POLITECNICO DI TORINO
Repository ISTITUZIONALE

Promise and pitfalls of acetogenic Clostridium species for hydrogen and carbon dioxide-driven gas fermentation

Original
Promise and pitfalls of acetogenic Clostridium species for hydrogen and carbon dioxide-driven gas fermentation / Raspone, R., Antonicelli, G., Pirri, F.C., Agostino, V.. - In: FRONTIERS IN MICROBIOLOGY. - ISSN 1664-302X. - 17:(2026), pp. 1-12. [10.3389/fmicb.2026.1953200]

Availability:
This version is available at: 11583/3017597 since: 2026-11-05T17:41:41Z

Publisher:
Frontiers Media

Published
DOI:10.3389/fmicb.2026.1953200

Terms of use:

This article is made available under terms and conditions as specified in the corresponding bibliographic description in the repository

Publisher copyright

(Article begins on next page)

19 November 2026

frontiers in Microbiology

TYPE Mini Review
PUBLISHED 02 November 2026
DOI 10.3389/fmicb.2026.1953200

Check for updates

OPEN ACCESS

EDITED BY
Maria Westerholm,
Swedish University of Agricultural Sciences, Sweden

REVIEWED BY
Hyun Ju Oh,
Korea Institute of Science and Technology (KIST), Republic of Korea
Naorem Bela Devi,
Indian Institute of Technology Guwahati, India
Vladimir Burov,
Harbin Institute of Technology, China

*CORRESPONDENCE
Giacomo Antonicelli
giacomo.antonicelli@iit.it;
agiacomo94@gmail.com

RECEIVED 30 July 2026
REVISED 20 October 2026
ACCEPTED 21 October 2026
PUBLISHED 02 November 2026

CITATION
Raspone R, Antonicelli G, Pirri FC and Agostino V (2026) Promise and pitfalls of acetogenic *Clostridium* species for hydrogen and carbon dioxide-driven gas fermentation.
Front. Microbiol. 17:1953200.
doi: 10.3389/fmicb.2026.1953200

COPYRIGHT
© 2026 Raspone, Antonicelli, Pirri and Agostino. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Promise and pitfalls of acetogenic *Clostridium* species for hydrogen and carbon dioxide-driven gas fermentation

Riccardo Raspone[1,2], Giacomo Antonicelli[1]*, Fabrizio Candido Pirri[1,2] and Valeria Agostino[1]

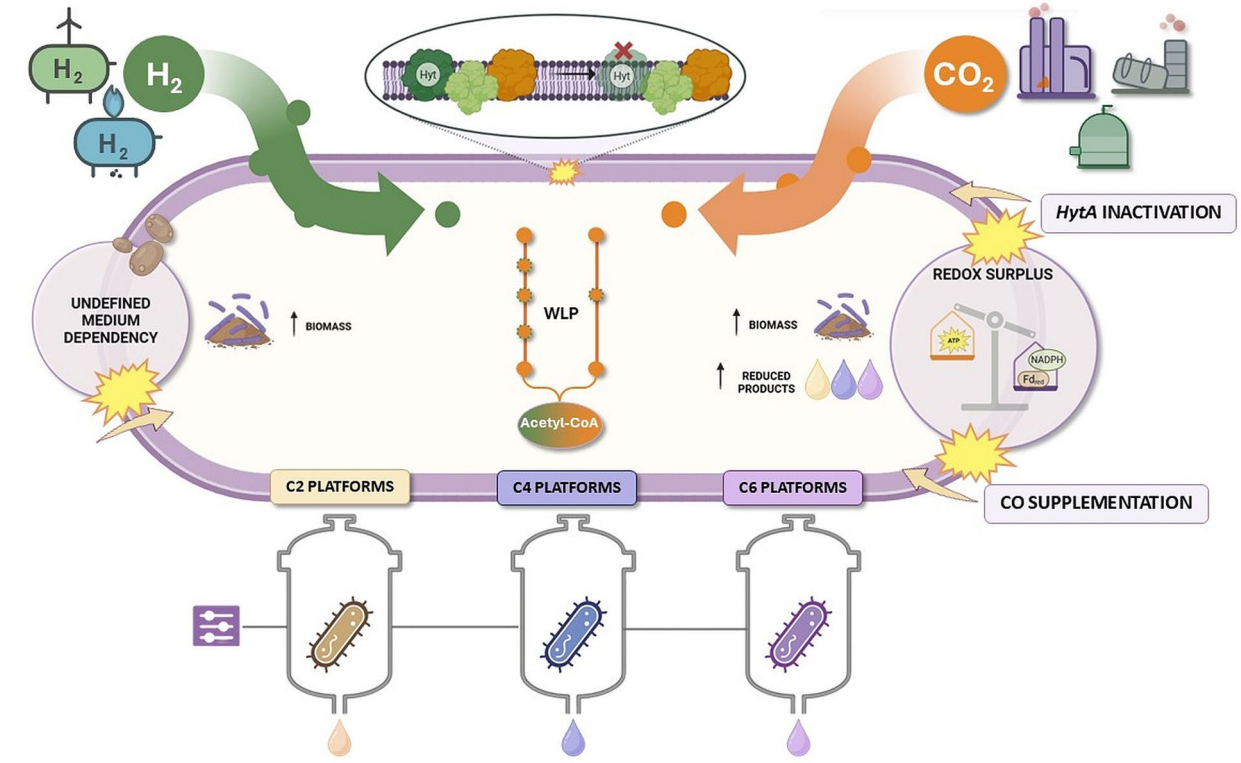
[1]Centre for Sustainable Future Technologies, Istituto Italiano di Tecnologia, Turin, Italy, [2]Department of Applied Science and Technology, Politecnico di Torino, Turin, Italy

Gas fermentation using anaerobic acetogenic bacteria represents a promising carbon capture and utilization strategy for converting C1 gases into valuable bioproducts. Among acetogens, *Clostridium* species have attracted significant attention due to their ability to produce native C2–C6 acids and alcohols through their versatile metabolism. While industrial applications have primarily focused on CO- and syngas-based fermentation, a large fraction of anthropogenic emissions originates from industrial sectors generating CO_2-rich gas streams with limited or negligible CO content. CO_2- fermentation, powered by renewable H_2, offers a potential route for the valorization of these emissions and the development of carbon-negative bioprocesses. However, research on CO_2/H_2-driven gas fermentation by *Clostridial acetogens* remains limited compared with CO- and syngas-based systems, and a comprehensive overview of their potential is still lacking. This mini-review summarizes the current state of research, highlighting the best laboratory-scale performances achieved for native product formation and discussing the key metabolic constraints affecting growth and productivity. Furthermore, it provides a framework for selecting suitable host species and operating conditions tailored to specific target molecules, while exploring recent advances in strain development and process optimization that may support the future scale-up of CO_2/H_2-driven gas fermentation and its application in sustainable biomanufacturing.

KEYWORDS
acetogenic *Clostridium* species, biomanufacturing, C2–C6 bioproducts, CO_2 valorization, gas fermentation, H_2/CO_2

1 Introduction

Among carbon capture and utilization technologies, gas fermentation by anaerobic acetogenic bacteria has emerged as a promising strategy for converting C1 gases into valuable bioproducts (Drake et al., 2008; Ragsdale and Pierce, 2008; Poehlein et al., 2025; Rodriguez et al., 2026). Within this group, *Clostridium* species have attracted particular interest because of their versatile native metabolic pathways, which enable the production of a broad range of products beyond acetate. These pathways include AOR-dependent routes that can favor ethanol production over acetate, as well as reverse β-oxidation pathways that enable the elongation of short-chain carboxylic acids into longer-chain products, including C4–C6 carboxylic acids and their corresponding alcohols (Köpke and Simpson, 2020).



GRAPHICAL ABSTRACT

Industrial development with acetogenic *Clostridium* species has so far focused primarily on carbon monoxide -rich waste gases, particularly steel mill off-gases, with the commercial-scale process for ethanol production established by LanzaTech using *Clostridium autoethanogenum* (Poehlein et al., 2025; Fackler et al., 2021). However, a significant share of anthropogenic emissions arises from industrial sectors generating CO_2-rich gas streams with low or negligible CO content. These concentrated point sources include: (i) cement & ammonia production, with ~1.5 Gt and 0.4–0.5 Gt CO_2 annually, respectively (Andrew, 2019; Mukherjee et al., 2026); (ii) fossil-based H_2 production via steam reforming, contributing ~0.83 Gt CO_2 per year (Global CCS Institute, 2025); (iii) biorefining & bioenergy plants, generating high purity biogenic CO_2 streams from bioethanol fermentation (~82–85 Mt./year) and biogas upgrading (~5–10 Mt./year) (International Energy Agency, 2024; Zitscher and Kaltschmitt, 2024).

Compared to CO-based fermentations, CO_2 conversion requires an external electron donor to provide the reducing power needed for biomass and product formation. The use of H_2 as an external electron donor enables acetogens to reduce CO_2 as the sole carbon source for acetyl-CoA formation, with no stoichiometric CO_2 generation in the overall acetogenic reaction. In contrast, CO-based metabolism involves the oxidation of CO to CO_2, resulting in net CO_2 generation during acetate formation (Bertsch and Müller, 2015). However, this metabolic advantage should not be interpreted as evidence of a net carbon-negative bioprocess. The overall climate impact of industrial gas fermentation depends on the complete carbon and energy balance of the process, including the source and production route of H_2, CO_2 capture and conditioning, energy requirements, downstream processing, and the fate of the carbon incorporated into the final products (Almeida Benalcázar et al., 2022; Kanso et al., 2025).

Despite their high potential, experimental works on H_2/CO_2 fermentation by acetogenic *Clostridia* remain limited compared to the more extensive literature on CO- and syngas-based fermentation (Khalid et al., 2024; Kim et al., 2023; Poehlein et al., 2025). To the best of the authors' knowledge, a review of *Clostridial acetogens* grown on H_2/CO_2 is still lacking. This gap reflects the lower growth rate and productivity of *C. acetogens* cultured on H_2 and CO_2, which relies on the thermodynamic constraints associated with using H_2 as an energy source instead of CO. Consequently, achieving industrially relevant performance requires rigorous process optimization, often combined with strain development through adaptive laboratory evolution and genetic engineering.

Therefore, this mini-review aims to provide an overview of the current research landscape, with a focus on the best laboratory-scale performance achieved for the production of C2–C6 acid and alcohols products. It also discusses the main metabolic challenges associated with H_2/CO_2-dependent growth in *C. acetogens*, as well as the most recent insights that offer promising perspectives for the industrial implementation of H_2/CO_2 gas fermentation.

2 Challenges and emerging insights into autotrophic H_2/CO_2-driven metabolism

2.1 CO versus H_2: unequal electron donors in acetogenic metabolism

Acetogenic *Clostridium* species grow autotrophically on H_2/CO_2, CO, and syngas using the Wood–Ljungdahl pathway (WLP), which fixes two molecules of CO_2 into acetyl-CoA and subsequently acetate. Although acetate formation generates one ATP via substrate-level

phosphorylation, this is offset by the ATP required for formate activation, resulting in essentially no net ATP gain from the WLP (Ragsdale and Pierce, 2008; Katsyv and Müller, 2020). Detailed descriptions of the WLP are available in previous reviews (Drake et al., 2008; Ragsdale and Pierce, 2008; Schuchmann and Müller, 2014; Debabov, 2021).

Autotrophic growth therefore strongly depends on the well-established chemiosmotic mechanism of energy conservation (Katsyv and Müller, 2020). The main player in this mechanism is the membrane-bound Rnf complex, which couples electron transfer to proton translocation, generating the proton motive force that drives ATP synthesis via the F_0F_1-ATP synthase (Köpke et al., 2010; Tremblay et al., 2013). Since Rnf is fueled by NAD^+ and reduced ferredoxin (Fd_{red}), maintaining an optimal pool of these cofactors is essential for sustaining chemiosmotic energy conservation. Accordingly, the pathways responsible for generating and balancing NAD(P)H and Fd_{red} represent a critical link between substrate oxidation and energy conservation. Specifically, Fd_{red} generation varies depending on electron donor used (H_2 or CO). During H_2/CO_2 fermentation, H_2 is mainly oxidized by the electron-bifurcating hexameric hydrogenase Hyt (HytABCDE1E2), which transfers electrons from the H^+/H_2 redox couple ($E^{0\prime} = -414$ mV) to both ferredoxin ($E^{0\prime} \approx -400$ to -500 mV) and the more favorable $NAD(P)^+$/NAD(P)H couple ($E^{0\prime} \approx -320$ mV) (Mock et al., 2015). This electron bifurcation enables Fd reduction but results in a limited yield of 0.5 mol Fd_{red} per mol H_2 (Wang et al., 2013a). In contrast, CO oxidation by carbon monoxide dehydrogenase (CODH) ($E^{0\prime} \approx -520$ mV) directly reduces ferredoxin, producing 1 mol Fd_{red} per mol CO (Wang et al., 2013a; Buckel and Thauer, 2018). This 2-fold difference in Fd_{red} availability impacts Rnf-driven ATP generation, making growth on H_2/CO_2 generally more challenging than on CO.

Fd_{red} availability under H_2/CO_2 fermentation depends also on the well-described transhydrogenase Nfn (Wang et al., 2010; Liang et al., 2019), which reversibly interconverts Fd_{red}, NADH and NADPH according to cellular demand. Under Fd_{red}-limiting conditions, Nfn consumes NADPH to regenerate Fd_{red}, prioritizing Rnf-driven ATP production over biosynthesis and product formation (Hermann et al., 2020). Other metabolic strategies may also contribute to maintaining the reduced ferredoxin (Fd_{red}) pool. For example, when grown on H_2/CO_2, *Clostridium ljungdahlii* activates a malate-oxidizing pathway and upregulates flavodoxin, providing an alternative route for electron transfer (Aklujkar et al., 2017).

Direct evidence for this Fd_{red}-driven limitation comes from comparative fermentation studies in the same organism under H_2/CO_2 with or without CO supplementation. In *C. autoethanogenum* grown on H_2/CO_2, adding 2% CO tripled steady-state biomass in chemostat reactor (3-fold, to 0.54 g_{CDW}/L), and a similar increase was observed in batch when CO was raised from 0 to 10% (1.7-fold, to 0.10 g_{CDW}/L) (Heffernan et al., 2020; Suazo et al., 2026). The same trend emerged in *Clostridium ragsdalei*, where continuous cultivation with 8% CO increased biomass 3.4-fold (to 0.84 g_{CDW}/L), and in *Clostridium muellerianum*, where CO supplementation nearly doubled biomass (1.7-fold, to ~0.85 g_{CDW}/L) while shifting metabolism from acidogenesis to solventogenesis (Thunuguntla et al., 2025, 2026).

Lastly, recent kinetic studies show that H_2 uptake follows first-order kinetics across the physiological range, indicating that hydrogenases operate below saturation and so that H_2 gas–liquid transfer is the major constraint given by its low solubility in water, considerably lower than that of CO (Philips, 2020). This presents a critical bottleneck for H_2/CO_2 fermentation, requiring optimized bioreactor design and process conditions to enhance the volumetric mass transfer coefficient (k_La), essential aspects for industrial implementation.

2.2 The paradox of hexameric hydrogenase HytA subunit inactivation

As described above, H_2/CO_2 performance critically depends on redox balance regulation, mainly controlled by hydrogenases. Acetogenic *Clostridium* species encode a structurally diverse hydrogenase repertoire, grouped into two metalloenzyme families (Greening et al., 2016). Electron-bifurcating hydrogenases include trimeric Group A3 [FeFe]-hydrogenases, dependent on both NAD^+ and ferredoxin, and hexameric Group A4 (Hyt) [FeFe]-hydrogenases, which similarly depend on $NADP^+$ and ferredoxin. In contrast, monomeric Group B [FeFe]-hydrogenases are non-bifurcating and exclusively ferredoxin-dependent, whereas Group 1a [NiFe]-hydrogenases couple periplasmic H_2 oxidation to proton-gradient generation via a membrane-bound cytochrome, independently of soluble cofactors (Wang et al., 2013b; Berrisford et al., 2016; Di Leonardo et al., 2022).

Despite this large repertoire, only the hexameric Hyt proved to be expressed and functionally relevant under H_2/CO_2 conditions, channeling H_2-derived electrons into the WLP (Mock et al., 2015). Hyt forms a complex with a formate dehydrogenase (FdhA), forming an H_2-dependent CO_2 reductase (HDCR) that couples H_2 oxidation to the reversible reduction of CO_2 to formate. This complex was first described in model acetogens *Acetobacterium woodii* and *Thermoanaerobacter kivui* (Schuchmann and Müller, 2013; Schwarz et al., 2018). In acetogenic *Clostridium* species, the corresponding complex is organized into a seven-subunit assembly (FdhA/HytABCDE1E2), encoded by a conserved gene cluster (*fdhA/hytABCDE1E2*) (Di Leonardo et al., 2022).

Paradoxically, inactivation of the catalytic subunit of hexameric hydrogenase (*hytA*), improves autotrophic *Clostridium* growth and production on H_2/CO_2. In *C. autoethanogenum*, ClosTron-mediated *hytA* disruption generated a clone (ΔCAETHG_2798) that achieved higher biomass and over 20-fold higher ethanol production under H_2/CO_2 than the wild-type (WT) strain (Nagaraju and Koepke, 2017). Recently, the same outcome was observed in *C. ljungdahlii*, where CRISPR/Cas9-mediated *hytA* deletion (ΔCLJU_RS03480) resulted in 3.4-fold higher biomass and raised the ethanol-to-acetate ratio ~18-fold (from 0.10 to 1.83) compared to the WT (Wen et al., 2026). Finally, in *C. carboxidivorans*, adaptive laboratory evolution (ALE) under H_2/CO_2 selective pressure spontaneously generated an adapted strain with a frameshift insertion in *hytA* (Ccar_RS16050), introducing a premature stop codon upstream of the H-cluster-coordinating motif. The resulting truncated and likely non-functional catalytic subunit increased biomass by 1.5-fold compared to the parental strain, while redirecting carbon flux toward hexanol production with remarkable selectivity (Antonicelli et al., 2023) (Figure 1).

These improved performances are likely the result of a rearrangement of redox cofactor pools. Following *hytA* disruption, cells compensate by upregulating alternative hydrogenases that reduce ferredoxin without NADPH generation, shifting H_2 uptake away from the Fd^+ $NADP^+$ dependence of Hyt complex. In *C. ljungdahlii*, this cofactor shift involves ~5-fold upregulation of the Fd-only Hyd (CLJU_RS18325, Group B), whereas in *C. autoethanogenum* it is driven by a ~5-fold compensatory upregulation of the multi-subunit,

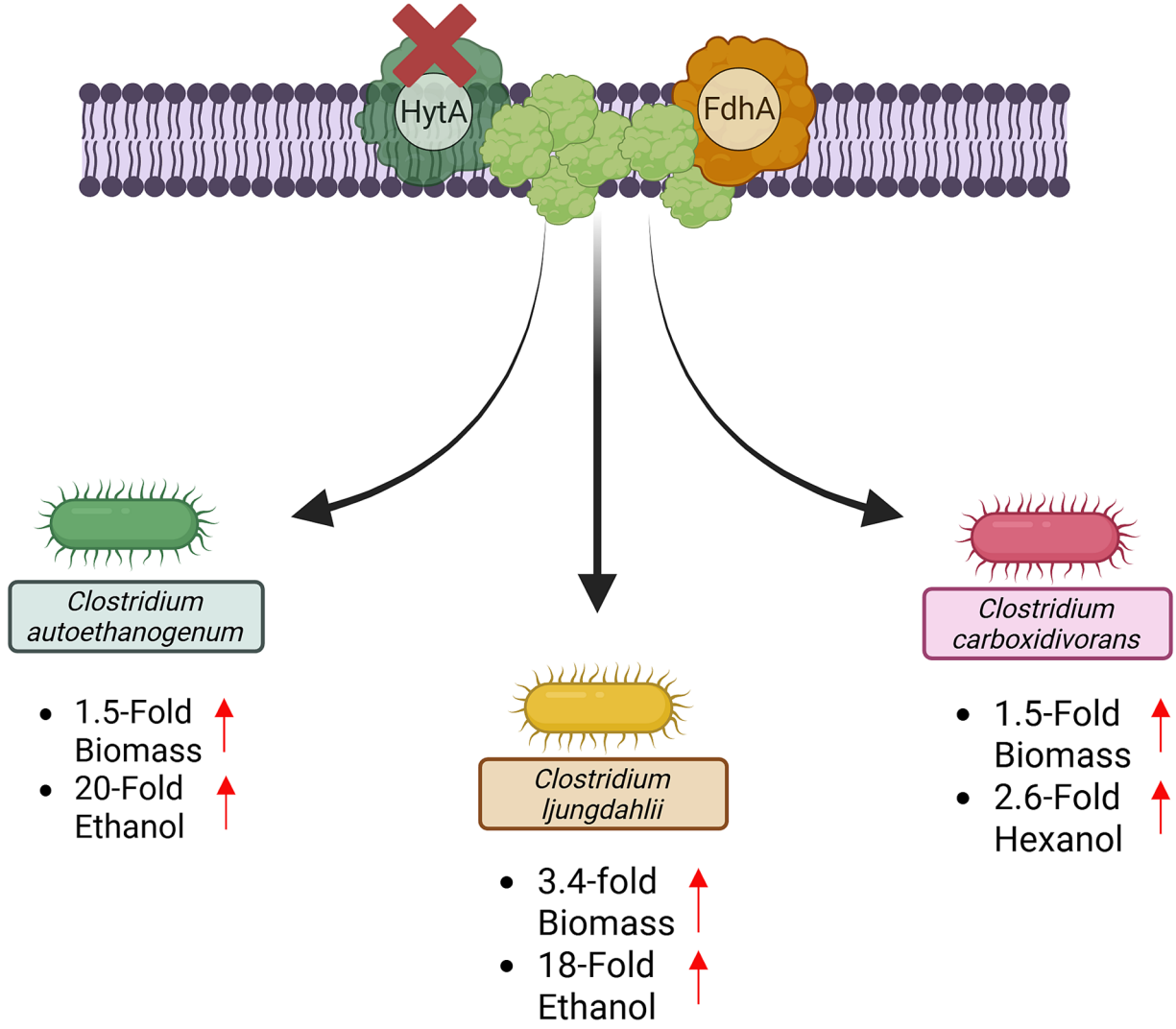


FIGURE 1
Schematic representation of the hexameric electron-bifurcating hydrogenase complex (Hyt), comprising the catalytic subunit HytA and the associated formate dehydrogenase (FdhA), before and after HytA inactivation. Loss of HytA activity generally redirects electron flow through compensatory single-subunit hydrogenases (Hyd), improving autotrophic performance in a species-specific manner. In *C. autoethanogenum*, disruption of hytA (CAETHG_2798) increased biomass and raised ethanol titers over 20-fold, driven by ~5-fold compensatory upregulation of the Hyd cluster (CAETHG_1576-78) together with the single-subunit hydrogenase CAETHG_0110 (Nagaraju and Koepke, 2017). In *C. ljungdahlii*, CRISPR/Cas9-mediated hytA (CLJU_RS03480) deletion increased biomass 3.4-fold and raised the ethanol-to-acetate ratio ~18-fold, a shift attributed to ferredoxin-only reduction by the compensatory Hyd (CLJU_RS18325) enzyme, which generates an ATP surplus via the Rnf complex and drives carbon flux toward ethanol through the AOR pathway (Wen et al., 2026). In *C. carboxidivorans*, a spontaneous frameshift mutation in *hytA* (Ccar_RS16050) arising during adaptive laboratory evolution (ALE) under H_2/CO_2 produced a truncated non-functional enzyme, increasing biomass 1.5-fold and redirecting carbon flux toward chain elongation, yielding a 2.6-fold increase in hexanol (Antonicelli et al., 2023). Created in Biorender.com.

NAD^+-and-Fd-dependent Hyd cluster (CAETHG_1576–78, Group A3) together with the single-subunit Hyd (CAETHG_0110) (Nagaraju and Koepke, 2017; Wen et al., 2026).

The mechanism proposed to explain the improved performance of *C. ljungdahlii* mutant focuses on Fd_{red} accumulation from the alternative Fd-only H_2 uptake, which enhances Rnf activity, increasing NADH levels and proton motive force. The loss of Hyt-derived NADPH is compensated by Nfn, which converts excess Fd_{red} and NADH into NADPH for WLP-dependent carbon fixation (Wen et al., 2026). Overall, this cofactor redistribution improves ATP and reducing power availability, favoring biomass formation and reduced product synthesis.

This positions hydrogenase engineering, and particularly HytA inactivation, as a rational and promising strategy to improve H_2/CO_2-driven gas fermentation processes with autotrophic *Clostridium* acetogens.

2.3 The ultimate energetic constraint: achieving full autotrophy

Achieving full autotrophy represents another major metabolic challenge under H_2/CO_2. Validating this capacity requires chemically defined media to confirm that acetogens can synthesize all essential biomass precursors and satisfy their metabolic energy

demands exclusively from inorganic substrates. Moreover, using defined media is also industrially more attractive due to their controlled composition, reduced batch-to-batch variability, lower contamination risk, and facilitated downstream product recovery, thus undefined components such as yeast extract (YE) are avoided (Zhang and Greasham, 1999). However, robust autotrophic growth in chemically defined media remains difficult to achieve in many acetogenic *Clostridium* species, as the limited ATP availability restricts the *de novo* biosynthesis of amino acids and cofactors required for growth. YE supplementation alleviates this constraint by directly supplying amino acids, some of which can also contribute to energy-conserving pathways. Glycine can enter the Reductive Glycine Pathway, operating alongside the WLP in *C. drakei* (Song et al., 2020), whereas L-arginine is catabolized through the Arginine Deiminase Pathway, generating ATP via substrate-level phosphorylation (Valgepea et al., 2017).

As a result, many strains remain dependent on nutrient-rich supplements. These include *C. drakei*, *C. scatologenes*, *C. luticellarii* and *C. magnum*, which require YE or tryptone for stable lithotrophic activity (Bomar et al., 1991; Küsel et al., 2000; Gößner et al., 2008; Mariën et al., 2024b). Recently, cost-effective alternatives to refined YE, such as corn steep liquor (CSL) and cotton seed extract (CSE), have shown potential as nutrient-rich supplements in H_2/CO_2 fermentation, influencing product formation in strains like *C. carboxidivorans*, *C. muellerianum* and *C. ragsdalei* (Ramachandriya et al., 2013; Thunuguntla et al., 2024).

So far, only few acetogens, including *C. ljungdahlii* (Klask et al., 2020; Ricci et al., 2021), *C. autoethanogenum* (Abrini et al., 1994) *C. coskatii* (Zahn, 2012) and *C. carboxidivorans* (Phillips et al., 2015; Lakhssassi et al., 2020), support growth without YE, although with lower biomass yields and slower growth rates than in YE-supplemented media.

Regarding strain development, only a few works have successfully addressed auxotrophies using targeted genetic engineering approaches. For instance, in *Clostridium* sp. AWRP, lithotrophic growth limited by auxotrophies for several B vitamins has recently been alleviated through genetic engineering (Lee et al., 2019; Kwon et al., 2024). On the other hand, ALE has proven essential in *C. autoethanogenum* to develop a superior strain able to bridge the performance gap between YE-supplemented and chemically defined cultivation (Ingelman et al., 2024). Subsequently, through reverse engineering the same authors identified and confirmed the genes involved in accelerated autotrophic growth in minimal medium without YE (Ingelman et al., 2025). Overall, this presents ALE as an effective tool for uncovering key mutations that can be engineered into other *C. acetogens* to overcome YE dependency.

3 Native product portfolio under H_2/CO_2

The following sections review acetogenic *Clostridium* pure-culture H_2/CO_2 fermentations, focusing on key strains for specific native products. Special emphasis is given to reactor-based studies, due to their relevance to process evaluation and scale-up (Table 1). In Supplementary material, Supplementary Table S1 provides a broader overview including works based on serum bottles and sealed tubes at laboratory scale, while Supplementary Table S2 reports the global market size of the acetogenic native products and their different industrial applications.

3.1 Acetate platforms

Acetate production follows two distinct strategies in acetogenic *Clostridium* species. Strictly homoacetogenic species channel all fixed carbon toward acetate, whereas solventogenic species distribute carbon flux between acetate and more reduced end-products (alcohols and diols) (Bengelsdorf et al., 2018).

Homoacetogens stand out for their gas-conversion efficiency. *C. aceticum* reached ~96% H_2 conversion with an acetate volumetric productivity (r_{Ac}) of 0.84 g/(L·h) in trickle-bed biofilm reactors, whereas *C. thailandense* upgraded biogas from 37.7 to 96.9% (v/v) biomethane using activated-carbon immobilization, achieving complete CO_2 and 78% H_2 conversion despite the presence of CH_4, an inert gas for most acetogens (Riegler et al., 2019; Chaikitkaew et al., 2022, 2024).

Conversely, solventogenic species can be redirected toward a homoacetogenic phenotype through process engineering. pH control at 6.0–6.5 increases acetate selectivity while limiting solventogenesis in *C. ljungdahlii* and preventing chain elongation in *C. luticellarii* (Klask et al., 2020; Mariën et al., 2024b). At neutral pH acetic acid (pKa of 4.75) remains almost completely dissociated, and therefore biologically unavailable to feed collateral pathways such as AOR route for solventogenesis, or the reverse β-oxidation for chain elongation (Mock et al., 2015; Lauer et al., 2022; Mariën et al., 2024a).

High dilution rates also favor acetate formation in *C. autoethanogenum* by supporting faster growth through the more ATP-efficient acetogenesis pathway (Heffernan et al., 2024). Since acetate production is growth-associated, maintaining active cell growth is critical for sustaining acetate productivity, making H_2 availability a key determinant of production performance. This has been demonstrated in both *C. ljungdahlii*, where increasing the H_2:CO_2 ratio enhanced acetate production in stirred-tank reactors even under relatively low-pH conditions, and *C. autoethanogenum*, where H_2 microsparging under low agitation rates likewise promoted acetate production (Heffernan et al., 2023; Schick et al., 2026).

Under these controlled reactor conditions, solventogenic strains can outperform dedicated homoacetogens in terms of acetate productivity. For instance, *C. autoethanogenum* and *C. ljungdahlii* cultivated both in continuous stirred tank reactor (CSTR) operating in cell-retention configuration achieved a r_{Ac} of 1.50 g/(L·h) (Mock et al., 2015) and 2.0 g/(L·h) (Schick et al., 2026), respectively. On the other hand, the homoacetogen *C. aceticum* cultivated in trickle bed reactor reported a r_{Ac} of 0.84 g/(L·h) (Riegler et al., 2019). Furthermore, *C. ljungdahlii* demonstrated the capability to reach acetate titers of up to 43 g/L (Schick et al., 2026) and relevant acetate carbon selectivity of 90–100% (Table 1) (Stoll et al., 2018; Emerson et al., 2019; Hermann et al., 2020).

3.2 Ethanol platforms

Currently, ethanol is the only gas fermentation product commercially produced through LanzaTech's anaerobic process based on a CO-rich gaseous waste stream (Ewing et al., 2022; Fackler et al., 2021). Ethanol biosynthesis proceeds through either a direct route, involving

TABLE 1 Bioreactor performance overview of acetogenic *Clostridium* species cultivated on H_2/CO_2.

Organism	Strain collection number	Reactor	Operating conditions	Growth performance		Product profile			Reference
				μ (h^{-1})	Max biomass (gDCW/L or OD)	Max products titer (g/L)	Max productivity (g/(L·h))	Carbon selectivity (C-moles/C-moles total %)	
Clostridium aceticum[£]	DSM 1496[T]	Trickle-bed biofilm (chemostat)	H_2:CO_2 4:1 + N_2·30 °C·pH 8·1–3.5 bar·τ 1–0.10 h^{-1}·YE	n.r.	8.2 g/L (fixed bed volume)[^]	(Ac) 9.07	0.84 (Ac)	n.r.	Riegler et al. (2019)
Clostridium autoethanogenum	JA1-1·DSM 10061	HFMBR	H_2:CO_2 2.8:1 + N_2 (contigas)·37 °C·pH 5.3·1 bar·D 0.02 h^{-1}·retentostat ·defined medium	0.021 β[1]	1.83 gDCW/L·OD_{600} 4.57 α[1]	(Ac) 7.48·(EtOH) 6.3	1.5 (Ac)·1.29 (EtOH)	n.r.	Mock et al. (2015)
	DSM 19630	CSTR (chemostat)	H_2:CO_2 2.9:1 + Ar/+2%CO (contigas)·37 °C·pH 5.0·1 bar·D 0.02–0.04 h^{-1}·defined medium	H_2:CO_2: 0.019	H_2:CO_2: 0.18 gDCW/L	H_2:CO_2: (Ac) 2.5·(EtOH) 2.4	H_2:CO_2: 0.05 (Ac)·0.05 (EtOH) π[2]	H_2:CO_2: (Ac) 42 (EtOH) 54 Ω[3]	Heffernan et al. (2020)
				+2%CO: 0.042	+2%CO: 0.54 gDCW/L	+2%CO: (Ac) 6.0·(EtOH) 9.7	+2%CO: 0.21 (Ac)·0.26 (EtOH) π[2]	+2%CO: (Ac) 30 (EtOH) 66 Ω[3]	
	DSM 19630	CSTR (chemostat)	H_2:CO_2 3.3:1 + CH_4/Ar (Contigas)·37 °C·pH 5 1 bar·D 0.02 h^{-1}·defined medium	0.021	0.29 gDCW/L	(Ac) 5.3·(EtOH) 2.8	0.11 (Ac)·0.06 (EtOH) π[2]	(Ac) 58 (EtOH) 38 Ω[3]	Heffernan et al. (2023)
	WT & evolved lineage D·DSM 19630	CSTR (chemostat)	H_2:CO_2 2.9:1 + Ar (contigas)·37 °C·pH 5.0·1 bar·D 0.02–0.04 h^{-1}·defined medium	WT: 0.027 β[1]	WT: 0.27 gDCW/L α[6]	WT: (Ac) 6.8·(EtOH) 2.5*[2]	WT: (Ac) 0.18·(EtOH) 0.05 π[3]	n.r.	Heffernan et al. (2024)
				Evolved D: 0.038 β[1]	Evolved: 0.21 gDCW/L α[6]	Evolved: (Ac) 7.6·(EtOH) 2.1*[2]	Evolved: (Ac) 0.36·(EtOH) 0.04 π[3]		
Clostridium carboxidivorans	hex21	Elevated-pressure STR	H_2:CO_2 2:1/4:1 (Fedbatch/Conti-gas) 25 °C·pH 5.0–5.6·1.8 bar·YE·OAL extraction	H_2:CO_2 2:1 (Fedbatch): 0.020 β[1]	H_2:CO_2 2:1 (Fedbatch): 1.05 gDCW/L	H_2:CO_2 2:1 (Fedbatch): (Hex) 1.6	H_2:CO_2 2:1 (Fedbatch): (Hex) 0.01	H_2:CO_2 2:1 (Fedbatch): (Hex) 32 Ω[3]	Antonicelli et al. (2025)
				H_2:CO_2 4:1 (Fedbatch/Conti-gas): 0.020 β[1]	4:1 (Fedbatch/Conti-gas): 1.10 gDCW/L	4:1 (Fedbatch/Conti-gas): (EtOH) 0.7·(ButOH) 1·(HexOH) 3.7	n.r	4:1 (Fedbatch/Conti-gas): (EtOH) 9·(ButOH) 15·(HexOH) 60 Ω[3]	

(Continued)

10.3389/fmicb.2026.1953200

TABLE 1 (Continued)

Organism	Strain collection number	Reactor	Operating conditions	Growth performance		Product profile			Reference
				μ (h^{-1})	Max biomass (gDCW/L or OD)	Max products titer (g/L)	Max productivity (g/(L·h))	Carbon selectivity (C-moles/C-moles total %)	
Clostridium ljungdahlii	PETC·DSM 13528	STR	H_2:CO_2 2:1 + N_2 (Contigas)·37 °C·pH 5.9·1–7 bar·Semi-batch·Defined Medium	n.r.	1 bar: OD_{600} 0.5	1 bar: (Ac) 6.1·(EtOH) 0.6·(Form) 0.1	n.r.	n.r.	Stoll et al. (2018)
					7 bar: OD_{600} 0.2	7 bar: (Ac) 0.8·(EtOH) >0.1·(form) 5.4	n.r	n.r.	
	PETC·DSM 13528	CSTR (chemostat)	H_2:CO_2 4:1 (Contigas)·37 °C·pH 4.5–6.0·1 bar·D 0.01 h^{-1}·Defined medium·NH_4^+/NO_3^-	NO_3: 0.01 β^1	NO_3: OD_{600} 1.36	NO_3: (Ac) 13.6·(EtOH) 5.0*[1]	NO_3: (Ac) 0.16·(EtOH) 0.06 π^3	n.r	Klask et al. (2020)
				NH_4: 0.01 β^1	NH_4: OD_{600} 0.9	NH_4: (Ac) 8.6·(EtOH) 2.0*[1]	NH_4: (Ac) 0.10·(EtOH) 0.02 π^3		
	PETC·DSM 13528	STR	H_2:CO_2 1:1 (Contigas)·37 °C·pH 5.9·1 bar·YE	0.024	0.26 gDCW/L	(Ac) 9.02; (EtOH) 0.14	(Ac) 0.15·(EtOH) >0.01 π^1	(Ac) 93·(EtOH) 2 Ω^3	Hermann et al. (2020)
	Mutants Hex#15Int & Hex#15Int Ccar1gInt	STR	H_2:CO_2 4:1 (Contigas)·30 °C·pH 6.0·1 bar·Semi-batch·Defined medium	Hex#15Int: n.r	Hex#15Int: OD_{600} 1.47	Hex#15Int: (ButOH) 0.4·(HexOH) 0.1	n.r.	n.r.	Lauer et al. (2022)
				Hex#15Int Ccar1gInt: n.r	Hex#15Int Ccar1gInt: OD_{600} 0.99	Hex#15Int Ccar1gInt: (ButOH) 0.1·(HexOH) 0.4	n.r.	n.r.	
	PETC WT & ΔhytA·DSM 13528	STR	H_2:CO_2 2.3:1 (Fedbatch)·37 °C·pH 5.8·2 bar·YE	WT: 0.01*	OD_{600} 2.0	(Ac) 22.8·(EtOH) 2.2	(Ac) 0.20·(EtOH) 0.05 π^1	n.r.	Wen et al. (2026)
				ΔhytA: 0.02*	OD_{600} 7.5	(Ac) 12.2·(EtOH) 22.3	(Ac) 0.12·(EtOH) 0.18 π^1		
	WT·DSM 13528	CSTR	H_2:CO_2 3:1 (Contigas)·37 °C·pH 5.5·1 bar·YE·D: 0.02 h^{-1}·Retentostat	0.02 β^1	OD_{600} 6.00	(Ac) 43	(Ac) 2	(Ac) 98	Schick et al. (2026)
	WT & ΔadhE1 DSM 13528	STR	H_2:CO_2 1.5:1 (Fedbatch)·37 °C·pH 6·2 bar·YE	WT: n.r	OD_{600} 1.6	(Ac) 34·(EtOH) 2 *[3]	(Ac) 0.35·(EtOH) n.r π^1	n.r	Liu et al. (2026)
Clostridium luticellarii	FW431·DSM 29923	HFMBR (chemostat)	H_2:CO_2 5:1 (Contigas)·37 °C·pH 6.5·1 bar·D 0.01 h^{-1}·YE	n.r.^	n.r^	(Ac) 8.93·(Iso-But) 1.5·(But) 1.6	(Ac) 0.09·(Iso-But&But) 0.03	(Ac) 63·(Iso-But) 18·(But) 19Ω^1	Mariën et al. (2024a)
Clostridium muellerianum	P21·DSM 111390	CSTR (Chemostat)	H_2:CO_2 2.5:1 (Contigas) + 8%CO·37 °C·pH 4.7–4.9·1 bar·D 0.012 h^{-1}·CSL	0.012 β^1	OD_{600} 2.48 0.9 gDCW/L α^5	(Ac) 6.0·(EtOH) 12.6·(ButOH) 2.5	(Ac) 0.07·(EtOH) 0.15·(ButOH) 0.03 π^2	n.r.	Thunuguntla et al. (2026)
Clostridium ragsdalei	P11·ATCC-PTA-7826	CSTR (chemostat)	H_2:CO_2 2.5:1 (Contigas) + CO·37 °C·pH 4.8·1 bar·D 0.01 h^{-1}·YE	0.012 β^1	OD_{600} 2.23 0.8 gDCW/L α^2	(EtOH) 19.83	0.18 (EtOH)	(EtOH) 43 Ω^2	Thunuguntla et al. (2025)

(Continued)

10.3389/fmicb.2026.1953200

10.3389/fmicb.2026.1953200

TABLE 1 (Continued)

Organism	Strain collection number	Reactor	Operating conditions	Growth performance		Max products titer (g/L)	Product profile		Reference
				μ (h^{-1})	Max biomass (gDCW/L or OD)		Max productivity (g/(L·h))	Carbon selectivity (C-moles/C-moles total %)	
Clostridium sp.	*AWRP WT & Adapted 46T-a*	STR	H_2:CO_2 4:1 (Contigas) 37 °C; pH 5.0-1 bar-Rich Medium +YE	n.r.	*46T-a*: OD_{600} 2.8	*46T-a*: (Ac) 8.1-(EtOH) 1.3	*46T-a*: (Ac) 0.15-(EtOH) 0.01 π^1	n.r.	Kwon et al. (2022)
					WT: OD_{600} 1.5	WT: (Ac) 8.4-(EtOH) 7.4	WT: (Ac) 0.23-(EtOH) 0.14 π^1		

The table summarizes reported performance data for Clostridium species cultivated on H_2/CO_2. Data are organized by species in alphabetic order to enable direct comparison of strain-, growth performance and products distribution on H_2/CO_2. Ac, acetate; But, butyrate; ButOH, n-butanol; EtOH, ethanol; Form, formate; Hex, hexanoate (caproate); HexOH, n-hexanol; Iso-But, isobutyrate. AC, activated carbon; CSE, cotton seed extract; CSL, corn steep liquor; OAL, oleyl alcohol (in situ extraction solvent); RCM, Reinforced Clostridial Medium; YE, yeast extract. ATCC, American Type Culture Collection; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen; CSTR, continuous stirred-tank reactor; HFMBR, hollow-fiber membrane bioreactor; STR, stirred-tank reactor. Contigas, continuous-gas feeding regime; D, dilution rate (h^{-1}); gDCW/L, grams dry cell weight per liter; μ, specific growth rate (h^{-1}); n.q., not quantified; n.r., not reported; OD, optical density; τ, hydraulic residence time; WT, wild type. Growth performance data: β, Growth rate data derived from the article when not declared on the text by looking at graphs and/or applying following formulas (β^p): $\beta^1\mu = D$; $\beta^2\mu = \ln(2)/t_d$; $\beta^3\mu = [\ln(OD2) - \ln(OD1)]/(t2 - t1)$; (α) = Optical Density and Biomass derived from the article when not declared on the text by looking at graphs and/or applying following formulas (α^n): α^1 *C. autoethanogenum* OD = Biomass (g/L)/0.4; α^2 *C. ragsdalei* P11 OD = (X cell + 0.003)/0.377; α^3 *C. carboxidivorans* P7: OD = (Xcell + 0.004) /0.337; α^4 *C. carboxidivorans* P20: OD = (X cell + 0.002)/0.364; α^5 *C. muellerianum* P21: OD = (X cell + 0.002)/0.343; α^6 *C. autoethanogenum* CDW (g/L) = OD × 0.21; α^7 *C. ljungdahlii* CDW (g/L) = OD × 0.30; Product profile: (π) = Maximum Product Titer derived from the article when not declared on the text by looking at graphs and/or applying following formulas (π^n); π^1 Max Titer (g/L) = Productivity/Dilution rate; π^2 Max Titer (g/L) = (Molecular Weight (g/mol)/ n° carbon product); π^3 Max Titer (g/L) = mM × Molecular Weight/1000; (*) = Maximum Product Productivity derived from the article when not declared on the text by looking at graphs and/or applying following formulas (^{*n}); *^{1} Productivity (g/(L·h)) = ($C_2 - C_1$)/($t_2 - t_1$); $t_2 - t_1$ chosen is the shortest as possible to obtain reliable productivity; *^{2} Productivity (g/(L·h)) = Steady-state concentration × Dilution rate; *^{3}Productivity (g/(L·h)) = (C-M L^{-1} d^{-1}/n° carbon product) × Molecular weight (g/mol)/24 h; (Ω) = Carbon Selectivity and corresponding formulas derived from the article (Ω^n) (basis as indicated); Ω^1 Selectivity(%) = (Total moles of product/Total moles of all acid products) × 100; Ω^2 Selectivity(%) = (Total moles of product/(Total CO_2 consumed−CO_2 consumed for other products))/(Theoretical Ratio) × 100; Ω^3 Selectivity(%) = (C-moles/C-moles total products) × 100; n.r., data not available; ^, data not available due to biofilm formation; £, recently re-named *Andreesenella acetica*.

ATP-dependent reduction of acetyl-CoA to ethanol with the bifunctional aldehyde/alcohol dehydrogenase (AdhE), or an indirect AOR-driven route. The latter is energetically favored, as it reduces acetate to acetaldehyde using $Fd_{red,}$ followed by reduction to ethanol via alcohol dehydrogenase (ADH), fully bypassing the ATP cost of acetyl-CoA activation (Mock et al., 2015; Nissen and Basen, 2019).

Among *Clostridium* species, AORs differ in isoform number, gene copy count, expression and activity. *C. ragsdalei* carries the largest cluster, with four AOR gene copies (two aor1 and two aor2 paralogs), while *C. autoethanogenum* and *C. ljungdahlii* carry two orthologous copies. In contrast, *C. carboxidivorans* bears a single copy, whereas *C. coskatii* entirely lacks AOR (Bengelsdorf et al., 2016). However, gene copy number and isoform multiplicity do not directly dictate ethanol production performance, as ethanol synthesis is governed by a complex interplay of metabolic factors, including enzyme expression levels and catalytic activity, isoform specificity, and intracellular redox conditions.

Concerning AOR isoforms, studies confirm that they can operate in reverse mode, influencing the ethanol-to-acetate (Et/Ac) ratio and redox balance. In *C. ljungdahlii*, targeted deletion of aor1 increased the Et/Ac ratio 8-fold on H_2/CO_2, identifying AOR1 as the key isoform channeling acetaldehyde oxidation toward acetate rather than ethanol formation (Baur et al., 2025). Moreover, recent studies showed that adhE deletion impairs its growth on H_2/CO_2. Under this condition, AdhE converts acetyl-CoA to acetaldehyde, which is then oxidized to acetate through AOR, regenerating Fd_{red} to support H_2/CO_2 growth (Liu et al., 2026).

At bioreactor scale, *C. ljungdahlii*, *C. ragsdalei*, and *C. autoethanogenum* are the most extensively characterized strains achieving high ethanol titer and productivity. The engineered *C. ljungdahlii* ΔhytA strain delivered a noteworthy ethanol concentration of 22.3 g/L in fed-batch stirred tank reactor with an Et/Ac ratio of 1.83 (Wen et al., 2026). Similarly, *C. ragsdalei* WT reached 19.8 g/L in CSTR with minor CO supplementation, but with a much higher Et/Ac ratio of 12.2 (Thunuguntla et al., 2025). However, beside acetate titers, continuous retentostat operation revealed crucial to enhance the r_{EtOH} on H_2/CO_2, reaching 1.3 g/(L·h) with *C. autoethanogenum* WT (Table 1) (Mock et al., 2015). Furthermore, *C. autoethanogenum* can concurrently transform biogas-derived CO_2 into ethanol and acetate, effectively upgrading the gas mixture into a CH_4-enriched stream (Heffernan et al., 2023).

3.3 C4–C6 acid and alcohol platforms

C4–C6 acids and alcohols are the most metabolically demanding, yet economically valuable, native products that can be synthesized from H_2/CO_2 (Angenent et al., 2016).

Chain elongation in acetogenic *Clostridium* species proceeds through reverse β-oxidation, where acetyl-CoA units are sequentially condensed onto a growing acyl-CoA chain. This pathway relies on a conserved *hcs* gene cluster encoding thiolase, 3-hydroxyacyl-CoA dehydrogenase, crotonase, and butyryl-CoA dehydrogenase coupled to electron-transferring flavoproteins (EtfAB). Its role was demonstrated by transferring the *C. carboxidivorans hcs1* gene cluster into *A. woodii*, enabling heterologous production of C4–C6 acids and alcohols. However, the presence of the cluster does not guarantee its expression or activity, as shown by the inactive second *hcs* cluster in *C. carboxidivorans* genome (Wirth and Dürre, 2021). In addition, each elongation cycle requires a balanced supply of Fd_{red} and NADH, which

is particularly challenging to maintain under H_2/CO_2 (Angenent et al., 2016). Accordingly, chain elongation from H_2/CO_2 has been demonstrated in only a few *Clostridium* species: *C. carboxidivorans* and *C. muellerianum*, which produce C4–C6 compounds (Antonicelli et al., 2023, 2025; Doyle et al., 2022; Thunuguntla et al. (2026), and *C. luticellarii*, the only acetogenic pure culture reported to produce branched isobutyrate directly from C1 substrates (Mariën et al., 2024a,b).

The production of butyrate, isobutyrate, and caproate requires sufficient acetate accumulation during an initial acidogenic phase, gas-limited conditions, and tight control of temperature, pH, and gas–liquid mass transfer (Antonicelli et al., 2023, 2025; Mariën et al., 2024a; Tiong et al., 2026).

In *C. luticellarii*, product distribution is mainly governed by gas composition and pH: C4-acid selectivity increases as the H_2:CO_2 ratio rises from 2.5 to 5.0, while acidic pH further promotes C4 acids, although methanol co-feeding unlocks C4 biosynthesis without requiring low pH (Mariën et al., 2024a, 2026). In *C. carboxidivorans*, lowering the temperature from 37 ° to 25 °C shifts metabolism toward C6 products (Antonicelli et al., 2023, 2025; Tiong et al., 2026).

Moreover, CO_2 limitation seems to enhance chain elongation in both *C. carboxidivorans* and *C. luticellarii*. This stimulatory effect could be rationalized as a mechanism of redox balance, whereby CO_2 limitation constrains WLP activity due to substrate scarcity, while electron-donor oxidation continues, leading to Fd_{red} and NADH accumulation. In this condition CO_2 cannot act as electron acceptor, thus the residual acetyl-CoA and acetate may act as electron sink feeding the reverse β-oxidation, ultimately redirecting carbon flux toward more reduced products. This mechanism is supported by observations in *C. luticellarii*, where low CO_2 triggers net acetate consumption resulting in enhanced butyrate and iso-butyrate production (Petrognani et al., 2026). A similar pattern has been observed in an evolved strain of *C. carboxidivorans*, where gas-limited operation proved effective for caproate production (Antonicelli et al., 2025). Conversely, the opposite trend seems to regulate *Clostridium kluyveri*, the model chain-elongator heterotrophic strain, as recent studies have shown that CO_2 availability is positively correlated with both growth and caproate production (Fernández-Blanco et al., 2024; Huo et al., 2026). Importantly, *C. kluyveri* does not fix CO_2 through the WLP. Thus, CO_2 does not serve as a terminal electron acceptor in this organism and cannot compete with reverse β-oxidation as an electron sink.

Finally, the use of complex undefined nitrogen sources such as CSL proved to amplify butyrate and hexanoate formation in *C. carboxidivorans* and *C. muellerianum* (Thunuguntla et al., 2024).

In terms of reactor performance (Table 1), *C. luticellarii* achieved remarkable butyrate and isobutyrate titers in a continuous hollow fiber membrane biofilm reactor (1.6 and 1.5 g/L respectively), while relevant caproate production performances were observed with *C. carboxidivorans* (1.7 g/L in Reinforced Clostridial Medium; 1.6 g/L in elevated-pressure fed-batch reactors with "hex21" strain) (Mariën et al., 2024a; Antonicelli et al., 2025; Tiong et al., 2026).

For the alcohols, butanol and hexanol, temperature is again a key parameter but species-specific: the optimum for hexanol production is 25 °C in *C. carboxidivorans* and 37 °C in *C. muellerianum* (Antonicelli et al., 2023; Thunuguntla et al., 2026; Tiong et al., 2026). Beyond process control, strain adaptation has proven equally critical. ALE of *C. carboxidivorans* generated the "hex21" strain, reaching 18% selectivity for butanol and a 40% hexanol selectivity (1.67 g/L, rHex = 0.031 g/(L·h)), while ethyl methanesulfonate mutagenesis produced a mutant with higher butanol formation than wild type (Lakhssassi et al., 2020; Antonicelli et al., 2023).

By looking at reactor studies, a relevant butanol titer (2.5 g/L) was obtained with *C. muellerianum* in a chemostat CSTR supplied with CSL and low CO; under H_2/CO_2 gas mixture, however, the same setup shifted to acidogenesis (Thunuguntla et al., 2026). On the other hand, the evolved *C. carboxidivorans*_hex21 strain achieved noteworthy hexanol titers using a two-stage elevated-pressure setup with in-situ oleyl-alcohol product extraction. Under these conditions, the strain reached a maximum titer of 3.6 g/L and a remarkable 60% carbon selectivity, although these results were not obtained in steady-state condition (Table 1) (Antonicelli et al., 2025).

4 Conclusion and outlook

This review highlights that promising results have already been achieved for ethanol and acetate production from H_2/CO_2 fermentation at lab scale, whereas longer-chain products still show lower titers and productivities, despite the increasing progress made in recent years. Overcoming these bottlenecks and expanding the product portfolio of acetogenic *Clostridium* species will continue to rely on targeted strain engineering and adaptive evolution. The effect of HytA inactivation on the performance of H_2/CO_2-cultivated strains is particularly promising in this respect, although further work across additional strains and growth conditions is needed to establish how broadly it applies within this group.

Finally, although beyond the scope of this review, the limited solubility of H_2 represents an important avenue for further research in CO_2-driven fermentation. Because H_2 availability limits the supply of reducing equivalents to the cells, it ultimately constrains the selectivities and productivities that can be achieved, regardless of strain performance. Improving H_2 transfer through reactor design and operation at elevated pressure therefore represents a key opportunity, as the concentration of dissolved H_2 increases with its partial pressure. This leads to a broader challenge for the field: strain and process optimization are still predominantly performed in atmospheric stirred-tank reactors, whose agitation requirements scale poorly and may limit their industrial applicability. Evaluating engineered and evolved strains under process conditions that more closely resemble those expected for scale-up, including gas-lift reactor configurations and pressurized operations, could yield phenotypes that are more predictive of industrial performance, thereby narrowing the gap between laboratory development and industrial implementation.

Author contributions

RR: Conceptualization, Visualization, Writing – original draft, Writing – review & editing. GA: Conceptualization, Writing – original draft, Writing – review & editing. FP: Funding acquisition, Writing – review & editing. VA: Conceptualization, Funding acquisition, Project administration, Writing – review & editing.

Funding

The author(s) declared that financial support was received for this work and/or its publication. This work is part of the GoodByO project. GoodByO is supported by the Circular Bio-based Europe Joint Undertaking and its members. Funded by the European Union under GA no 101156486. This work is also part of the "Technologies for Sustainability" Flagship program of the Istituto Italiano di Tecnologia.

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that Generative AI was used in the creation of this manuscript. Google Gemini AI was used during manuscript preparation exclusively to improve language clarity and readability. The authors independently reviewed and finalized all text and arguments, retaining full responsibility for the scientific content, literature citations, and overall integrity of the manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Author disclaimer

Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or Circular Bio-based Europe Joint Undertaking. Neither the European Union nor the granting authority can be held responsible for them.

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2026.1953200/full#supplementary-material

References

Abrini, J., Naveau, H., and Nyns, E.-J. (1994). *Clostridium autoethanogenum*, sp. nov., an anaerobic bacterium that produces ethanol from carbon monoxide. *Arch. Microbiol.* 161, 345–351. doi: 10.1007/BF00303591

Aklujkar, M., Leang, C., Shrestha, P. M., Shrestha, M., and Lovley, D. R. (2017). Transcriptomic profiles of *Clostridium ljungdahlii* during lithotrophic growth with syngas or H_2 and CO_2 compared to organotrophic growth with fructose. *Sci. Rep.* 7:12712. doi: 10.1038/s41598-017-12712-w

Almeida Benalcázar, E., Noorman, H., Maciel Filho, R., and Posada, J. A. (2022). Decarbonizing ethanol production via gas fermentation: impact of the $CO/H_2/CO_2$ mix source on greenhouse gas emissions and production costs. *Comput. Chem. Eng.* 159:107670. doi: 10.1016/j.compchemeng.2022.107670

Andrew, R. M. (2019). Global CO_2 emissions from cement production, 1928–2018. *Earth Syst. Sci. Data* 11, 1675–1710. doi: 10.5194/essd-11-1675-2019

Angenent, L. T., Richter, H., Buckel, W., Spirito, C. M., Steinbusch, K. J. J., Plugge, C. M., et al. (2016). Chain elongation with reactor microbiomes: open-culture biotechnology to produce biochemicals. *Environ. Sci. Technol.* 50, 2796–2810. doi: 10.1021/acs.est.5b04847

Antonicelli, G., Ricci, L., Tarraran, L., Fraterrigo Garofalo, S., Re, A., Vasile, N. S., et al. (2023). Expanding the product portfolio of carbon dioxide and hydrogen-based gas fermentation with an evolved strain of *Clostridium carboxidivorans*. *Bioresour. Technol.* 387:129689. doi: 10.1016/j.biortech.2023.129689

Antonicelli, G., Vasile, N., Piro, E., Fraterrigo Garofalo, S., Menin, B., Verga, F., et al. (2025). Harnessing an adapted strain of *Clostridium carboxidivorans* to unlock hexanol production from carbon dioxide and hydrogen in elevated-pressure stirred tank reactors. *Bioresour. Technol.* 418:131966. doi: 10.1016/j.biortech.2024.131966

Baur, S. T., Schulz, S., McCluskey, J. B., Velázquez Gómez, J. A., Angenent, L. T., and Molitor, B. (2025). Deletion of aldehyde:ferredoxin oxidoreductase-encoding genes in *Clostridium ljungdahlii* results in changes in product spectrum with various carbon sources. *Bioresour. Technol.* 431:132596. doi: 10.1016/j.biortech.2025.132596

Bengelsdorf, F. R., Beck, M. H., Erz, C., Hoffmeister, S., Karl, M. M., Riegler, P., et al. (2018). Bacterial anaerobic synthesis gas (syngas) and $CO_2 + H_2$ fermentation. *Adv. Appl. Microbiol.* 103, 143–221. doi: 10.1016/bs.aambs.2018.01.002

Bengelsdorf, F. R., Poehlein, A., Linder, S., Erz, C., Hummel, T., Hoffmeister, S., et al. (2016). Industrial acetogenic biocatalysts: a comparative metabolic and genomic analysis. *Front. Microbiol.* 7:1036. doi: 10.3389/fmicb.2016.01036

Berrisford, J. M., Baradaran, R., and Sazanov, L. A. (2016). Structure of bacterial respiratory complex I. *Biochim. Biophys. Acta Bioenerg.* 1857, 892–901. doi: 10.1016/j.bbabio.2016.01.012

Bertsch, J., and Müller, V. (2015). Bioenergetic constraints for conversion of syngas to biofuels in acetogenic bacteria. *Biotechnol. Biofuels* 8:210. doi: 10.1186/s13068-015-0393-x

Bomar, M., Hippe, H., and Schink, B. (1991). Lithotrophic growth and hydrogen metabolism by *Clostridium magnum*. *FEMS Microbiol. Lett.* 83, 347–350. doi: 10.1111/j.1574-6968.1991.tb04488.x

Buckel, W., and Thauer, R. K. (2018). Flavin-based electron bifurcation, ferredoxin, flavodoxin, and anaerobic respiration with protons (Ech) or NAD^+ (Rnf) as electron acceptors: a historical review. *Front. Microbiol.* 9:401. doi: 10.3389/fmicb.2018.00401

Chaikitkaew, S., In-Chan, S., Singkhala, A., Tukanghan, W., Mamimin, C., Reungsang, A., et al. (2022). *Clostridium thailandense* sp. nov., a novel CO_2-reducing acetogenic bacterium isolated from peatland soil. *Int. J. Syst. Evol. Microbiol.* 72:005254. doi: 10.1099/ijsem.0.005254

Chaikitkaew, S., Wongfaed, N., Mamimin, C., O-Thong, S., and Reungsang, A. (2024). Conversion of carbon dioxide in biogas into acetic acid by *Clostridium thailandense* immobilized on porous support materials. *Heliyon* 10:e26378. doi: 10.1016/j.heliyon.2024.e26378

Debabov, V. G. (2021). Acetogens: biochemistry, bioenergetics, genetics, and biotechnological potential. *Microbiol.* 90, 273–297. doi: 10.1134/S0026261721030024

Di Leonardo, P. F., Antonicelli, G., Agostino, V., and Re, A. (2022). Genome-scale mining of acetogens of the genus *Clostridium* unveils distinctive traits in [FeFe]- and [NiFe]-hydrogenase content and maturation. *Microbiol. Spectrum* 10:e01019-22. doi: 10.1128/spectrum.01019-22

Doyle, D. A., Smith, P. R., Lawson, P. A., and Tanner, R. S. (2022). Clostridium muellerianum sp. nov., a carbon monoxide-oxidizing acetogen isolated from old hay. *Int. J. Syst. Evol. Microbiol.* 72:005297. doi: 10.1099/ijsem.0.005297

Drake, H. L., Gößner, A. S., and Daniel, S. L. (2008). Old acetogens, new light. *Ann. N. Y. Acad. Sci.* 1125, 100–128. doi: 10.1196/annals.1419.016

Emerson, D. F., Woolston, B. M., Liu, N., Donnelly, M., Currie, D. H., and Stephanopoulos, G. (2019). Enhancing hydrogen-dependent growth of and carbon dioxide fixation by *Clostridium ljungdahlii* through nitrate supplementation. *Biotechnol. Bioeng.* 116, 294–306. doi: 10.1002/bit.26847

Ewing, T. A., Nouse, N., van Lint, M., van Haveren, J., Hugenholtz, J., and van Es, D. S. (2022). Fermentation for the production of biobased chemicals in a circular economy: a perspective for the period 2022–2050. *Green Chem.* 24, 6373–6405. doi: 10.1039/d1gc04758b

Fackler, N., Heijstra, B. D., Rasor, B. J., Brown, H., Martin, J., Ni, Z., et al. (2021). Stepping on the gas to a circular economy: accelerating development of carbon-negative chemical production from gas fermentation. *Annu. Rev. Chem. Biomol. Eng.* 12, 439–470. doi: 10.1146/annurev-chembioeng-120120-021122

Fernández-Blanco, C., Veiga, M. C., and Kennes, C. (2024). Carbon dioxide as key player in chain elongation and growth of *Clostridium kluyveri*: insights from batch and bioreactor studies. *Bioresour. Technol.* 394:130192. doi: 10.1016/j.biortech.2023.130192

Global CCS Institute (2025) Global status of CCS 2025: staying the course. Available online at: https://www.globalccsinstitute.com/resources/global-status-report/ (Accessed July 26, 2026)

Gößner, A. S., Picardal, F., Tanner, R. S., and Drake, H. L. (2008). Carbon metabolism of the moderately acid-tolerant acetogen *Clostridium drakei* isolated from peat. *FEMS Microbiol. Lett.* 287, 236–242. doi: 10.1111/j.1574-6968.2008.01313.x

Greening, C., Biswas, A., Carere, C. R., Jackson, C. J., Taylor, M. C., Stott, M. B., et al. (2016). Genomic and metagenomic surveys of hydrogenase distribution indicate H_2 is a widely utilised energy source for microbial growth and survival. *ISME J.* 10, 761–777. doi: 10.1038/ismej.2015.153

Heffernan, J., Garcia Gonzalez, R. A., Mahamkali, V., McCubbin, T., Daygon, D., Liu, L., et al. (2024). Adaptive laboratory evolution of *Clostridium autoethanogenum* to metabolize CO_2 and H_2 enhances growth rates in chemostat and unravels proteome and metabolome alterations. *Microb. Biotechnol.* 17:e14452. doi: 10.1111/1751-7915.14452

Heffernan, J. K., Lai, C.-Y., Gonzalez-Garcia, R. A., Nielsen, L. K., Guo, J., and Marcellin, E. (2023). Biogas upgrading using *Clostridium autoethanogenum* for value-added products. *Chem. Eng. J.* 452:138950. doi: 10.1016/j.cej.2022.138950

Heffernan, J. K., Valgepea, K., Lemgruber, R. S. P., Casini, I., Plan, M., Tappel, R., et al. (2020). Enhancing CO_2-valorization using *Clostridium autoethanogenum* for sustainable fuel and chemicals production. *Front. Bioeng. Biotechnol.* 8:204. doi: 10.3389/fbioe.2020.00204

Hermann, M., Teleki, A., Weitz, S., Niess, A., Freund, A., Bengelsdorf, F. R., et al. (2020). Electron availability in CO_2, CO and H_2 mixtures constrains flux distribution, energy management and product formation in *Clostridium ljungdahlii*. *Microb. Biotechnol.* 13, 1831–1846. doi: 10.1111/1751-7915.13625

Huo, W., Ye, R., Hu, T., Shao, Y., Zheng, Z., Zhang, R., et al. (2026). A novel mechanistic insight into CO_2 effects on chain elongation in *Clostridium kluyveri* based on transcriptomic and 13C-targeted metabolomic analyses. *Water Res.* 289:125007. doi: 10.1016/j.watres.2025.125007

Ingelman, H., Heffernan, J. K., Harris, A., Brown, S. D., Shaikh, K. M., Saqib, A. Y., et al. (2024). Autotrophic adaptive laboratory evolution of the acetogen *Clostridium autoethanogenum* delivers the gas-fermenting strain LAbrini with superior growth, products, and robustness. *New Biotechnol.* 83, 1–15. doi: 10.1016/j.nbt.2024.06.002

Ingelman, H., Shaikh, K. M., and Valgepea, K. (2025). Reverse-engineered gas-fermenting acetogen strains recover enhanced phenotypes from autotrophic adaptive laboratory evolution. *Microb. Biotechnol.* 18:e70208. doi: 10.1111/1751-7915.70208

International Energy Agency (2024) Energy efficiency 2024. Available online at: https://www.iea.org/reports/energy-efficiency-2024 (Accessed July 26, 2026)

Kanso, M., Robles-Rodriguez, C. E., and Tiruta-Barna, L. (2025). CO_2 as an alternative carbon source for ethanol production: a simulation-based technical and environmental analysis. *J. CO_2 Util.* 96:103108. doi: 10.1016/j.jcou.2025.103108

Katsyv, A., and Müller, V. (2020). Overcoming energetic barriers in acetogenic C1 conversion. *Front. Bioeng. Biotechnol.* 8:621166. doi: 10.3389/fbioe.2020.621166

Khalid, H., Amin, F. R., Gao, L., Chen, L., Chen, W., Javed, S., et al. (2024). Syngas conversion to biofuels and biochemicals: a review of process engineering and mechanisms. *Sustain. Energy. Fuels* 8, 9–28. doi: 10.1039/d3se00916e

Kim, J. Y., Lee, M., Oh, S., Kang, B., Yasin, M., and Chang, I. S. (2023). Acetogen and acetogenesis for biological syngas valorization. *Bioresour. Technol.* 384:129368. doi: 10.1016/j.biortech.2023.129368

Klask, C.-M., Kliem-Kuster, N., Molitor, B., and Angenent, L. T. (2020). Nitrate feed improves growth and ethanol production of *Clostridium ljungdahlii* with CO_2 and H_2, but results in stochastic inhibition events. *Front. Microbiol.* 11:724. doi: 10.3389/fmicb.2020.00724

Köpke, M., Held, C., Hujer, S., Liesegang, H., Wiezer, A., Wollherr, A., et al. (2010). *Clostridium ljungdahlii* represents a microbial production platform based on syngas. *Proc. Natl. Acad. Sci. USA* 107, 13087–13092. doi: 10.1073/pnas.1004716107

Köpke, M., and Simpson, S. D. (2020). Pollution to products: recycling of "above ground" carbon by gas fermentation. *Curr. Opin. Biotechnol.* 65, 180–189. doi: 10.1016/j.copbio.2020.02.017

Küsel, K., Dorsch, T., Acker, G., Stackebrandt, E., and Drake, H. L. (2000). *Clostridium scatologenes* strain SL1 isolated as an acetogenic bacterium from acidic sediments. *Int. J. Syst. Evol. Microbiol.* 50, 537–546. doi: 10.1099/00207713-50-2-537

Kwon, S. J., Lee, J., and Lee, H. S. (2022). Metabolic changes of the acetogen *Clostridium* sp. AWRP through adaptation to acetate challenge. *Front. Microbiol.* 13:982442. doi: 10.3389/fmicb.2022.982442

Kwon, S. J., Lee, J., and Lee, H. S. (2024). Exploring auxotrophy and engineering vitamin B6 prototrophy in the acetogen *Clostridium* sp. AWRP. *Appl. Environ. Microbiol.* 90:e01160-24. doi: 10.1128/aem.01160-24

Lakhssassi, N., Baharlouei, A., Meksem, J., Hamilton-Brehm, S. D., Lightfoot, D. A., Meksem, K., et al. (2020). Ems-induced mutagenesis of *Clostridium carboxidivorans* for increased atmospheric CO_2 reduction efficiency and solvent production. *Microorganisms* 8:1239. doi: 10.3390/microorganisms8081239

Lauer, I., Philipps, G., and Jennewein, S. (2022). Metabolic engineering of *Clostridium ljungdahlii* for the production of hexanol and butanol from CO_2 and H_2. *Microb. Cell Factories* 21:85. doi: 10.1186/s12934-022-01802-8

Lee, J., Lee, J. W., Chae, C. G., Kwon, S. J., Kim, Y. J., Lee, J. H., et al. (2019). Domestication of the novel alcohologenic acetogen *Clostridium* sp. AWRP: from isolation to characterization for syngas fermentation. *Biotechnol. Biofuels* 12:228. doi: 10.1186/s13068-019-1570-0

Liang, J., Huang, H., and Wang, S. (2019). Distribution, evolution, catalytic mechanism, and physiological functions of the flavin-based electron-bifurcating NADH-dependent reduced ferredoxin: $NADP^+$ oxidoreductase. *Front. Microbiol.* 10:373. doi: 10.3389/fmicb.2019.00373

Liu, Z.-Y., Zhang, K.-D., Shi, P.-H., Fan, Y.-X., Zha, X.-S., Li, L.-P., et al. (2026). Aldehyde dehydrogenase inactivation triggers metabolic reprogramming in *Clostridium ljungdahlii*. *Green Carbon.* doi: 10.1016/j.greenca.2026.03.002

Mariën, Q., Flores-Alsina, X., Aslam, U., Gernaey, K. V., Regueira, A., and Ganigué, R. (2024a). Isobutyric and n-butyric acid production from H_2 and CO_2 by *Clostridium luticellarii* in a hollow fiber membrane biofilm reactor: reactor operation and process modelling. *Chem. Eng. J.* 495:153216. doi: 10.1016/j.cej.2024.153216

Mariën, Q., Regueira, A., and Ganigué, R. (2024b). Steerable isobutyric and butyric acid production from CO_2 and H_2 by *Clostridium luticellarii*. *Microb. Biotechnol.* 17:e14321. doi: 10.1111/1751-7915.14321

Mariën, Q., Regueira, A., Petrognani, C., Scarborough, M., and Ganigué, R. (2026). Enhancing carbon capture and utilization: mixotrophic growth of *Clostridium luticellarii* using methanol and hydrogen for efficient CO_2 reduction. *Bioresour. Technol.* 444:133886. doi: 10.1016/j.biortech.2025.133886

Mock, J., Zheng, Y., Mueller, A. P., Ly, S., Tran, L., Segovia, S., et al. (2015). Energy conservation associated with ethanol formation from H_2 and CO_2 in *Clostridium autoethanogenum* involving electron bifurcation. *J. Bacteriol.* 197, 2965–2980. doi: 10.1128/JB.00399-15

Mukherjee, T., Kopperi, H., Kumari, P., Javvadi, Y., Katakojwala, R., Venkata Mohan, S., et al. (2026). India's ammonia industry: balancing growth with sustainability. *RSC Sustain.* 4, 2993–3007. doi: 10.1039/d5su00936g

Nagaraju, S., and Koepke, M. (2017). *Microorganism with Modified Hydrogenase Activity*. Geneva: World Intellectual Property Organization.

Nissen, L. S., and Basen, M. (2019). The emerging role of aldehyde:ferredoxin oxidoreductases in microbially-catalyzed alcohol production. *J. Biotechnol.* 306, 105–117. doi: 10.1016/j.jbiotec.2019.09.005

Petrognani, C., Mariën, Q., De Vos, L., van Oijen, M., Boon, N., and Ganigué, R. (2026). CO_2 availability as process tool to enhance isobutyric acid production in methanol fermentation by *Clostridium luticellarii*. *Bioresour. Technol.* 459:135255. doi: 10.1016/j.biortech.2026.135255

Philips, J. (2020). Extracellular electron uptake by acetogenic bacteria: does H_2 consumption favor the H_2 evolution reaction on a cathode or metallic iron? *Front. Microbiol.* 10:2997. doi: 10.3389/fmicb.2019.02997

Phillips, J. R., Atiyeh, H. K., Tanner, R. S., Torres, J. R., Saxena, J., Wilkins, M. R., et al. (2015). Butanol and hexanol production in *Clostridium carboxidivorans* syngas fermentation: medium development and culture techniques. *Bioresour. Technol.* 190, 114–121. doi: 10.1016/j.biortech.2015.04.043

Poehlein, A., Zeldes, B., Flaiz, M., Böer, T., Lüschen, A., Höfele, F., et al. (2025). Advanced aspects of acetogens. *Bioresour. Technol.* 427:131913. doi: 10.1016/j.biortech.2024.131913

Ragsdale, S. W., and Pierce, E. (2008). Acetogenesis and the Wood-Ljungdahl pathway of CO_2 fixation. *Biochim. Biophys. Acta Proteins Proteom.* 1784, 1873–1898. doi: 10.1016/j.bbapap.2008.08.012

Ramachandriya, K. D., Kundiyana, D. K., Wilkins, M. R., Terrill, J. B., Atiyeh, H. K., and Huhnke, R. L. (2013). Carbon dioxide conversion to fuels and chemicals using a hybrid green process. *Appl. Energy* 112, 289–299. doi: 10.1016/j.apenergy.2013.06.017

Ricci, L., Agostino, V., Fino, D., and Re, A. (2021). Screening of gas substrate and medium effects on 2,3-butanediol production with *C. ljungdahlii* and *C. autoethanogenum* aided by improved autotrophic cultivation technique. *Fermentation* 7:264. doi: 10.3390/fermentation7040264

Riegler, P., Bieringer, E., Chrusciel, T., Stärz, M., Löwe, H., and Weuster-Botz, D. (2019). Continuous conversion of CO_2/H_2 with *Clostridium aceticum* in biofilm reactors. *Bioresour. Technol.* 291:121760. doi: 10.1016/j.biortech.2019.121760

Rodriguez, K., Joshi, J., Greening, C., and Marcellin, E. (2026). Model acetogens as chassis for CO_2-driven bioproduction. *Curr. Opin. Biotechnol.* 97:103423. doi: 10.1016/j.copbio.2025.103423

Schick, B., Poduschnick, L., Engelmann, A., and Magnus, J. B. (2026). Design of experiments based optimization of pH and carbon dioxide to hydrogen ratio for enhanced acetate and biomass formation by *Clostridium ljungdahlii*. *Bioresour. Technol.* 459:135165. doi: 10.1016/j.biortech.2026.135165

Schuchmann, K., and Müller, V. (2013). Direct and reversible hydrogenation of CO_2 to formate by a bacterial carbon dioxide reductase. *Science* 342, 1382–1385. doi: 10.1126/science.1244758

Schuchmann, K., and Müller, V. (2014). Autotrophy at the thermodynamic limit of life: a model for energy conservation in acetogenic bacteria. *Nat. Rev. Microbiol.* 12, 809–821. doi: 10.1038/nrmicro3365

Schwarz, F. M., Schuchmann, K., and Müller, V. (2018). Hydrogenation of CO_2 at ambient pressure catalyzed by a highly active thermostable biocatalyst. *Biotechnol. Biofuels* 11:237. doi: 10.1186/s13068-018-1236-3

Song, Y., Lee, J. S., Shin, J., Lee, G. M., Jin, S., Kang, S., et al. (2020). Functional cooperation of the glycine synthase-reductase and Wood-Ljungdahl pathways for autotrophic growth of *Clostridium drakei*. *Proc. Natl. Acad. Sci. USA* 117, 7516–7523. doi: 10.1073/pnas.1912289117

Stoll, K. I., Herbig, S., Zwick, M., Boukis, N., Sauer, J., Neumann, A., et al. (2018). Fermentation of H_2 and CO_2 with *Clostridium ljungdahlii* at elevated process pressure—first experimental results. *Chem. Eng. Trans.* 64, 151–156. doi: 10.3303/CET1864026

Suazo, A., Olm, I. P., Martínez-Ruano, J., Conejeros, R., Aroca, G., and Sousa, D. Z. (2026). Influence of H_2:CO_2 ratio and low CO supplementation on product distribution and carboxylate reduction in *Clostridium autoethanogenum*. *Chem. Eng. J.* 509:172982. doi: 10.1016/j.cej.2026.172982

Thunuguntla, R., Atiyeh, H. K., Huhnke, R. L., and Tanner, R. S. (2024). CO_2-based production of C2-C6 acids and alcohols: the potential of novel *Clostridia*. *Bioresour. Technol. Rep.* 25:101713. doi: 10.1016/j.biteb.2023.101713

Thunuguntla, R., Tanner, R. S., and Atiyeh, H. K. (2025). Harnessing *Clostridium ragsdalei* for conversion of CO_2 into ethanol: insights from batch and continuous fermentation. *Bioresour. Technol.* 421:132133. doi: 10.1016/j.biortech.2025.132133

Thunuguntla, R., Tanner, R. S., and Atiyeh, H. K. (2026). Medium composition, temperature, and CO supplementation control CO_2 conversion to C2–C6 alcohols by *Clostridium muellerianum* P21. *Bioresour. Technol.* 456:134887. doi: 10.1016/j.biortech.2026.134887

Tiong, Y. W., Yong, A., Nalaparaju, A., Nguyen, H.-H., Koh, L. Q., Wong, R. J., et al. (2026). Experimental and multiphysics simulations study of *Clostridium carboxidivorans* for acid and alcohol production in CO_2/H_2. *Fuel* 411:137867. doi: 10.1016/j.fuel.2025.137867

Tremblay, P. L., Zhang, T., Dar, S. A., Leang, C., and Lovley, D. R. (2013). The Rnf complex of *Clostridium ljungdahlii* is a proton-translocating ferredoxin: NAD+ oxidoreductase essential for autotrophic growth. *mBio* 4:e00406-12. doi: 10.1128/mBio.00406-12

Valgepea, K., Loi, K. Q., Behrendorff, J. B., Lemgruber, R. S. P., Plan, M., Hodson, M. P., et al. (2017). Arginine deiminase pathway provides ATP and boosts growth of the gas-fermenting acetogen *Clostridium autoethanogenum*. *Metab. Eng.* 41, 202–211. doi: 10.1016/j.ymben.2017.04.007

Wang, S., Huang, H., Kahnt, J., Mueller, A. P., Köpke, M., and Thauer, R. K. (2013a). NADP-specific electron-bifurcating [FeFe]-hydrogenase in a functional complex with formate dehydrogenase in *Clostridium autoethanogenum* grown on CO. *J. Bacteriol.* 195, 4373–4386. doi: 10.1128/JB.00678-13

Wang, S., Huang, H., Kahnt, J., and Thauer, R. K. (2013b). A reversible electron-bifurcating ferredoxin- and NAD-dependent [FeFe]-hydrogenase (HydABC) in *Moorella thermoacetica*. *J. Bacteriol.* 195, 1267–1275. doi: 10.1128/JB.02158-12

Wang, S., Huang, H., Moll, J., and Thauer, R. K. (2010). $NADP^+$ reduction with reduced ferredoxin and $NADP^+$ reduction with NADH are coupled via an electron-bifurcating enzyme complex in *Clostridium kluyveri*. *J. Bacteriol.* 192, 5115–5123. doi: 10.1128/JB.00612-10

Wen, Z. Q., Li, Y. Z., Su, H. J., Zhang, J. Z., Liu, Z. Y., Wang, S. N., et al. (2026). Deletion of electron-bifurcating [FeFe]-hydrogenase enhances H_2-driven CO_2 fixation and ethanol production in *Clostridium ljungdahlii*. *Bioresour. Technol.* 453:134636. doi: 10.1016/j.biortech.2026.134636

Wirth, S., and Dürre, P. (2021). Investigation of putative genes for the production of medium-chained acids and alcohols in autotrophic acetogenic bacteria. *Metab. Eng.* 66, 296–307. doi: 10.1016/j.ymben.2021.04.010

Zahn, J. A. (2012) *Novel ethanologenic Clostridium species, Clostridium coskatii*. U.S. Patent No 8143037. Washington, DC: U.S. Patent and Trademark Office

Zhang, J., and Greasham, R. (1999). Chemically defined media for commercial fermentations. *Appl. Microbiol. Biotechnol.* 51, 407–421. doi: 10.1007/s002530051411

Zitscher, T., and Kaltschmitt, M. (2024). Sustainable carbon utilization for a climate-neutral economy–framework necessities and assessment criteria. *Energies* 17:4118. doi: 10.3390/en17164118