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Harnessing acetogenic bacteria for one-carbon valorization toward sustainable chemical production

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The pressing climate change issues have intensified the need for a rapid transition towards a bio-based circular carbon economy. Harnessing acetogenic bacteria as biocatalysts to convert C1 compounds such as CO₂, CO, formate, or methanol into value-added multicarbon chemicals is a promising solution for both carbon capture and utilization, enabling sustainable and green chemical production. Recent advances in the metabolic engineering of acetogens have expanded the range of commodity chemicals and biofuels produced from C1 compounds. However, producing energy-demanding high-value chemicals on an industrial scale from C1 substrates remains challenging because of the inherent energetic limitations of acetogenic bacteria. Therefore, overcoming this hurdle is necessary to scale up the acetogenic C1 conversion process and realize a circular carbon economy. This review overviews the acetogenic bacteria and their potential as sustainable and green chemical production platforms. Recent efforts to address these challenges have focused on enhancing the ATP and redox availability of acetogens to improve their energetics and conversion performances. Furthermore, promising technologies that leverage low-cost, sustainable energy sources such as electricity and light are discussed to improve the sustainability of the overall process. Finally, we review emerging technologies that accelerate the development of high-performance acetogenic bacteria suitable for industrial-scale production and address the economic sustainability of acetogenic C1 conversion. Overall, harnessing acetogenic bacteria for C1 valorization offers a promising route toward sustainable and green chemical production, aligning with the circular economy concept.

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1 Introduction

The imminent threat of climate change is pressing humanity to reduce global greenhouse gas (GHG) emissions.¹ A circular carbon economy has emerged as a promising framework in the quest for sustainable solutions to mitigate climate change.² The circular carbon economy aims to minimize carbon emissions by closing the carbon loop, wherein carbon is continuously recycled and reused rather than being released into the atmosphere. This initiative has driven the development of sustainable carbon-negative manufacturing technologies for chemical production.

One strategy to achieve this goal is adopting carbon capture and utilization (CCU) technologies, which capture carbon dioxide (CO₂) at the point of emission or after emission and utilize

it as a feedstock for producing value-added compounds such as commodity chemicals and fuels.³ By decreasing both direct CO₂ emissions and the reliance of the chemical industry on fossil fuels as a carbon source, CCU offers routes to carbon-negative manufacturing and a circular economy.⁴ Among various CCU technologies, biocatalysts represent a greener alternative to chemical catalyst-based CCUs, offering several advantages.⁵ They are biodegradable, safe, and nontoxic, operating under mild conditions, which leads to less energy-intensive processes, unlike chemical catalysts that operate under extreme conditions and contain toxic, harmful compounds. Therefore, conforming to 10 of the 12 principles of green chemistry,⁶ biocatalysts play a vital role in developing sustainable and green CCU technologies.

One promising approach for a greener CCU involves harnessing acetogenic bacteria (acetogens) as biocatalysts for sustainable chemical production from waste carbon sources. Acetogens possess a unique capability to convert one-carbon (C1) compounds, such as CO₂, CO, formate, or methanol, into value-added chemicals *via* the Wood-Ljungdahl pathway (WLP), known as the most

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energetically efficient CO_2 fixation pathway in nature.^{7,8} Given the natural abundance, low production cost, and availability of C1 compounds as industrial waste by-products, leveraging acetogens to valorize these substrates offers a sustainable and green alternative for chemical synthesis.^{9,10} With advancements in genetic tools developed for acetogens, over 50 different chemicals have been produced from C1 compounds.^{7,11,12} While some of these chemicals have already achieved industrially relevant performance levels, they are limited to short-chain compounds such as ethanol. Because of the growing interest in longer-chain compounds owing to their higher market value, it is imperative to realize industrial-scale production of these energy-demanding high-value chemicals from C1 substrates. However, this remains challenging because of the inherent energetic limitations of acetogens, which operate at the thermodynamic limit of life.^{13,14} In pursuit of addressing this challenge, numerous studies have recently been conducted, aiming to fully exploit the potential of acetogens as green chemical production platforms.

This review overviews acetogens and their potential as sustainable, green chemical production platforms. Recent efforts to overcome the challenges hindering their industrial-scale application have focused on improving their energetics regarding ATP and redox availability. Furthermore, promising technologies that leverage low-cost and sustainable energy sources such as electricity and light are discussed to enhance both the performance and sustainability of the acetogenic C1 conversion process. Finally, emerging technologies for the development of high-performance strains and the economic sustainability of acetogenic C1 bioconversion are reviewed to accelerate advancements in this field.

2 One-carbon valorization *via* acetogenic bacteria

2.1 The Wood-Ljungdahl pathway and energy conservation system

Acetogens are strictly anaerobic bacteria capable of fixing CO_2 *via* the WLP, recognized as the most energetically efficient carbon fixation pathway in nature, as it requires only one mole of adenosine triphosphate (ATP) for carbon fixation.¹⁵ In the WLP, two moles of CO_2 are reduced to one mole of two-carbon (C2) acetyl-CoA through a series of stepwise reactions catalyzed by metalloenzymes, with tetrahydrofolate (THF) as a C1-carrier (Fig. 1). These reactions occur in two branches: the methyl-branch and carbonyl-branch, which generate methyl and carbonyl groups, respectively, contributing to the formation of acetyl-CoA.¹⁶ In the methyl branch, CO_2 is first reduced to formate by formate dehydrogenase (Fdh). Subsequently, formate is activated to formyl-THF by formyl-THF synthetase (Fhs), driven by ATP hydrolysis. Formyl-THF is further reduced to methyl-THF through a series of reduction reactions catalyzed by formyl-THF cyclohydrolase (Fch), methenyl-THF dehydrogenase (Mthfd), and methylene-THF reductase (Mthfr). Finally, methyl-THF condenses with CO derived from the carbonyl

branch and CoA on CO dehydrogenase/acetyl-CoA synthase (CODH/ACS), resulting in acetyl-CoA (Fig. 1).^{16,17}

This stepwise reduction of two CO_2 molecules to acetyl-CoA *via* the WLP requires one ATP molecule and eight electrons. These electrons are provided by intracellular electron carriers such as ferredoxin (Fd) and nicotinamide adenine dinucleotide (NADH), which serve as reducing equivalents. As CO_2 is fully oxidized, H_2 or CO must be utilized as an energy source to provide these reducing equivalents. When H_2 is the energy source, electron-bifurcating hydrogenase (Hyd) oxidizes H_2 , yielding reduced Fd (Fd_{red}) and NADH.¹⁸ Despite the higher redox potential of H_2 ($E^{\circ'} = -414$ mV) compared to that of Fd ($E^{\circ'} = -500$ mV), reduction of Fd with H_2 becomes possible through the energy bifurcation mechanism of Hyd. This mechanism couples the exergonic electron flow from H_2 to NAD^+ ($E^{\circ'} = -320$ mV) with the endergonic electron flow from H_2 to Fd.¹³ On the other hand, when CO is utilized as the energy source, direct reduction of Fd with CO is feasible due to the low redox potential of the CO_2/CO couple ($E^{\circ'} = -520$ mV). CODH oxidizes CO to CO_2 and produces Fd_{red} .¹³ The reducing equivalents generated by Hyd or CODH are then supplied to the WLP. In certain acetogens, NADPH serves as a cofactor for the WLP operation. NADPH is generated from the obtained Fd_{red} and NADH through the action of NADH-dependent $\text{Fd}_{\text{red}}\text{:NAD}^+$ oxidoreductase (Nfn) or *Sporomusa*-type Nfn (Stn) complex (Fig. 1).^{19,20}

The resulting acetyl-CoA is converted to acetate *via* acetyl-phosphate, providing one ATP molecule *via* substrate-level phosphorylation (SLP) in the acetate kinase reactions to compensate for one ATP molecule consumed in the WLP. This results in a net-zero ATP yield for the overall process.²¹ However, in energy-limited environments where acetogens grow on gaseous substrates, they must generate additional ATP to sustain life. This is accomplished through an energy conservation system in which Fd-driven membrane-bound respiratory enzymes and ATP synthases cooperate to synthesize chemiosmotic ATP (Fig. 1). Acetogens possess one of two types of respiratory enzymes found in acetogens: Rnf (*Rhodobacter* nitrogen fixation) and energy-converting hydrogenase (Ech), which are classified based on their final electron acceptor. The Rnf complex is an ion-translocating Fd: NAD^+ oxidoreductase that transfers electrons from Fd_{red} to NAD^+ .^{22,23} The energy released in this exergonic reaction is used to generate a transmembrane proton (H^+) or sodium (Na^+) gradient. The Rnf complex plays a crucial role in the acetogenic metabolism, serving both as a redox-balancing and energy-conserving module.²⁴ In contrast, the Ech complex acts as an Fd: H^+ oxidoreductase, transferring electrons from Fd_{red} to H^+ to establish a transmembrane H^+ gradient. This ion gradient drives ATP formation *via* ATP synthase, leading to chemiosmotic ion gradient-driven phosphorylation (Fig. 1). Given that the overall WLP process yields net-zero ATP, the energy conservation system is primarily responsible for ATP production during acetogenic C1 conversion.¹³ Although the final ATP yields depend on the energy systems present in acetogens,^{14,25} for example, the Rnf-containing, Na^+ -dependent acetogen *Acetobacterium woodii* yields 1 mole of ATP per 3.3 Na^+ translocation.



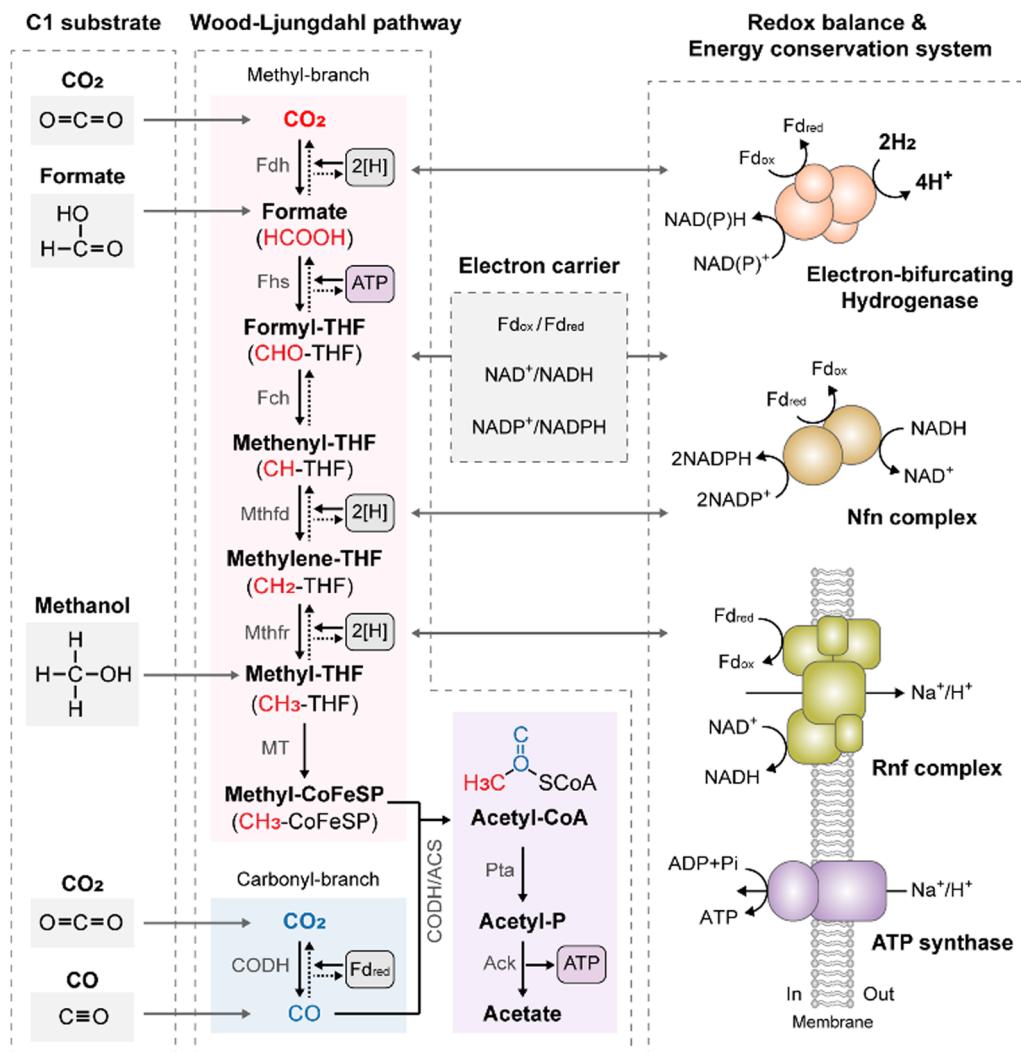


Fig. 1 The Wood–Ljungdahl pathway and energy conservation system in acetogens. Fdh, formate dehydrogenase; Fhs, formyl-THF synthetase; Fch, formyl-THF cyclohydrolase; Mthfd, methenyl-THF dehydrogenase; Mthfr, methylene-THF reductase; MT, methyltransferase; CODH/ACS, CO dehydrogenase/acetyl-CoA synthase; Pta, phosphotransacetylase; Ack, acetate kinase; $[H]$, reducing equivalent ($[H] = 1e^- + 1H^+$).

This resulted in 0.3 moles of ATP per mole of acetate from CO_2/H_2 .

2.2 Upgrading one-carbon to multicarbon chemicals via acetogens

Acetogens comprise over 100 species isolated from diverse habitats (e.g., soils, sediments, sludge, and the intestinal tracts of numerous animals), exhibiting both phylogenetic and metabolic versatility. They are capable of growing on a wide range of substrates, including sugars, carboxylic acids, aldehydes, and CO_2 .^{16,24} Despite their extensive diversity, only a subset of acetogenic species is currently the focus of active investigation and application as biocatalysts for C1 bioconversion (Table 1). This selective attention is due to their notable features, which include the ability to utilize diverse C1 compounds (e.g., CO_2 , CO, formate, and methanol), genetic accessibility to available genome sequences and genetic tools, and relatively well-characterized physiology compared with other less-explored

acetogens. These ten representative acetogens and their characteristics are listed in Table 1.

Depending on their genetic and metabolic features, acetogens can convert C1 substrates into various multicarbon chemicals other than acetate (Table 1 and Fig. 2). Acetogens such as *A. woodii*, *Sporomusa ovata*, *Moorella thermoacetica*, and *Thermoanaerobacter kivui* have been reported to exclusively produce acetate as the sole end-product of acetogenic C1 conversion,^{27,28,44,45,47,49} while others are capable of producing ethanol, lactate, 2,3-butanediol, butyrate, butanol, caproate, and hexanol. Among them, ethanol production from waste gases utilizing *Clostridium autoethanogenum* has already been commercialized by LanzaTech.⁷ *Clostridium carboxidivorans* stands out as an intriguing acetogen due to its broad product profile, encompassing acetate, ethanol, butyrate, butanol, caproate, and hexanol.³⁴ Of particular interest are the longest-chain compounds, caproate and hexanol (C6 compounds), which exhibit significantly higher energy densities than

Table 1 Characteristics of representative acetogenic bacteria applied for C1 bioconversion

| Acetogenic bacteria | C1 substrates | Temp. (°C) | pH | Natural product | Genetic Ref. /entry> | |
|---|---|------------|---------|--|----------------------|-----------|
| <i>Acetobacterium woodii</i> | CO ₂ , methanol, formate | 30 | 7.0–7.4 | Acetate | 26 | 27 and 28 |
| <i>Butyribacterium methylotrophicum</i> | CO ₂ , CO, methanol, formate | 37 | 7.5 | Acetate, ethanol, lactate, butyrate, butanol | 29 | 30 |
| <i>Clostridium autoethanogenum</i> | CO ₂ , CO | 37 | 5.8–6.0 | Acetate, ethanol, lactate, 2,3-butanediol | 31 | 32 |
| <i>Clostridium carboxidivorans</i> | CO ₂ , CO | 37 | 5.0–7.0 | Acetate, ethanol, butyrate, butanol, caproate, hexanol | 33 | 34 and 35 |
| <i>Clostridium ljungdahlii</i> | CO ₂ , CO | 37 | 6.0 | Acetate, ethanol, lactate, 2,3-butanediol | 36 | 32 |
| <i>Clostridium</i> sp. AWRP | CO ₂ , CO | 37 | 6.0 | Acetate, ethanol, 2,3-butanediol | 37 | 38 |
| <i>Eubacterium limosum</i> | CO ₂ , CO, methanol, formate | 37 | 7.0 | Acetate, lactate, butyrate, butanol, caproate | 39 | 40–42 |
| <i>Sporomusa ovata</i> | CO ₂ , methanol, formate | 34 | 6.3 | Acetate | 43 | 44 and 45 |
| <i>Moorella thermoacetica</i> | CO ₂ , CO, methanol, formate | 55 | 6.5–6.8 | Acetate | 46 | 47 |
| <i>Thermoanaerobacter kivui</i> | CO ₂ , CO, formate | 66 | 6.4 | Acetate | 48 | 49 |

short-chain acids and alcohols. This feature makes them promising platform chemicals for producing biodiesel and jet fuels.^{50,51} A recently discovered acetogenic species, *Clostridium leticellarii*, has been found to produce a novel compound within the acetogen isobutyrate from CO₂/H₂, adding to the diversity of chemicals generated by acetogens.^{52,53}

Despite the promising capabilities of the *Clostridium* species in producing various acids and alcohols, their utilization as C1 substrates is limited to gaseous forms (CO₂ and CO). Expanding the substrate range, *Butyribacterium methylotrophicum* and *Eubacterium limosum* emerge as promising biocatalysts with the ability to efficiently utilize liquid C1 substrates (methanol and formate) alongside C1 gases.^{30,40} Methanol and formate are promising liquid C1 substrates, bypassing the gas–liquid mass transfer issue posed by gaseous substrates and providing higher energy efficiency than CO₂/H₂ or CO.⁵⁴ These substrates can be directly incorporated into the methyl branch of the WLP, where reducing equivalents can be generated by operating the WLP in the reverse direction (Fig. 1).^{30,40} *B. methylotrophicum* and *E. limosum* are known for butyrate production, and in some instances, they can produce more reduced butanol when supplied with methanol.

Extensive research has focused on metabolic engineering to expand the product spectrum of acetogens with recent advancements in genetic tools developed for acetogens (Table 1). Table 2 lists the chemicals produced by engineered acetogens reported within the last five years. Fifteen chemicals were tested and produced during this period in acetogens starting with acetyl-CoA as the precursor (Fig. 2). *A. woodii* and *M. thermoacetica*, which were initially capable of producing only acetate, were engineered to synthesize ethanol, lactate, acetone, isopropanol, butyrate, isobutanol, poly-3-hydroxybutyrate (PHB), and caproate (Table 2). These were achieved by introducing the corresponding synthesis pathways from other organisms or disrupting endogenous genes without pathway introduction. The latter approach was particularly intriguing as it unraveled novel metabolic traits of acetogens. For instance, disrupting methylene-THF reductase in *A. woodii* enables the production of ethanol and lactate alongside formate, molecular hydrogen, and acetate.⁵⁵ Similarly, deleting hydrogenases in *A. woodii* produces lactate from fructose or methyl groups + CO.⁵⁶ Although these compounds are not produced solely on C1 substrates, these findings suggest the

potential to engineer strains that convert fructose or even C1 compounds into reduced products, such as ethanol or lactate. However, numerous efforts have focused on introducing heterologous pathways in *A. woodii*, leading to acetone synthesis,⁵⁷ isopropanol,^{58,59} butyrate,⁶⁰ isobutanol,⁶¹ PHB,⁶² and caproate.⁶³ In comparison, only the ethanol,⁶⁴ acetone,^{65,66} and isopropanol⁶⁷ pathways have been demonstrated in *M. thermoacetica*.

Engineering *E. limosum* and *B. methylotrophicum* have also been successful, albeit restricted to the chemical production of C3 or C4, including acetone,⁷¹ acetoin,⁴¹ 2,3-butanediol,⁷⁴ and butanol^{30,71} (Table 2). On the other hand, *Clostridium* species have been actively engineered to produce various non-native chemicals and fuels, including ethylene glycol,⁶⁸ acetone,⁷⁰ isopropanol,^{72,73} butyrate,⁷⁵ isobutanol,^{61,76} 3-hydroxybutyrate (3-HB),⁷² PHB,⁷⁸ ethyl acetate,⁷⁹ and hexanol⁸⁰ (Table 2). Of these, ethylene glycol⁶⁸ and ethyl acetate⁷⁹ production demonstrated in *C. autoethanogenum* is the first report of acetogens, as they are novel compounds not naturally produced in acetogens. Notably, acetone,⁷⁰ isopropanol,⁷⁰ and 3-HB⁷⁷ have recently been produced at relatively high levels from C1 gases, reaching productivities of up to 2.5 g L⁻¹ h⁻¹, 3 g L⁻¹ h⁻¹, and 1.5 g L⁻¹ h⁻¹, respectively, in *C. autoethanogenum*. These results demonstrate the potential of the acetogenic C1 conversion as a platform for the sustainable production of chemicals and fuels.

2.3 Challenges in acetogenic C1 conversion

Engineered acetogens have demonstrated the ability to produce various multicarbon chemicals; however, their product titers and productivities remain limited, typically reaching only mg L⁻¹ or mg L⁻¹ h⁻¹ scales on C1 substrates (Table 2). Although high-level production of 3-HB, acetone, and isopropanol has been achieved in *C. autoethanogenum*, there is still room for improvement in higher carbon production, given the increasing interest for longer-chain products, such as butyrate, caproate, and hexanol due to their substantially higher market value (e.g., butyrate has 3–5 times higher value than acetate).^{51,53,81} While *C. carboxidivorans* can naturally produce caproate and hexanol, its product titer, yield, and selectivity are insufficient for large-scale production due to its predominant production of C2 compounds (acetate and ethanol) with small amounts of C4 and C6 products.^{34,35} Despite engineering efforts in *A. woodii* and *C. ljungdahlii* showing potential for caproate and hexanol production, respectively, titers



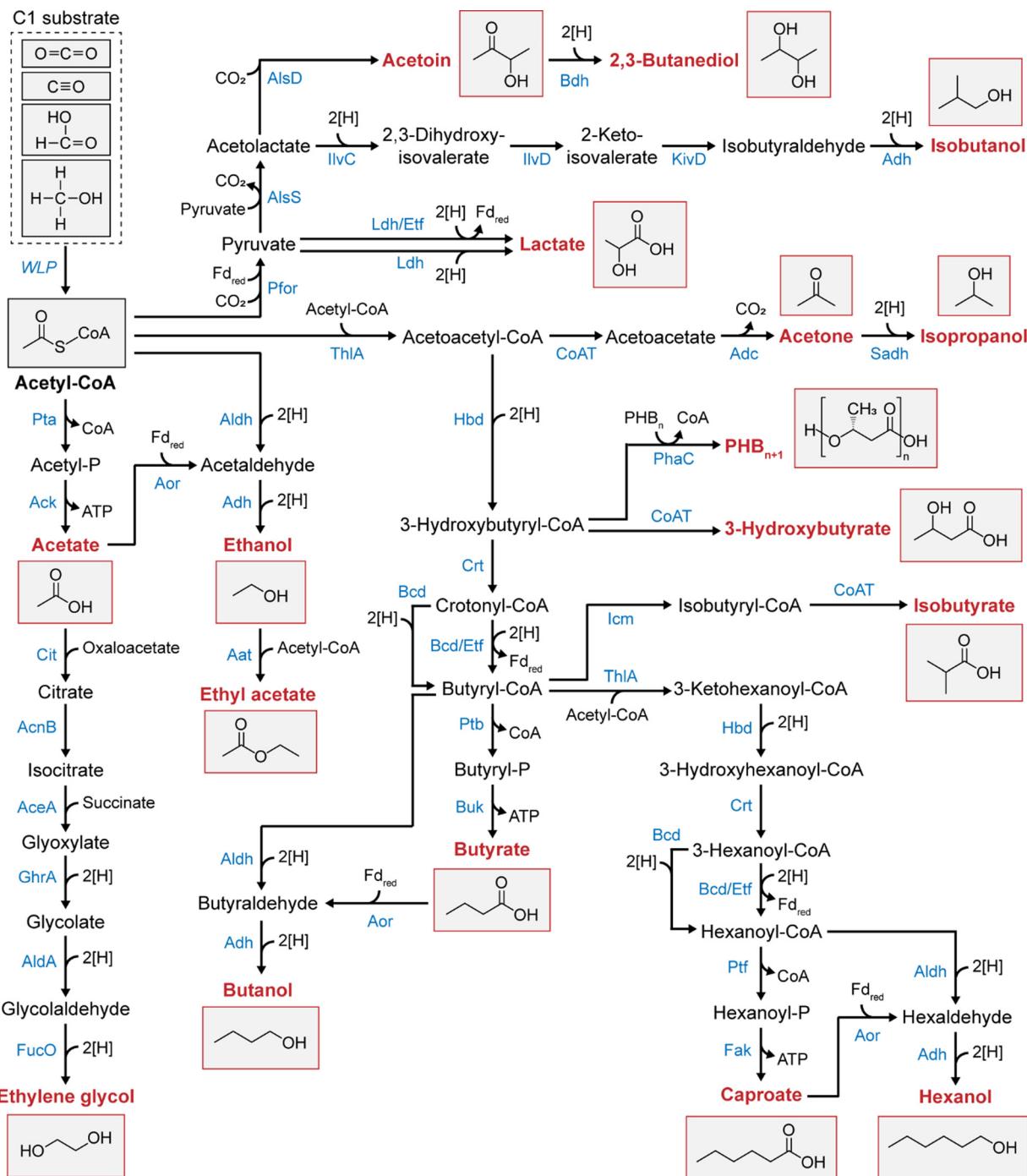


Fig. 2 Biosynthesis pathways of multicarbon chemicals converted from acetyl-CoA in wild-type and engineered acetogens. Abbreviations: CoA, coenzyme A; PHB, poly-3-hydroxybutyrate; Pfor, pyruvate:ferredoxin oxidoreductase; AlsS, acetolactate synthase; AlsD, acetolactate decarboxylase; BdH, 2,3-butanediol dehydrogenase; Ldh, lactate dehydrogenase; Ldh/Etf, bifurcating Ldh; IlvC, ketol-acid reductoisomerase; IlvD, dihydroxy-acid dehydratase; KivD, ketoisovalerate decarboxylase; Pta, phosphotransacetylase; Ack, acetate kinase; Aor, aldehyde:ferredoxin oxidoreductase; Aldh, aldehyde dehydrogenase; Adh, alcohol dehydrogenase; Aat, alcohol acetyltransferase; ThlA, thiolase; Hbd, 3-hydroxybutyryl-CoA dehydrogenase; Crt, crotonase; Bcd, butyryl-CoA dehydrogenase; Bcd/Etf, bifurcating Bcd; PtB, phosphotransbutyrylase; Btk, butyrate kinase; Ptf, phosphotransferase; Fak, fatty acid kinase; Icm, isobutyryl-CoA mutase; CoAT, CoA transferase; PhaC, polyhydroxyalkanoate synthase; Adc, acetoacetate decarboxylase; Sadh, primary secondary alcohol dehydrogenase; Cit, citrate lyase; AcnB, aconitase; AceA, isocitrate lyase; GhrA, glyoxylate reductase; AldA, glycolaldehyde dehydrogenase; FucO, lactaldehyde reductase.

remain very low (Table 2).^{63,80} Overcoming these limitations in upgrading C1 to value-added multicarbon chemicals is crucial for

ultimately replacing the fossil-based chemical industry with an acetogenic C1 conversion platform.

Table 2 A list of chemicals produced by engineered acetogens reported within the last 5 years

| Chemical | Acetogenic bacteria | Substrate | Product titer (g L ⁻¹) | Productivity | Production scale | Ref. |
|---|----------------------------|--|------------------------------------|---|----------------------|------|
| Ethanol (C ₂ H ₆ O) | <i>M. thermoacetica</i> | Fructose/CO ₂ | 0.810 | — | Serum bottle | 64 |
| | <i>A. woodii</i> | Fructose | 0.138 | — | Resting cell | 55 |
| Ethylene glycol (C ₂ H ₆ O ₂) | <i>C. autoethanogenum</i> | Fructose | 0.394 | — | Serum bottle | 68 |
| | <i>A. woodii</i> | Fructose | 0.522 | — | Resting cell | 55 |
| Lactate (C ₃ H ₆ O ₃) | <i>A. woodii</i> | Glycine betaine + 10% CO | 1.838 | — | Resting cell | 56 |
| | <i>A. woodii</i> | CO ₂ /H ₂ | 1.693 | — | Serum bottle | 69 |
| Acetone (C ₃ H ₆ O) | <i>A. woodii</i> | CO ₂ /H ₂ | 1.249 | — | Serum bottle | 57 |
| | <i>M. thermoacetica</i> | CO ₂ /H ₂ | 0.232 | — | Serum bottle | 65 |
| Isopropanol (C ₃ H ₈ O) | <i>M. thermoacetica</i> | CO/H ₂ | 0.192 | 0.09 g gDCW ⁻¹ h ⁻¹ | Serum bottle | 66 |
| | <i>C. autoethanogenum</i> | CO/H ₂ /CO ₂ /N ₂ (50/10/30/10) | — | 2.5 g L ⁻¹ h ⁻¹ | 2-L CSTR | 70 |
| Isopropanol (C ₃ H ₈ O) | <i>E. limosum</i> | MeOH | 0.092 | — | Serum bottle | 71 |
| | <i>A. woodii</i> | CO ₂ /H ₂ | 0.871 | — | 2-L CSTR | 58 |
| Isopropanol (C ₃ H ₈ O) | <i>A. woodii</i> | CO ₂ /H ₂ | 0.834 | — | Serum bottle | 59 |
| | <i>M. thermoacetica</i> | CO/H ₂ | 0.120 | 0.03 g gDCW ⁻¹ h ⁻¹ | Serum bottle | 67 |
| Isopropanol (C ₃ H ₈ O) | <i>C. autoethanogenum</i> | CO/H ₂ /CO ₂ /N ₂ (50/10/30/10) | — | 3 g L ⁻¹ h ⁻¹ | 2-L CSTR | 70 |
| | <i>C. ljungdahlii</i> | CO/CO ₂ /H ₂ /N ₂ (56/20/9/15) | 13.4 | — | 2-L CSTR | 72 |
| Isopropanol (C ₃ H ₈ O) | <i>C. ljungdahlii</i> | CO/CO ₂ /H ₂ (70/20/10) | 2.4 | — | 2-L CSTR | 73 |
| | <i>E. limosum</i> | CO/CO ₂ /H ₂ (44/22/2) | 0.070 | — | Serum bottle | 41 |
| Acetoin (C ₄ H ₈ O ₂) | <i>E. limosum</i> | CO/CO ₂ /H ₂ (66/22/2) | 1.370 | 0.0026 g L ⁻¹ h ⁻¹ | 1-L gas-lift reactor | 74 |
| 2,3-Butanediol (C ₄ H ₁₀ O ₂) | <i>A. woodii</i> | CO ₂ /MeOH | 0.015 | — | Serum bottle | 60 |
| Butyrate (C ₄ H ₈ O ₂) | <i>C. ljungdahlii</i> | CO ₂ /CO | 1.010 | — | Serum bottle | 75 |
| Butanol (C ₄ H ₁₀ O) | <i>B. methylotrophicum</i> | MeOH/formate | 0.111 | — | Serum bottle | 30 |
| Isobutanol (C ₄ H ₁₀ O) | <i>E. limosum</i> | MeOH | 0.044 | — | Serum bottle | 71 |
| Isobutanol (C ₄ H ₁₀ O) | <i>A. woodii</i> | Fructose | 0.007 | — | Serum bottle | 61 |
| | <i>C. ljungdahlii</i> | CO/CO ₂ /H ₂ (50/5/45) | 0.007 | — | Serum bottle | 61 |
| Isobutanol (C ₄ H ₁₀ O) | <i>C. ljungdahlii</i> | CO/CO ₂ /H ₂ (55/5/30) | 0.130 | — | 3-L CSTR | 76 |
| | <i>C. ljungdahlii</i> | CO/CO ₂ /H ₂ /N ₂ (56/20/9/15) | 3.0 | — | 2-L CSTR | 72 |
| 3-Hydroxybutyrate (C ₄ H ₈ O ₃) | <i>C. autoethanogenum</i> | CO/CO ₂ /H ₂ (50/40/10) | 14.6 | 1.5 g L ⁻¹ h ⁻¹ | 1.5-L CSTR | 77 |
| | <i>C. autoethanogenum</i> | CO/CO ₂ /H ₂ (50/20/20) | 0.027 | 0.00113 g L ⁻¹ h ⁻¹ | Continuous reactor | 78 |
| Ethyl acetate (C ₄ H ₈ O ₂) | <i>C. autoethanogenum</i> | CO ₂ /H ₂ | 0.024 | — | Serum bottle | 62 |
| Caproate (C ₆ H ₁₂ O ₂) | <i>A. woodii</i> | CO ₂ /H ₂ | 0.181 | — | Serum bottle | 63 |
| Hexanol (C ₆ H ₁₄ O) | <i>C. ljungdahlii</i> | CO ₂ /H ₂ | 0.393 | — | 3.7-L CSTR | 80 |

3 Overcoming energetic limitations in acetogenic C1 conversion

3.1 Impacts of electron donors on the feasibility of acetogenic C1 conversion

The low product titers and yields in acetogens are attributable to the thermodynamic feasibility of acetogenic C1 conversion, which requires the substrate to provide sufficient energy for cellular maintenance and growth.⁸² The ATP yield varies depending on the electron donors utilized (H₂, CO, or methanol) and the final products, which range from -0.4 ATP to +4.4 ATP per mol of product (Table 3).^{14,83} Notably, employing H₂ as an electron donor presents the highest ATP demands, as it results in negative ATP yields. Thus, producing chemicals other than acetate (e.g., ethanol, lactate, acetone, and butanol) under CO₂/H₂ conditions poses significant challenges. For instance, while acetate production from CO₂/H₂ is feasible with 0.3 ATP/acetate, ethanol or butanol production may not be viable because of negative ATP yields (Table 3). Under a CO₂/H₂ environment, acetogens operate their metabolism at the thermodynamic limit of feasibility.¹³ Due to the inherent energetic limitations, engineering acetogens to redirect carbon flow from acetate to other chemicals often leads to ATP shortage unless the chemical biosynthetic pathway generates ATP. Consequently, only the biosynthesis pathways generating ATP can

serve as significant products (e.g., acetate and ethanol). In contrast, ATP-demanding products (negative ATP yields) can only be produced at a higher yield if generated concurrently with other by-products that yield ATP, leading to a low product yield and selectivity.

In contrast, utilizing CO and methanol as electron donors yielded positive ATP yields for all products (Table 3), as they are more reduced substrates than CO₂/H₂. The utilization of these reduced substrates has been shown to favor the production of more reduced products (e.g., butyrate, ethanol, and butanol) by altering the metabolism of acetogens and thereby shifting the product profiles towards reduced products.^{30,40,84} *E. limosum* exhibited butyrate production when grown with methanol but not with other C1 substrates such as CO, CO₂, or formate.⁸⁴ Moreover, butanol production in *E. limosum* was only demonstrated when methanol and formate were utilized as substrates at a ratio of 7.5 : 1, resulting in butyrate as the major product.⁴⁰ Similarly, supplying both CO and methanol induced butanol formation and ethanol and lactate production in *B. methylotrophicum*, whereas methanol/CO₂ and only CO conditions did not trigger butanol formation.³⁰ These findings emphasize the significance of supplying CO or methanol as electron donors, as they play a critical role in shifting the product spectrum towards more reduced products and determining the feasibility of desired chemical production.

Table 3 Theoretical ATP yield for chemical production with H₂, CO, or methanol as an electron donor, calculated based on energetics of *A. woodii*^{14,83}

| Product | Equation | Electron donor | Metabolic pathway | ATP yield (mol ATP/mol product) |
|---------|--|----------------|-------------------|---------------------------------|
| Acetate | 2CO ₂ + 4H ₂ → CH ₃ COOH + 2H ₂ O | H ₂ | Ack | 0.3 |
| | 4CO + 2H ₂ O → CH ₃ COOH + 2CO ₂ | CO | Ack | 1.5 |
| | 4CH ₃ OH + 2CO ₂ → 3CH ₃ COOH + 2H ₂ O | MeOH | Ack | 0.8 |
| Ethanol | 2CO ₂ + 6H ₂ → C ₂ H ₅ OH + 3H ₂ O | H ₂ | Aldh-Adh | -0.1 |
| | 6CO + 3H ₂ O → C ₂ H ₅ OH + 4CO ₂ | CO | Aldh-Adh | 1.7 |
| | 6CH ₃ OH → 3C ₂ H ₅ OH + 3H ₂ O | MeOH | Aldh-Adh | 0.7 |
| Lactate | 3CO ₂ + 6H ₂ → C ₃ H ₆ O ₃ + 3H ₂ O | H ₂ | Ldh/Etf | -0.1 |
| | 6CO + 3H ₂ O → C ₃ H ₆ O ₃ + 3CO ₂ | CO | Ldh/Etf | 1.7 |
| | 6CH ₃ OH + 3CO ₂ → 3C ₃ H ₆ O ₃ + 3H ₂ O | MeOH | Ldh/Etf | 0.7 |
| Acetone | 3CO ₂ + 8H ₂ → C ₃ H ₆ O + 5H ₂ O | H ₂ | Adc | -0.4 |
| | 8CO + 3H ₂ O → C ₃ H ₆ O + 5CO ₂ | CO | Adc | 2.0 |
| | 4CH ₃ OH + CO ₂ → C ₃ H ₆ O + CH ₃ COOH + 3H ₂ O | MeOH | Adc | 0.5 |

Despite the higher energy potential of methanol and CO compared to H₂, their utilization is restricted to a few acetogens, such as carboxydrophic acetogens capable of catalyzing and resistant to CO,⁸⁵ and methylotrophic acetogens harboring a methyltransferase for methanol assimilation (Table 1).⁸³ Hence, a fundamental solution for enhancing intracellular ATP availability, even when using H₂ as an electron donor, is imperative.

3.2 Boosting chemiosmotic ATP synthesis with energy-conserving electron acceptors

One strategy for enhancing ATP production involves boosting ion gradient-driven phosphorylation (IGP). Given that most ATP is generated through the ion gradient established by membrane-bound respiratory enzymes (Rnf or Ech), the overexpression or engineering of these enzymes could stimulate ion gradient generation, thereby augmenting IGP. However, adopting this direct approach can be challenging because membrane-bound proteins are often difficult to express in heterologous systems.^{86,87} Instead of directly manipulating the respiratory enzyme, a preferable alternative approach is indirectly increasing the ion gradient by supplying additional terminal electron acceptors other than CO₂.

Appropriate electron acceptors can facilitate IGP-dependent ATP synthesis independent of SLP through H₂ oxidation. Studies have demonstrated the effectiveness of electron acceptors such as dimethyl sulfoxide (DMSO),^{64,65,88} thiosulfate,⁶⁵ nitrate,^{89,90} and caffate^{91–93} as energy-conserving electron acceptors for several acetogens. Enhanced ATP production increases biomass and product formation, accompanied by reduced acetate production.^{65,88,89}

As low-cost electron acceptors, DMSO and nitrate enable cost-effective processes and successfully enhance product synthesis. For instance, *M. thermoacetica* can utilize DMSO alongside CO₂ as an electron acceptor (Fig. 3A).⁸⁸ It has been reported that DMSO increases intracellular ATP levels 2-fold and reduces acetate production by half, indicating a redirection of electron flow away from acetogenesis towards DMSO reduction in *M. thermoacetica*.⁶⁵ The study also confirmed that DMSO acts solely as an electron acceptor, not as a carbon source, despite being an organic compound containing two methyl groups. Moreover, supplying DMSO to engineered *M. thermoacetica* restored both growth and

acetone production under CO₂/H₂ conditions, which could not be achieved without DMSO supplementation because of its inability to grow solely on CO₂/H₂.^{65,66} As shown in Table 3, acetone production under CO₂/H₂ conditions required ATP because of the absence of ATP yields. Consequently, the engineered *M. thermoacetica* strain, which includes the partial deletion of the acetate pathway and introduction of the acetone production pathway, encounters challenges in CO₂/H₂ environments owing to ATP shortage, resulting in no growth or acetone production. However, DMSO supplementation restored the autotrophic growth and enabled acetone production. This positive effect was attributed to the increased intracellular ATP levels in the DMSO-supplemented strain.⁶⁵ Since *M. thermoacetica* possesses DMSO reductase, menaquinone (MQ), and the NADH dehydrogenase complex, it is assumed that ATP production could be augmented through anaerobic DMSO respiration, a mechanism akin to DMSO respiration in *E. coli*, where H₂ is the electron source.⁶⁵ Through the oxidation of H₂ coupled with DMSO respiration in *M. thermoacetica*, a proton gradient is generated, subsequently driving ATP synthase for IGP-coupled chemiosmotic ATP synthesis (Fig. 3A). However, a detailed understanding of the mechanism underlying H₂ oxidation coupled with DMSO reduction has yet to be achieved.

Similarly, nitrate supplementation has been reported to enhance H₂-dependent growth in *C. ljungdahlii*.⁸⁹ With the necessary genes for nitrate assimilation, *C. ljungdahlii* simultaneously reduces CO₂ and nitrate using electrons derived from H₂. In contrast to DMSO, which is presumed to promote ion gradient generation directly, nitrate acts as an electron sink for reducing equivalents derived from the Rnf complex, thus indirectly promoting chemiosmotic ATP generation. The proposed mechanism relies on electron bifurcation by hydrogenase to couple nitrate reduction with ATP production. Since reduced Fd is utilized for chemiosmotic ATP synthesis rather than CO₂ reduction, nitrate reduction yields more ATP than acetate production from CO₂/H₂ (1.5 ATP for nitrate and 0.63 ATP for CO₂ reduction).⁸⁹ The study also found a significant increase in the ATP/ADP ratio and acetyl-CoA pools, indicating that nitrate reduction is coupled to ATP generation. In order to employ this mechanism, the effect of nitrate supplementation on ethanol production by *C. ljungdahlii* was investigated.⁹⁰ Indeed,



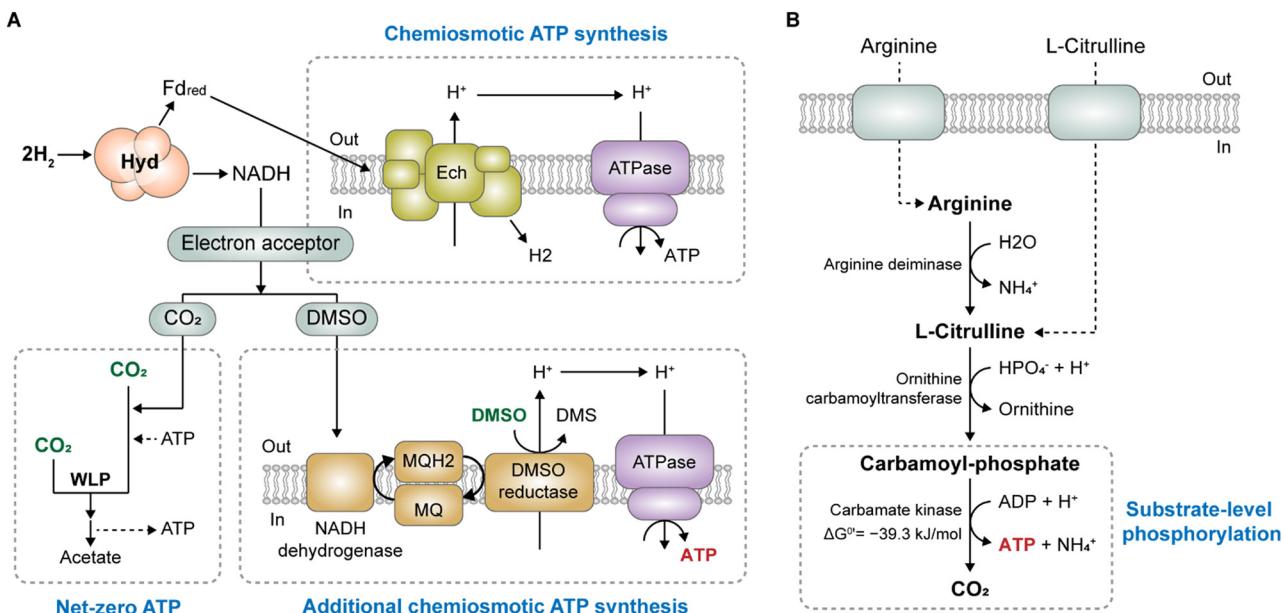


Fig. 3 Strategies to increase intracellular ATP availability in acetogens. (A) A hypothetical mechanism for additional ATP synthesis *via* energy-conserving electron acceptor DMSO proposed in *M. thermoacetica*. (B) Arginine deiminase pathway for substrate-level phosphorylation coupled ATP synthesis.

supplying nitrate improved its growth by up to 62% and ethanol production by up to 3-fold from CO_2/H_2 . However, this positive effect was observed only in pH-controlled bioreactors and not in serum bottle experiments. This occurred because the ammonium produced during nitrate reduction increased the pH of the medium, leading to inhibition of biomass formation. Variations in biomass, acetate, and ethanol production were observed across all nitrate-supplemented bioreactors. Because of stochastic inhibition events stemming from the interplay between undissociated acetic acid and ammonium production, this study emphasizes the need for further investigation at both physiological and bioprocessing levels to effectively harness the potential of nitrate as an electron acceptor to overcome ATP limitations.

While caffeoate has been shown to enhance growth yield and ATP levels in *A. woodii* through caffeoate respiration linked to energy conservation *via* Rnf and ATP synthase, its application for improving desired product synthesis has not been demonstrated.^{91–93} This is likely due to the toxicity of caffeoate to cells and its high cost, making this approach economically less favorable.¹⁴ To leverage additional ATP production *via* electron acceptors, a metabolic system must be in place that couples ATP synthesis with the electron acceptor reduction. Supplying electron acceptors may not significantly affect growth and could even lead to growth inhibition, as demonstrated in *M. thermoacetica*, where nitrate supplementation inhibited WLP and acetogenic metabolism.⁹⁴ Nevertheless, these findings suggest that supplying electron acceptors can be a beneficial strategy for producing ATP-intensive heterologous products from acetyl-CoA by decoupling ATP production from CO_2 fixation and redirecting carbon flux from acetate to the desired product.

3.3 Employing SLP-coupled reactions to gain additional ATP

Another strategy for enhancing ATP availability involves leveraging additional SLP. Katsyv *et al.* (2020) described several reactions coupled to SLP, which have a phosphoryl group transfer potential with more negative free energy than $-31.8 \text{ kJ mol}^{-1}$, enabling ADP phosphorylation.¹⁴ Among these reactions, the carbamate kinase reaction ($\Delta G^\circ = -39.3 \text{ kJ mol}^{-1}$) has been reported to be effective for ATP production in several acetogens. As part of the arginine deiminase (ADI) pathway, carbamate kinase generates one ATP molecule with arginine as a supplement (Fig. 3B). The pathway was initially discovered in *C. autoethanogenum*, where arginine supplementation increased growth rate and abolished acetate production during autotrophic growth on syngas (a mixture of CO , CO_2 , and H_2).⁹⁵ The observed growth-stimulating effect of arginine was attributed to the increased ATP production from SLP during the catabolism of arginine by carbamate kinase in the ADI pathway.

In addition, supplementation of citrulline promoted ATP production in *C. carboxidivorans* P7, leading to increased biomass and specific growth rate under both heterotrophic and autotrophic conditions.⁹⁶ Specifically, the intracellular ATP level and biomass were improved by 80.5% and 31.6%, respectively, under syngas conditions. Moreover, the ethanol yield increased by 18.6%, and the alcohol-to-acid production ratio increased by 60.3%, likely because of the extra ATP generated from the citrulline supply.

Based on these findings, leveraging the ADI pathway for SLP *via* the carbamate kinase reaction could be a strategy for overcoming ATP limitations in acetogens. Validating this approach, the heterologous expression of the ADI pathway in *A. woodii* led to a 69% increase in biomass and a 60% reduction in the acetate yield per biomass.⁹⁷ Further research is needed to assess the



effectiveness of this strategy for producing ATP-intensive chemicals. In addition, exploring various SLP-coupled reactions for ATP synthesis in acetogens is necessary.

3.4 Utilizing acetate as a precursor for alcohol production

In addition to ATP supply through additional SLP- or IGP-coupled reactions, utilizing acetate as a precursor for the desired chemical production can be helpful for ATP production without relying on acetate kinase reactions to obtain ATP. In *Clostridium* species of acetogens, such as *C. autoethanogenum* and *C. ljungdahlii*, two pathways for ethanol production exist: the aldehyde:Fd oxidoreductase (Aor)-alcohol dehydrogenase (Adh) pathway and the bifunctional aldehyde/alcohol dehydrogenase pathway (Aldh-Adh) (Fig. 2). The Aor-Adh pathway is preferred to the Aldh-Adh pathway because it retains the acetate kinase reaction, enabling ATP generation during ethanol production.^{31,98–100} In the Aor-Adh pathway, Aor utilizes acetate as a substrate and reduces it to acetaldehyde using reduced Fd, which is then converted to ethanol by Adh using NADH. Conversely, the Aldh-Adh pathway converts acetyl-CoA into ethanol using acetaldehyde as an intermediate, resulting in ATP loss by bypassing the acetate kinase reaction (Fig. 2). Therefore, ethanol production *via* the Aor-Adh pathway yields more ATP than that *via* the Aldh-Adh pathway; for example, when CO is a substrate, providing 2.1 ATP and 1.7 ATP, respectively (Table 3). Based on this approach, introducing Aor into non-Aor-containing acetogens such as *A. woodii* enables ethanol production without ATP loss.

Since its discovery, Aor has garnered attention as the only enzyme to catalyze a thermodynamically challenging reverse reaction: reducing nonactivated acids to aldehydes using a low-redox-potential electron donor. Additionally, its wide substrate range has expanded its applications in reducing various organic acids (*e.g.*, acetate, butyrate, and caproate) to alcohols (*e.g.*, ethanol, butanol, and hexanol).¹⁰¹ As such, Aor can be heterologously expressed in butyrate-producing acetogens, such as *E. limosum* and *B. methylotrophicum*, to produce butanol by reducing butyrate production. This would benefit ATP yield, as butanol production using the Aor route results in greater ATP acquisition than non-Aor routes (Table 3). However, the heterologous expression of Aor remains to be explored. To date, only homologous expression of Aor in acetogens has been demonstrated in *C. carboxidivorans* and *C. autoethanogenum*, where the over-expression of Aor effectively reduced acetate accumulation and improved ethanol production.^{33,72}

In addition to the role of intracellular acetate in alcohol production, supplying exogenous acetate has been shown to affect the production of reduced acetogen products positively. Specifically, CO gas fermentation supplemented with acetate resulted in increased ethanol production by *C. ljungdahlii*,¹⁰² *C. autoethanogenum*,^{103,104} and *Clostridium* sp. AWRP.¹⁰⁵ In *C. ljungdahlii*, supplementation with 15 mM acetate boosted the ethanol production rate by over 2.4-fold, along with enhanced cell density and selectivity toward ethanol, achieving an ethanol-to-acetate ratio of 93.0.¹⁰² This effect was observed at a slightly acidic pH, ensuring an excess of both CO and undissociated acetic acid for optimal Aor activity, thereby

promoting higher selectivity for the more reduced product, ethanol. Acetate supplementation has been proposed as a simple and effective approach to alleviate CO inhibition in *Clostridium* sp. AWRP.¹⁰⁶ This is because CO oxidation is coupled with acetate reduction to ethanol, potentially lowering the intracellular CO levels below inhibitory levels, thereby mitigating CO inhibition. In this acetogen, CO fermentation with exogenous acetate enhanced the maximum cell density by 2-fold, overall alcohol production (2.9- and 2.3-fold higher titers of ethanol and 2,3-BDO, respectively), and specific growth rates (2.6-fold) compared to CO fermentation without acetate supplementation. Similarly, ethanol production in *C. autoethanogenum* was improved in the presence of exogenous acetate.¹⁰³ A recent study found that the addition of exogenous acetate increased the concentration of undissociated acetic acid, which in turn regulated the ethanol yield and production rates, presumably to counteract the inhibition caused by undissociated acetic acid.¹⁰⁴ These results highlight the potential of acetate as both a precursor and accelerator for alcohol production. Therefore, acetate, often regarded as a waste product, can be effectively utilized for alcohol production *via* the acetate kinase reaction without sacrificing ATP synthesis.

4 Redox supply for powering energetics in a sustainable manner

4.1 Optimizing redox balance for efficient acetogenic C1 conversion

The balance of the reducing equivalents is important for both growth and product synthesis in acetogens. Redox recycling enzymes, such as Rnf, Hyd, Nfn, and Aor, along with most enzymes in the WLP, are pivotal for maintaining redox balance.^{24,25,64,100} Particularly, during syngas fermentation and alcohol production, Aor activity is crucial for consuming reduced Fd and regulating redox balance and thus metabolic homeostasis.¹⁰⁰ Ethanol production is predominantly governed by thermodynamics and redox rather than gene or protein expression levels in acetogens, as indicated by several studies.^{100,107,108} Notably, *C. autoethanogenum* employs metabolic oscillations to manage redox imbalances arising from co-metabolizing H₂ and CO, ensuring effective homeostatic regulation.¹⁰⁰

However, the engineering of acetogens can lead to redox imbalances, resulting in low product yields and poor growth. For instance, excessive intracellular NADH levels caused by H₂-supplemented mixotrophy inhibited the growth of engineered ethanol-producing *M. thermoacetica*.⁶⁴ Although the authors applied H₂-supplemented mixotrophy (CO₂/H₂/fructose) to enhance ethanol yield, H₂ supply prevented the strain from H₂ formation with reversible hydrogenase activity, which is necessary to balance the redox by oxidizing excess electrons generated from fructose catabolism. This inhibition of H₂ formation leads to increased intracellular NADH levels and, hence, a redox imbalance, hampering growth and ethanol production. In contrast to the ethanol production pathway, introducing the acetone production pathway into *M. thermoacetica* successfully increased



acetate and acetone production. Because the acetone production pathway from acetyl-CoA does not require a reducing equivalent, H₂-supplemented mixotrophy provided positive effects without redox imbalance issues. Therefore, this study suggests that reversible hydrogenase activity enables *M. thermoacetica* to flexibly balance its intracellular redox state, emphasizing the need to fine-tune the redox balance to benefit from H₂-supplemented mixotrophy.⁶⁴

Balancing intracellular NADPH levels is crucial, especially for isopropanol production. The reduction of acetone to isopropanol by primary-secondary alcohol dehydrogenase is NADPH-dependent, and a redox imbalance may lead to incomplete conversion.¹⁰⁹ Therefore, NADPH supply and balance are essential for efficient acetone reduction. For example, engineered *M. thermoacetica* achieved complete acetone reduction to isopropanol from C1 substrates, whereas engineered *A. woodii* exhibited incomplete reduction, leaving one-third of the acetone unconsumed.⁶⁷ This difference is likely due to the presence of Nfn, an NADPH-dependent hydrogenase in *M. thermoacetica*, facilitating direct NADPH production from H₂. These findings underscore the importance of the redox balance and supply in achieving successful acetogenic C1 conversion with high yields and selectivity.

The intracellular redox pool must be increased to balance redox reactions and improve energetics. This can be achieved through the addition of H₂ or reduced substrates such as methanol and CO. This approach has been proven effective in increasing butyrate production in recombinant *A. woodii*⁶⁰ and ethanol and PHB production in *C. autoethanogenum*^{78,110} because these substrates can generate additional reducing equivalents. However, employing this approach becomes challenging when considering carbon-negative production because utilizing CO as the sole carbon and energy source theoretically results in unwanted CO₂ emissions with a loss of two-thirds of the carbon, lowering product yields. Given the current production method and cost of H₂, acetogenic C1 conversion seems less favorable than the photoautotrophic pathway, which uses renewable light at no cost.^{111,112} Therefore, exploring alternative renewable redox suppliers for powering acetogenic C1 conversion is necessary to achieve complete sustainability. There is growing interest in leveraging abundant, low-cost, and renewable energy sources such as electricity and light. Abiotic electrocatalysts and photocatalysts with robust stability and high catalytic efficiency are crucial materials for sustainably powering microbial growth and chemical biosynthesis.¹¹³ This approach offers promising routes for achieving a sustainable and renewable energy supply through reducing equivalents derived from light and electricity.

4.2 Electricity as a sustainable redox supplier

By adopting microbial electrosynthesis (MES), reducing equivalents can be supplied in unlimited quantities, either through direct electron transfer or indirectly via H₂ generated from the cathode surface (Fig. 4A). Electrotropic acetogens such as *S. ovata*,^{114–118} *C. ljungdahlii*,^{119–122} *T. kivui*,¹²³ and *Clostridium scatologenes*,¹²⁴ are harnessed for MES because they can take up electrons to reduce CO₂ into various multicarbon chemicals (Table 4). Notably, MES allows the production of reduced chemicals, such as ethanol,

lactate, and butyrate, from CO₂ when a sufficient redox supply is achieved,^{119–121,124} which is not achievable with chemoautotrophic growth on CO₂/H₂ owing to energetic limitations and negative ATP yields (Table 3).

MES has several advantages over chemoautotrophy with externally supplied H₂ gas. A recent study demonstrated that CO₂ fixation by *S. ovata* with H₂ generated from the cathode (electroautotrophy) was more efficient than externally supplied H₂ (chemoautotrophy).¹²⁹ The measured electron efficiencies were 99% and 71% under electroautotrophic and chemoautotrophic conditions, respectively, resulting in an increase in the efficiency of reducing equivalent utilization and CO₂ fixation into acetate from less than 80% with chemoautotrophy to over 95% under electroautotrophic conditions. This improvement was attributed to metabolomic rewiring and the regulation of the membrane electrochemical potential in *S. ovata* during MES, leading to enhanced proton transmembrane transport and boosted chemiosmotic ATP generation, consequently elevating CO₂ fixation rates.¹²⁹

Furthermore, recent research has emphasized the crucial role of H₂ availability in MES for the metabolic activity and productivity of *C. ljungdahlii*, which determine the efficiency of the MES process.¹²² By controlling inoculation density, two distinct conditions were established in MES: planktonic-dominant (high cell density) and biofilm-dominant (low cell density), characterized by high and low H₂ availability, respectively. The biofilm-dominant conditions resulted in significant glycine and ethanolamine production, likely due to a redox imbalance from low H₂ availability. In contrast, increased H₂ availability in the planktonic-dominant condition augmented redox cofactor pools, and thereby promoted faster turnover of redox-based metabolic activities and a larger driving force for CO₂ reduction, resulting in a higher acetate titer of 6.06 g L^{−1}, among the highest reported for MES from CO₂ with pure culture *Clostridium* species.¹²²

Enhancements in the production of reduced chemicals, such as ethanol and lactate, have been achieved through an additional supply of reducing equivalent in MES, facilitated by decreasing a cathodic potential (CP) and developing cathode materials to promote H₂ evolution, thereby increasing CO₂ conversion rates.^{119–121,124} Decreasing the CP from −0.6 V to −1.2 V (vs. Ag/AgCl) increased current density and product titers, including ethanol production in *C. scatologenes*.¹²⁴ Similarly, higher acetate product titers were achieved at a CP of −1.0 V, with lactate and formate production, which were unattainable at CPs of −0.6 V and −0.8 V, yielding only acetate.¹¹⁹ Additionally, newly developed cathode materials accelerated H₂ evolution on the electrode. For instance, modifying the cathode with nickel phosphide (Ni-P), a catalyst for the H₂ evolution reaction, increased the H₂ evolution rate and hence enhanced the C1-to-C4 conversion in MES using engineered butyrate-producing *C. ljungdahlii*.¹²⁰ The butyrate production rate was 2.5-fold higher in MES with Ni-P than in MES without Ni-P. Syngas-mediated MES has recently been demonstrated using a novel porous 3D cathode incorporating cobalt phthalocyanine catalysts to produce syngas (CO and H₂) from CO₂.



The newly developed cathode achieved stable CO production and acetate (5.1 g L^{-1}) and ethanol (1.2 g L^{-1}) production titers, outperforming similar MES studies using *C. ljungdahlii*.¹²¹ This syngas-mediated MES system provided a promising approach for CO₂ conversion to high-value, energy-demanding chemicals through sustainable syngas generation from electrodes, providing more reducing equivalents than H₂.

The MES performance, including product profile and production capacity, varies among acetogens owing to their distinct characteristics. Among them, with its high productivity and efficiency, *S. ovata* is the best biocatalyst for acetate production in MES.^{130,131} *S. ovata* exhibits the highest conversion rates ($51.1 \text{ g m}^{-2} \text{ d}^{-1}$) in pure culture.¹³¹ Through the development of cathode materials and optimization of the MES reactor configuration to increase H₂ availability,^{115,117,118} as well as bio-printing of synthetic biofilm to reduce the time for biofilm formation on the electrode,^{114,116} acetate production rates and titers have been significantly improved. The highest acetate titer reached 11 g L^{-1} ,¹¹⁵ with an outstanding acetate production rate of $1697.6 \text{ mmol m}^{-2} \text{ d}^{-1}$ from CO₂ (Table 4).¹¹⁸ Despite numerous efforts, acetate production remains the predominant product in *S. ovata*, with no by-products other than acetate, likely due to its metabolic features and the absence of genetic tools for metabolic engineering to produce chemicals. Nevertheless, owing to its high MES productivity and efficiency, engineered *S. ovata* holds promise for expanding the product spectrum and outperforming other pure cultures in various chemical production processes. Recent advancements in the genetic tools available for *S. ovata*⁴³ lay the groundwork for future metabolic engineering endeavors to diversify product outputs beyond acetate in *S. ovata*.

Using pure cultures, MES successfully converted CO₂ into a C4 acid (butyrate) and a C2 alcohol (ethanol). With the aid of chain elongating microbes in mixed cultures, these short-chain carboxylic acids can be further transformed into longer-chain carboxylic acids and alcohols, such as caproate and hexanol (Table 4).^{125–128} Mixed culture MES has demonstrated the production of acetate, butyrate, isobutyrate, caproate, and their corresponding alcohols, including ethanol, butanol, isobutanol, and hexanol, from CO₂ through chain elongation *via* reverse β -oxidation.¹²⁶ Maintaining the culture pH around 5 in MES was crucial, as the mildly acidic pH promoted dominance

of *Clostridium* species, enabling reverse β -oxidation for chain elongation and production of butyrate and caproate. Furthermore, the successful utilization of alternative substrates other than pure CO₂ has been demonstrated in mixed-culture MES. Direct utilization of industrial CO₂ with low impurities for acetate production *via* MES resulted in higher acetate production rates and titers (1.8 g L^{-1} , $0.26 \text{ g L}^{-1} \text{ d}^{-1}$) than pure culture.¹²⁵ The mixed culture outperformed the pure culture in chemical production from industrial CO₂, likely because of its robustness and diverse functionality in coping with industrial gas impurities. Another study showed that waste C1 gas, as an alternative to pure CO₂, enhanced the generation of C4 and C6 carboxylates in MES.¹²⁷ Using a CO/CO₂ mixture as the substrate in MES favored simultaneous acetogenesis, solventogenesis, and chain elongation, resulting in the production of acetate, butyrate, caproate, propanol, butanol, and hexanol. Recently, the highest acetate titer of 16.0 g L^{-1} was achieved in lab-scale fed-batch reactors, representing one of the highest acetate titers reported in the literature.¹²⁸

4.3 Light-driven electrons

The integration of semiconductor nanomaterials with acetogens is another efficient strategy for facilitating sustainable chemical production from C1 substrates by harnessing light-driven electrons (Fig. 4B). Employing light-capturing photocatalysts such as cadmium sulfide nanoparticles or gold nanoparticles, several studies have demonstrated light-driven chemical production in acetogens *M. thermoacetica*,^{132,133} *S. ovata*,¹³⁴ and *C. autoethanogenum*.¹³⁵ Developing efficient, highly biocompatible, low-cost photocatalysts is crucial to improving conversion efficiency and establishing high-performance artificial photosynthesis platforms. In this pursuit, recent advancements have focused on twinning Cd_{0.8}Zn_{0.2}S nanoparticles with long-range ordered homojunction, successfully utilized for the first time in constructing a biohybrid system in *S. ovata*.¹³⁶ This system achieved a notable production yield of 49.33 mM of acetate at a rate of 8.22 mM per day, with a quantum efficiency of 16.82% under illumination, surpassing previous biotic–abiotic hybrid systems. However, current light-driven chemical production is primarily limited to acetate production, necessitating further enhancement of artificial photosynthetic systems using acetogens to broaden the range of synthesized products.

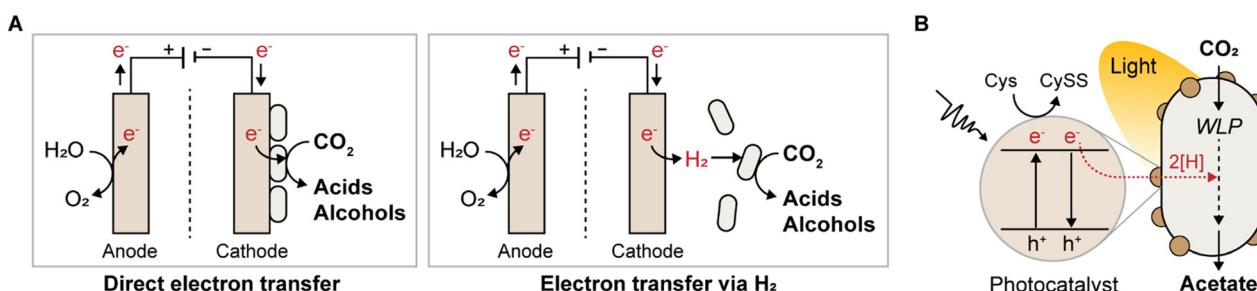


Fig. 4 Scheme of supplying redox power through electricity or light. (A) Microbial electrosynthesis by direct electron transfer or indirectly via H₂ generated from the cathode surface. (B) Acetogenic CO₂ conversion using light-driven energy sources.



Table 4 A comparative overview of chemical production via MES using acetogenic bacteria

| Species | Cathode material | Cathode potential (vs. Ag/AgCl) | Product | Maximum titer (g L ⁻¹) | Maximum productivity | Ref. |
|---|--|---------------------------------|--------------|------------------------------------|---|------|
| <i>S. ovata</i> | Bio-printed carbon cloth | -0.8 V | Acetate | 3.45 | 0.68 g L ⁻¹ d ⁻¹ | 114 |
| <i>S. ovata</i> | Bio-printed Ti mesh | -0.8 V | Acetate | 9.4 | 0.84 g L ⁻¹ d ⁻¹ | 116 |
| <i>S. ovata</i> | Copper foam coated with reduced graphene oxide | -1.2 V | Acetate | — | 1697.6 mmol m ⁻² d ⁻¹ | 118 |
| <i>S. ovata</i> | Porous nickel hollow fiber | -0.9 V | Acetate | — | 247 mmol m ⁻² d ⁻¹ | 117 |
| <i>S. ovata</i> | Dual cathode (graphite rods and titanium mesh) | -0.9 V | Acetate | 11 | 0.7 g L ⁻¹ d ⁻¹ | 115 |
| <i>T. kivui</i> | Ni-Mo coated graphite rod | NA | Acetate | 6.0 | 3.36 g L ⁻¹ d ⁻¹ | |
| <i>C. scatologenes</i> | Carbon felt | -0.6 V | Acetate | 0.03 | — | 124 |
| <i>C. scatologenes</i> | Carbon felt | -1.2 V | Acetate | 0.44 | — | 124 |
| <i>C. scatologenes</i> | Carbon felt | -1.2 V | Butyrate | 0.01 | — | |
| <i>C. scatologenes</i> | Carbon felt | -1.2 V | Ethanol | 0.015 | — | |
| <i>C. ljungdahlii</i> (planktonic-dominant condition) | Graphite block | -0.9 V | Acetate | 6.06 | 0.11 g L ⁻¹ d ⁻¹ | 122 |
| <i>C. ljungdahlii</i> (biofilm-dominant condition) | Graphite block | -0.9 V | Acetate | 1.01 | — | 122 |
| <i>C. ljungdahlii</i> | Graphite block | -0.8 V | Glycine | 0.39 | — | |
| <i>C. ljungdahlii</i> | Graphite block | -1.0 V | Ethanolamine | 0.14 | — | |
| <i>C. ljungdahlii</i> | Graphite block | -0.8 V | Acetate | 0.053 | — | 119 |
| <i>C. ljungdahlii</i> | Graphite block | -1.0 V | Acetate | 0.328 | — | 119 |
| <i>C. ljungdahlii</i> | Cobalt phthalocyanine incorporated into porous cathode | -1.2 V | Formate | 0.28 | — | |
| <i>C. ljungdahlii</i> | Nickel phosphide modified carbon felt | -1.1 V | Lactate | 0.069 | — | |
| <i>C. ljungdahlii</i> | Graphite plate | -1.2 V | Acetate | 5.1 | — | 121 |
| Mixed culture | Graphite plate | -1.0 V | Ethanol | 1.2 | — | |
| Mixed culture | Graphite granules | -0.8 V | Acetate | 1.18 | 0.17 g L ⁻¹ d ⁻¹ | 120 |
| | | | Butyrate | 0.67 | 0.1 g L ⁻¹ d ⁻¹ | |
| | | | Acetate | 1.14 | 0.138 g L ⁻¹ d ⁻¹ | 125 |
| | | | Ethanol | 1.3 | — | |
| | | | Butanol | 0.8 | — | |
| | | | Isobutanol | 0.2 | — | |
| | | | Hexanol | 0.2 | — | |
| Mixed culture | Carbon felt | -1.0 V to -1.3 V | Acetate | 4.9 | 0.26 g L ⁻¹ d ⁻¹ | 125 |
| | | | Butyrate | 3.1 | — | |
| | | | Isobutyrate | 1.6 | — | |
| | | | Caproate | 1.2 | — | |
| | | | Ethanol | 1.3 | — | |
| | | | Butanol | 0.8 | — | |
| | | | Isobutanol | 0.2 | — | |
| | | | Hexanol | 0.2 | — | |
| Mixed culture | Nickel foam–carbon felt | -1.1 V | Acetate | 5.47 | — | 127 |
| | | | Propanol | 0.04 | — | |
| | | | Butanol | 0.08 | — | |
| | | | Hexanol | 0.02 | — | |
| | | | Butyrate | NA | — | |
| | | | Caproate | NA | — | |
| | | | Acetate | 16.0 | — | 128 |
| | | | Butyrate | 2.87 | — | |
| | | | Formate | NA | — | |

Interestingly, a recent study revealed that visible light alone can stimulate autotrophic and heterotrophic ethanol production by *C. autoethanogenum* via photoexcitation-induced stress, even without the assistance of photocatalytic semiconductors.¹³⁷ Illumination of *C. autoethanogenum* resulted in 1.8- and 2.2-fold increases in ethanol production from C1 gases and fructose, respectively. This study revealed, for the first time, the direct impact of light on the metabolism of acetogens in the absence of photocatalytic semiconductors, which was a previously unknown phenomenon. It was found that *C. autoethanogenum* activates all ethanol production pathways upon energization with visible light, partly to accelerate acetate turnover, thereby mitigating cellular exposure to multifactorial stress. Although further investigation is required to fully understand this phenomenon, unassisted stimulation of autotrophic ethanol production by visible light offers insights into the direct utilization of light as

an electron source for sustainable chemical production *via* acetogens.

5 Emerging technologies to accelerate strain development

Significant advancements have been made in the acetogenic C1 conversion, resulting in an expanded product spectrum and improved energetics with increased ATP and redox availability. However, it remains challenging to understand acetogens and their limited tolerance to toxic compounds (e.g., CO and methanol) or impurities in gas mixtures remains challenging. Additionally, the oxygen-sensitive nature and slow growth of acetogens pose significant hurdles to developing efficient platform strains, impeding the transition of this system to an



industrial scale. Fortunately, advanced strategies that offer practical solutions to these challenges are available.

5.1 Adaptive laboratory evolution for enhancing strain tolerance and capability

Adaptive laboratory evolution (ALE) is a powerful approach for readily obtaining beneficial mutant strains through artificially applied selective pressure (Fig. 5A).^{138,139} Relying on the natural selection mechanism, ALE requires no genetic tools or engineering strategies to develop the strain. Among acetogens, ALE has proven successful in improving growth and production performance.

Although CO and methanol are favorable electron donors because of their high energy potentials, supplying them at high concentrations can inhibit growth owing to their toxicity.^{41,74,140} Adopting ALE in acetogens has proven effective in obtaining exceptional strains with high tolerance to C1 substrates and production capabilities. For instance, ALE techniques adapted *S. ovata* to methanol, significantly increasing its acetate production rate by 6.5-fold.¹⁴⁰ Similarly, adaptive evolution of *E. limosum* ATCC 8486 under syngas containing 66% CO enhanced its tolerance to high concentrations of CO, resulting in an 8-fold increase in growth rates. Remarkably, the resulting mutant strains exhibited 6.5-fold higher productivity of 2,3-butanediol than the wild-type under CO 66% syngas fed-batch fermentation.^{41,74}

Apart from C1 substrates, ALE has been applied to improve tolerance towards impurities present in syngas, such as cyanide in *C. ljungdahlii*,¹⁴¹ benzene, toluene, and xylenes in

C. autoethanogenum,¹⁴² and oxygen in *S. ovata*.¹⁴³ These impurities inhibit cell growth and enzyme activities, lowering process productivity. However, adapted strains with improved robustness and tolerance to impurities can overcome these inhibitory effects. Enhancing the oxygen tolerance of *S. ovata* improved its performance in MES, in which oxygen was generated by the anode and diffused into the cathode chamber, thereby serving as an inhibitor and adapting *S. ovata* to tolerate up to 5% oxygen improved robustness during MES, resulting in a 1.5-fold higher acetate production compared to the wild-type.¹⁴³ Moreover, yeast extract is another prohibitive supplement for economically favorable scale-up,¹⁴⁴ which complicates experimental analysis due to its unquantified sources of vitamins, nutrients, and trace elements. However, because most acetogens rely on yeast extract for growth, efforts have been made to eliminate this requirement. Continuous bioreactor cultivation has been employed to adapt *C. autoethanogenum* and *C. ljungdahlii*, enabling their growth without yeast extract in defined media.^{102,145}

Recently, ALE was used in *C. carboxidivorans* to expand its gas fermentation product spectrum from CO₂/H₂.¹⁴⁶ This study demonstrated, for the first time, direct hexanol production from energy-limited CO₂/H₂ conditions, achieving a maximum hexanol productivity of 0.031 g L⁻¹ h⁻¹, the highest reported to date in the literature using *C. carboxidivorans*. Furthermore, traces of valerate, pentanol, heptanol, and octanol, products not typically reported to be naturally generated by pure culture acetogens, were detected. Therefore, ALE has emerged as a potent strategy for developing highly tolerant and industrially relevant acetogens and expanding the product spectrum.

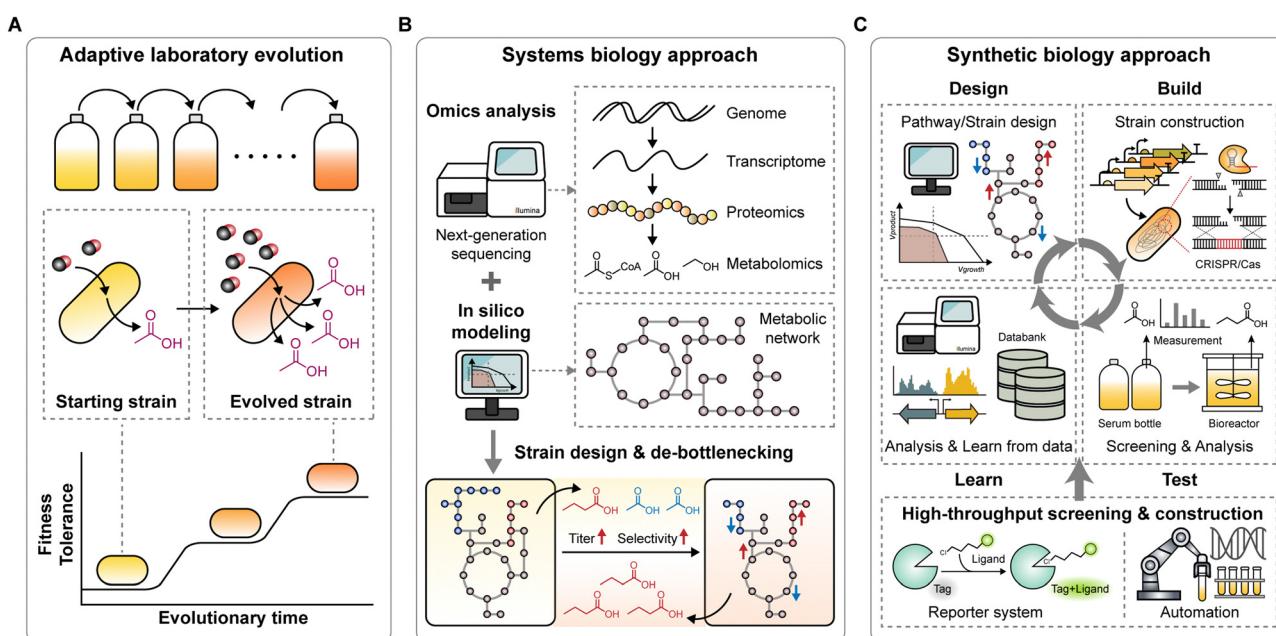


Fig. 5 Emerging technologies for accelerating the development of acetogenic strains with high performance. (A) Adaptive laboratory evolution generates mutant strains that possess enhanced tolerance to substrates or improved production capability. (B) A systems biology approach serves as a guide for understanding and designing improved strains, with the aid of omics analysis and *in silico* modelling. (C) A synthetic biology approach, employing a design-build-test-learn (DBTL) cycle as a framework, supports the construction, screening and designing of strains with desired functionalities. The DBTL cycle can be automated and accelerated with high-throughput screening and construction workflows.



5.2 Systems biology: a guide for understanding and designing improved strains

The systems biology approach serves as a guide for fundamentally understanding acetogens, unraveling associated complex regulatory systems, and designing efficient, high-performance platform strains (Fig. 5B). Recent advances in next-generation sequencing (NGS) technologies have facilitated numerous omics studies, including genomics, transcriptomics, metabolomics, proteomics, and metabolomics in acetogens, which have been reviewed in several papers.^{147,148} These studies have significantly increased our molecular and system understanding by elucidating the regulatory mechanisms of carbon and energy metabolism in acetogens. For example, studies have revealed the role of redox control in metabolic robustness in *C. autoethanogenum*¹⁰⁰ and the significance of the Rnf and Nnf complex in maintaining the redox and ATP pool at constant levels.¹⁴⁹ Additionally, translational regulation has been revealed in *A. woodii*,¹⁵⁰ *E. limosum*,¹⁵¹ and *C. ljungdahlii*,¹⁵² confirming its control over resource allocation in energy-deprived environments, given the energy-expensive nature of translation.

Omics analyses have also been applied to understand the mechanisms of MES and artificial photosynthesis systems.^{122,129,134–136} These studies investigated the reasons behind the superior CO₂ fixation performance of MES compared to chemoautotrophic conditions in *S. ovata*,¹²⁹ and explored the direct effects of light on metabolism in *C. autoethanogenum* in the absence of a photocatalyst.¹³⁷ These findings provide insights for developing high-performance MES and artificial photosynthesis systems to increase the CO₂-fixing efficiency.

In conjunction with the CRISPR–Cas or transposon systems, NGS has enabled functional genomic studies of acetogens. Transposon insertion sequencing in *C. autoethanogenum*¹⁵³ and genome-wide CRISPRi screening in *E. limosum*¹⁵⁴ have revealed gene essentiality and genotype–phenotype relationships under autotrophic and heterotrophic growth conditions. These datasets further expand our knowledge of acetogens and guide strain engineering.

Enabled by the growing pool of omics data, prediction models, such as genome-scale metabolic and kinetic ensemble models, have been developed for several acetogens and have provided a deeper understanding of metabolism and regulation.¹⁴⁷ Furthermore, these models help estimate the production yields of various metabolites and pathway feasibility, aiding in the design of efficient strains and pathways. Model-guided rational strain or pathway design streamlines the selection of target metabolic pathways and offers insights into their effects on the metabolic network (Fig. 5B).¹⁴⁸

Systematic analysis of engineered strains, based on kinetic ensemble modeling and omics data (e.g., proteomics, metabolomics), can identify potential bottlenecks in production pathways. For example, systemic analysis of engineered *C. ljungdahlii* identified bottlenecks in isobutanol production pathways as an NADPH limitation and thereby guided further engineering strategies such as replacing the cofactor dependency on NADPH by

NADH to improve the desired chemical production.⁷⁶ For efficient acetone production in *C. autoethanogenum*, fine-tuning enzyme levels relieved the identified pathway bottlenecks, optimizing the flux to acetone production and enhancing its productivity.⁷⁰ Alternatively, thermodynamic and kinetic modeling guided pathway optimization for isopropanol production, resulting in significant improvements in the engineered strain.⁷³

5.3 Synthetic biology and high-throughput screening for accelerating strain development

Considerable advances in synthetic biology tools have paved the way for accelerating the development of next-generation strains.^{155,156} CRISPR–Cas genome engineering tools represent a breakthrough technology, offering new engineering opportunities for previously difficult-to-engineer acetogens.⁷ CRISPR–Cas systems have been developed and applied in several acetogens, including *C. autoethanogenum*, *C. ljungdahlii*, and *E. limosum*, for gene disruption and transcriptional repression to efficiently manipulate carbon fluxes.^{39,156–158} Specifically, genes encoding phosphotransacetylase (*pta*), aldehyde:Fd oxidoreductase (*aor1* and *aor2*), or bifunctional alcohol/aldehyde dehydrogenases (*adhE1* and *adhE2*) have been targeted in *C. ljungdahlii*.^{36,159–161} This genetic manipulation redirected carbon flux towards desired products, successfully improving the production of acetate,¹⁵⁹ ethanol,¹⁶⁰ butyrate,¹⁶¹ and 3-HB.³⁶ While these studies focused on targeting genes directly related to biochemical synthesis pathways, a recent genome-wide CRISPRi screening in *E. limosum* identified three novel genes (ELIM_c2976 encoding putative Fe–S binding protein, ELIM_c1759 encoding Rho protein, and ELIM_c1868 encoding putative transcription regulator) whose repression improved autotrophic growth.¹⁵⁴ The simultaneous repression of these three genes resulted in a 1.5-fold increase in acetoin production in *E. limosum*.

Development of efficient production strains requires exploring a large design space, which involves screening hundreds to thousands of different pathways and strains. However, the slow growth and oxygen-sensitive nature of acetogens hinder this process, making screening and strain construction time-consuming and labor-intensive. This challenge can be overcome using synthetic biology tools with high-throughput workflows for strain construction and screening (Fig. 5C).

A suitable reporter system for acetogens is an initial requirement for high-throughput screening. Commonly used systems such as GFP or mRFP require oxygen for chromophore maturation, limiting their application to acetogens growing under strictly anaerobic conditions. As an alternative, oxygen-independent reporter systems such as HaloTag, SNAP-tag, and fluorescence-activating and absorption-shifting tag (FAST) have been developed and applied to the acetogens *C. ljungdahlii*,^{162–164} *E. limosum*,^{71,165} *A. woodii*,⁶⁹ and *T. kivui*¹⁶⁶ to study co-culture dynamics, screen genetic bioparts, and track heterologous protein production. The deployment of these reporter systems enables high-throughput screening of acetogens.

Cell-free prototyping, a cutting-edge synthetic biology tool, allows *in vitro* pathway prototyping to inform *in vivo* design to expedite strain engineering. This technology accelerates the process by testing hundreds to thousands of enzyme variants



and combinations in weeks rather than months.⁷ Cell-free prototyping based on the iPROBE platform has been utilized in developing efficient strains for producing 3-HB and butanol, evaluating 54 different pathway combinations for 3-HB production and optimizing 205 pathways for butanol production.⁷⁷ With a strong correlation between cell-free and *in vivo* cellular performance, this approach significantly improved production capabilities, showing a 20-fold increase in 3-HB titer, reaching up to 15 g L⁻¹ at rates of over 1.5 g L⁻¹ h⁻¹ in *C. autoethanogenum*. This result demonstrates its potential for industrial-scale applications.

The various tools mentioned above can be combined to construct highly efficient platform strains capable of producing high-energy compounds on an industrial scale. Recent achievements in this field have demonstrated the feasibility of applying synthetic biology tools to genome-scale optimization of acetogens for sustainable and scalable biomanufacturing.⁷⁰ Leveraging genome mining, genome engineering tools, omics analysis, kinetic modeling, cell-free prototyping, and high-throughput screening of nearly 300 strains, highly engineered *C. autoethanogenum* capable of carbon-negative production of isopropanol and acetone from industrial waste gases was developed. This successful scale-up to 120 L demonstrated a production rate of ~3 g L⁻¹ h⁻¹ and ~90% selectivity.

The design-build-test-lean (DBTL) cycle, a framework in synthetic biology for developing strains with desired functionalities, can be automated and streamlined in a biofoundry to accelerate strain development at high throughput, increasing speed, efficiency, cost-effectiveness, and consistency.¹⁶⁷ Biofoundries utilize advanced automation, synthetic biology, and computational tools to accelerate design of new strains, and their construction, and testing. Despite the challenges posed by the requirement for anaerobic conditions and gaseous substrates, LanzaTech's world-first anaerobic biofoundry for acetogens is rapidly showing the possibilities by generating and screening thousands of strains per cycle.¹¹ With synthetic biology technologies and automated workflows in biofoundries, engineering acetogens for industrial-scale production of high-energy compounds is now feasible. Implementing these technologies would significantly accelerate the development of sustainable and green, industrially relevant acetogenic C1 conversion platform strains.

6 Economic sustainability of acetogenic C1 conversion

To industrialize acetogenic C1 conversion and achieve a circular carbon economy, assessing its economic sustainability is crucial, which can be evaluated by the techno-economic assessment (TEA) and life-cycle analysis (LCA).¹⁶⁸ Recent TEA and LCA studies on acetogenic C1 conversion have demonstrated its economic and environmental viability, which depends on several factors such as feedstock costs and final products.^{70,82,168–170}

Recent TEA of CO₂ utilization *via* biological conversion has revealed that PHB production is cost-competitive, projecting a minimum selling price (MSP) of \$1.36 per kg, which falls below

its market price of \$2.40–3.30 per kg.¹⁷¹ Another assessment covering 11 products synthesized *via* various C1 conversion routes suggested focusing research efforts on products such as butyrate and butanol, whose production costs are near or below current market prices.¹⁶⁸ This strategic focus on high-value market products is crucial for ensuring economic competitiveness against current fossil fuel-based production.

Although ethanol and acetone have lower market prices compared to the aforementioned C4 products (PHB, butyrate, and butanol), their production processes can still be economically viable with strain improvements and process optimization aimed at increasing production efficiency and thereby reducing costs.¹⁷⁰ TEA and LCA studies can identify major cost contributors and research needs, guiding strain design and process optimization to enhance product yield, productivity, and titer. For instance, syngas fermentation with the best-engineered strain and optimized process achieved cost-competitive production of acetone and ethanol.¹⁷⁰ The cash cost of production (CCOP) was reduced by over 60% compared to the start of the project, enabling biofuel production at or below DOE's target of \$3 per gallon gasoline equivalent. Moreover, LCA indicated significant reductions in GHG emissions in the process, achieving reductions of over 180% for acetone and 90% for ethanol, compared to conventional processes.

Feedstock cost is a primary factor affecting the economic viability of the process.¹⁷² A comparative analysis of the economic feasibility of acetone production from various sources of syngas using *M. thermoacetica* identified syngas derived from a basic oxygen furnace (BOF) to be more economically feasible than syngas from natural gas and corn stover, which have higher feedstock costs.⁸² Another study reported that using reformed biogas is more favorable for sustainable and economically viable chemical production compared to utilizing steel mill off-gas.¹⁶⁸ Given that steel production is a major source of GHG emissions,¹⁷³ using steel mill off-gas results in higher emissions compared to reformed biogas, albeit achieving 60% lower emissions compared to conventional gasoline.¹⁷⁴ Nevertheless, *via* strain and process optimization, acetone and isopropanol production from such industrial waste gases can be economically viable. This was demonstrated by achieving a negative carbon footprint of -1.78 kgCO₂e per kg for the produced acetone and -1.17 kgCO₂e per kg for the produced isopropanol, which is lower than traditional petrochemical production that emits substantial GHGs (2.55 kgCO₂e per kg for acetone and 1.85 kgCO₂e per kg for isopropanol).⁷⁰

With its promises of economic sustainability, several companies have already established pilot and commercial plants for acetogenic C1 conversion (Table 5). Scaling up and commercializing gas fermentation for ethanol production have been pursued by several companies, including INEOS Bio (acquired by Jupeng Bio), Coskata (acquired by Synata Bio), Genomatica, and LanzaTech (Table 5).^{175,176} However, INEOS Bio and Coskata are no longer operational due to financial and operational challenges. To enhance product yields and improve process economics, White Dog Labs developed mixotrophic fermentation utilizing both syngas and sugars for the production of acetone and isopropanol.^{175,177}



Table 5 A list of companies involved in acetogenic C1 conversion processes

| Company | C1 conversion technology | Final product(s) | Ref. |
|----------------------|---|---|------|
| LanzaTech | Syngas or industrial waste gas fermentation | Ethanol, 2,3-butanediol, acetone, isopropanol, and others | 178 |
| Sekisui Bio-Refinery | Syngas fermentation | Ethanol | 179 |
| ArcelorMittal | Industrial blast furnace gas fermentation | Ethanol | 180 |
| Jupeng Bio | Syngas fermentation | Ethanol | 181 |
| Genomatica | Syngas fermentation | Ethanol | 182 |
| White Dog Labs | Mixotrophic fermentation (syngas + sugars) | Acetone, isopropanol | 177 |
| Evonik, Siemens | CO ₂ electrolysis (Siemens) + syngas fermentation (Evonik) | Butanol, hexanol, 2-hydroxyisobutyric acid | 183 |
| VITO | Microbial electrosynthesis (CO ₂ , electricity) | Ethanol, ethylene | 184 |

LanzaTech is a leading company in acetogenic C1 conversion. Their various pilot projects and commercial installations have demonstrated positive results (*e.g.*, ethanol production from C1 gases) in terms of both economic and environmental benefits, thereby proving the economic sustainability of their acetogenic C1 conversion technologies.^{11,185} By partnering with companies across the global supply chain, including Sekisui Bio-Refinery, ArcelorMittal, Zara, and BASF, LanzaTech is paving the way for a circular carbon economy and advancing its carbon recycling technology.¹⁸⁶

Although MES holds promise with the potential use of renewable electricity, its commercialization is hindered by technological, economic, and scalability challenges. These include the high cost and low scalability of CO₂ electrolyser, as well as the low electron consumption rate of microbial biofilms.^{168,171,187} One suggested approach to make the MES process economically viable is to decouple the surface-dependent abiotic process for electron delivery from the volume-dependent biotic carbon fixation process.¹⁶⁸ Additionally, research efforts to decrease electrolysis costs are also necessary. Currently, a few companies have explored MES for industrializing CO₂ conversion to chemical production using acetogens (Table 5). For example, VITO has conducted research on MES for producing ethanol and ethylene.¹⁸⁴ Joint research led by Evonik and Siemens utilizes a CO₂ electrolyser and syngas fermentation of a microbial consortium of acetogens and chain-elongating bacteria to convert CO₂ to butanol and hexanol.¹⁸³

Methanol is another promising C1 feedstock. The cost for butanol production using methanol was estimated at \$930 per t, close to the current market price. This estimate considers non-renewable methanol, which has a lower market price (\$300 per t) than renewable methanol (\$560 per t).^{168,188} Despite its economic feasibility, methanol is not currently used as a feedstock for industrial-scale acetogenic C1 conversion. Given that methylotrophic growth of acetogens can potentially achieve higher product yields, growth rates, and energy efficiency, using methanol as a feedstock is expected to become economically viable with strain improvements and process optimization.

7 Conclusions and future perspectives

Harnessing acetogenic bacteria for one-carbon valorization presents a promising route towards sustainable and green

chemical production aligned with the circular economy concept. Recent advancements in genetic tools developed for acetogens have enabled the production of over 50 different chemicals from engineered acetogens.^{11,12} Several of these, including ethanol, 2,3-butanediol, acetone, and isopropanol, have already reached industrially relevant performance levels, with production rates in the g L⁻¹ h⁻¹ range and titers at the tens of g L⁻¹ level.^{7,70,170} As a frontrunner in global carbon recycling, LanzaTech is actively commercializing its technology for acetogenic C1 conversion, providing actual proof of its potential value to the carbon-producing industry.⁸¹ However, they are currently limited to native products of acetogens or non-native short-chain compounds. The production of energy-demanding long-chain or non-native chemicals from C1 substrates remains a challenge, with most research focused mainly on low technology readiness levels (TRL) 2 to 3, corresponding to basic and applied research.⁸¹ The major obstacle in this regard is the inherent energetic limitations of acetogens.

Overcoming the energetic limitations of acetogens is crucial for achieving high product titers, yields, and productivities sufficient for advancing to higher TRLs (TRL 7–9 for commercial development). Several studies have focused on improving energetics by increasing ATP and redox availability. These studies employed effective strategies such as enhancing ATP availability through alternative electron donors, boosting chemiosmotic or SLP-coupled ATP synthesis, and leveraging acetate as a precursor. Furthermore, the cost and sustainability of the overall process are critical considerations for developing a green chemical production platform based on acetogenic C1 conversion. The availability and cost of C1 substrates and the bioconversion process efficiency significantly influence economic viability. In this regard, efforts to utilize low-cost and sustainable redox suppliers such as electricity and light offer promising paths to enhance the energetics and sustainability of the acetogenic C1 conversion process. In particular, the electricity use of MES has advanced considerably, allowing energy-demanding chemical production from CO₂. Despite its potential, its TRL currently corresponds to the lab scale.¹⁸⁹ Efforts are underway to improve its performance and bring MES technology towards commercialization.^{187,190,191}

The deployment of advanced technologies, including adaptive evolution, systems biology, and synthetic biology, with high-throughput workflows facilitates the design and construction of acetogenic strains with improved productivity, robustness, and efficiency. In particular, integrating biofoundries will accelerate the development of next-generation acetogenic platform strains,



revealing the full potential of acetogenic C1 conversion for sustainable and green chemical synthesis.

In conclusion, acetogenic C1 conversion holds great promise for transforming the fossil-based chemical industry towards more sustainable and greener chemical production. By overcoming existing challenges and embracing emerging technologies, acetogenic C1 conversion can play a pivotal role in achieving a bio-based circular economy, reducing the reliance on fossil fuels, and creating a greener society.

Author contributions

Jiyun Bae: writing – original draft, writing – review & editing, visualization. Chanho Park: writing – original draft. Hyunwoo Jung: writing – original draft. Sangrak Jin: writing – review & editing. Byung-Kwan Cho: conceptualization, supervision, writing – review & editing.

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

Conflicts of interest

There are no conflicts to declare.

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