2021) 124737.
[91] Y. Zheng, J.C. Chen, Y.M. Ma, et al., Engineering biosynthesis of polyhydroxyalkanoates (PHA) for diversity and cost reduction, Metab. Eng. 58 (2020) 82–93.
[92] M. Kumar, R. Rathour, R. Singh, et al., Bacterial polyhydroxyalkanoates: opportunities, challenges, and prospects, J. Clean. Prod. 263 (2020) 121500.
[93] G.Q. Chen, X.R. Jiang, Engineering bacteria for enhanced polyhydroxyalkanoates (PHA) biosynthesis, Synth. Syst. Biotechnol. 2 (3) (2017) 192–197.
[94] S. Obruča, P. Dvořák, P. Sedláček, M. Moller, K. Sedlář, I. Pernicová, D. Šafránek, Polyhydroxyalkanoates synthesis by halophiles and thermophiles: towards sustainable production of microbial bioplastics, Biotechnol. Adv. 58 (2022) 107906.
[95] J. Chen, W. Li, Z.Z. Zhang, et al., Metabolic engineering of *Escherichia coli* for the synthesis of polyhydroxyalkanoates using acetate as a main carbon source, Microb. Cell Fact. 17 (1) (2018) 102.
[96] L.L. Shi, Y.Y. Da, W.T. Zheng, et al., Production of polyhydroxyalkanoate from acetate by metabolically engineered *Aeromonas hydrophilia*, J. Biosci. Bioeng. 130 (3) (2020) 290–294.
[97] R. Sawangkeaw, S. Ngamprasertsith, A review of lipid-based biomasses as feedstocks for biofuels production, Renew. Sust. Energ. Rev. 25 (2013) 97–108.
[98] Z. Gong, W. Zhou, H. Shen, et al., Co-fermentation of acetate and sugars facilitating microbial lipid production on acetate-rich biomass hydrolysates, Bioresour. Technol. 207 (2016) 102–108.
[99] L. Liu, Y. Zhao, X. Jiang, et al., Lipid accumulation of *Chlorella pyrenoidosa* under mixotrophic cultivation using acetate and ammonium, Bioresour. Technol. 262 (2018) 342–346.
[100] P. Hu, S. Chakraborty, A. Kumar, et al., Integrated bioprocess for conversion of gaseous substrates to liquids, Proc. Natl. Acad. Sci. U. S. A. 113 (14) (2016) 3773–3778.
[101] W. Zhang, J. Wu, Y.J. Zhou, et al., Enhanced lipid production by *Rhodotorula glutinis* CGMCC 2.703 using a two-stage pH regulation strategy with acetate as the substrate, Energy Sci. Eng. 7 (5) (2019) 2077–2085.
[102] X. Qian, O. Gorte, L. Chen, et al., Continuous self-provided fermentation for microbial lipids production from acetate by using oleaginous yeasts *Cryptococcus podzolicus* and *Trichosporon porosum*, Renew. Energ. 146 (2020) 737–743.
[103] Y. Tashiro, S.H. Desai, S. Atsumi, Two-dimensional isobutyl acetate production pathways to improve carbon yield, Nat. Commun. 6 (1) (2015) 7488.
[104] E.R. Marella, C. Holkenbrink, V. Siewers, et al., Engineering microbial fatty acid metabolism for biofuels and biochemicals, Curr. Opin. Biotech. 50 (2018) 39–46.
[105] Y. Xiao, Z. Ruan, Z. Liu, et al., Engineering *Escherichia coli* to convert acetic acid to free fatty acids, Biochem. Eng. J. 76 (2013) 60–69.
[106] K. Jing, S. He, T. Chen, et al., Enhancing beta-carotene biosynthesis and gene transcriptional regulation in *Blakeslea trispora* with sodium acetate, Biochem. Eng. J. 114 (2016) 10–17.
[107] J. Yang, Q. Nie, Engineering *Escherichia coli* to convert acetic acid to beta–caryophyllene, Microb. Cell Fact. 15 (2016) 74.
[108] M. Jo, M.H. Noh, H.G. Lim, et al., Precise tuning of the glyoxylate cycle in *Escherichia coli* for efficient tyrosine production from acetate, Microb. Cell Fact. 18 (1) (2019) 57.
[109] H. Yang, B. Huang, N. Lai, et al., Metabolic engineering of *Escherichia coli* carrying the hybrid acetone-biosynthesis pathway for efficient acetone biosynthesis from acetate, Microb. Cell Fact. 18 (1) (2019) 6.